This thesis is in a form
acceptable for
the award of the degree.

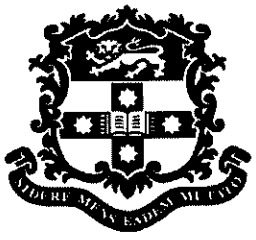
EFFECTS OF SPRINT TRAINING ON METABOLIC AND IONIC REGULATION DURING INTENSE EXERCISE IN SUBJECTS WITH AND WITHOUT TYPE 1 DIABETES MELLITUS

Alison Rosemary Harmer

BAppSc (Physiotherapy) (with Distinction)

A thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy



School of Exercise and Sport Science, Faculty of Health Sciences

The University of Sydney

April, 2001

CANDIDATE'S CERTIFICATE

I, Alison Rosemary Harmer, certify that this thesis has not been submitted to another university or institution as a part or whole requirement for a higher degree. The work contained herein is original and is solely that of the author, except as otherwise acknowledged.

..... Alison Rosemary Harmer

ACKNOWLEDGEMENTS

I am indebted to my original supervisors, the late Professor John R. Sutton (The University of Sydney) and Associate Professor Michael McKenna (Victoria University of Technology). Professor Sutton passed away on February 7th, 1996, shortly before the commencement of the second study. Professor Sutton was an inspirational, gifted and generous man. His enthusiasm for science, excellent medical skills, good sense of humour and his adventurous approach to life were always evident, and helped much in putting subjects at ease during testing. I am indeed privileged to have had such a remarkable man as my supervisor and mentor. The scientific papers arising from this thesis are dedicated to the memory of Professor John Robert Sutton.

Associate Professor McKenna provided both excellent academic input and innumerable hours of assistance with data collection and assays in the first study, throughout which he demonstrated a meticulous scientific approach. I am also very appreciative of his extremely thorough and constructive critical review of sections of the thesis, both for the first and second studies. His input has been invaluable.

I thank Professor Len Storlien (University of Wollongong) who acted as interim supervisor after Professor Sutton's death.

I am grateful to Associate Professor Martin Thompson (The University of Sydney), who then assumed the role of supervisor, for many hours of assistance with insertion of venous lines and blood sampling, and for reading and commenting on the thesis.

I am very appreciative of the assistance provided by my associate supervisor, Professor Don Chisholm of the Garvan Institute of Medical Research, Sydney. Professor Chisholm has greatly honoured his friendship with Professor Sutton in providing me with invaluable clinical advice, critically reviewing the thesis, and generously providing for the insulin and glucagon assays.

I thank all my subjects for their magnificent efforts during testing and training. I am especially thankful to my sister Carolyn, who volunteered as a subject in the second study. My subjects have richly demonstrated that "grey is all theory, but ever green is the tree of life" (Goethe).

I am highly appreciative of the friendship, and of the invaluable assistance in the biochemistry laboratory, of Patricia Ruell, Professional Officer and fellow postgraduate. Pat has very high scientific standards and was generous and patient enough to share her wealth of biochemical knowledge on numerous occasions. In addition, she meticulously performed the catecholamine assays and set-up and helped execute the vanadate-facilitated [^{3}H]ouabain binding site assay.

I am very grateful for the many, many (early) hours of assistance in collecting and handling blood and muscle samples, supporting my subjects, and for the friendship extended to me by my then fellow postgraduates, Dr Sandra Hunter, Jeanette Thom, Dr Norm Morris, John Booth, and Dr Regina Crameri. I am also very grateful to fellow postgraduate Justine Naylor for stimulating discussions and unflagging friendship. I am grateful to Tom Gwinn for advice and discussions on physiology at various stages. Thanks to postgraduates Xanne Janse de Jong and Chris Hill who also afforded friendship and helped during testing.

I am grateful to Drs Grace Bryant and James Harrison, who skilfully performed the muscle biopsies and assisted with blood collection in the second study; and to Dr Greg Bennett, who inserted venous lines and sampled blood on several occasions, and provided me with employment for several years.

I am very appreciative of the assistance of Dr Rod Snow, Deakin University, who gave advice and practical help in the muscle assays (especially with regard to HPLC analysis), and who carefully read and criticized sections of the thesis. I thank Associate Professor Michael Carey (VUT), for allowing me access to the biochemistry laboratory and for generously providing consumables for, and advice on, muscle assays. I thank Chris Stathis (VUT) for assistance in the laboratory.

I am very appreciative of the excellent technical assistance of Nadine Mackay and Diane Eager, who spent many hours with me in the laboratory, assisting with blood collection and processing. Nadine also provided support to my subjects throughout testing and training, for which I am very grateful.

I thank Kuet Li and Donna Wilks of the Garvan Institute, who carefully performed the insulin and glucagon assays.

I thank Janet Bryson, Department of Biochemistry (The University of Sydney), for skilfully performing the PDH, HK and CS assays.

I thank Ray Patton and Tim Turner for excellent technical assistance at various stages.

I am also grateful to the undergraduate students who assisted with data collection and subject training, especially Fiona MacPhee and Fiona Griffin.

I thank Drs Jeff Flack, Andrew Krzyszton (Bankstown-Lidcombe Diabetes Centre), and Rob Coles for assisting with recruiting subjects with type 1 diabetes. I thank Dr Flack for arranging for the HbA_{1c} and progesterone assays to be performed at Liverpool Hospital.

I am very grateful to Dr Marg Torode for her assistance in the final stages of the candidature.

I thank Dr Roger Adams (School of Physiotherapy) for statistical advice.

I am fortunate to have been the recipient of a Sydney University Postgraduate Research Award. I am very grateful to the American College of Sports Medicine Foundation and the Gatorade Sports Science Institute (Australasia) for awarding me Doctoral Student Research Grants, without which many assays could not have been performed. Similarly, I am very grateful to the School of Exercise and Sport Science, The University of Sydney, for generous provision of funds throughout the duration of the thesis.

I am very appreciative of the encouragement and interest of family and friends throughout the course of the candidature.

Finally, I am eternally thankful to the Lord, God, through whom all things were made, for sustaining me throughout the thesis, and for giving me light and salvation.

DEDICATION

This thesis is dedicated to my husband, Philip Lloyd Harmer,
and to my parents, David and Rosemary Badman.

Phil has been wonderfully encouraging and enduringly loving and patient throughout the protracted duration of the thesis. I could not have completed the thesis without him. My parents have always encouraged me, provided loving support, and have been ever interested in the thesis. I cannot sufficiently express my love and gratitude to Phil and my parents, except to say that I am eternally indebted to all three.

ABSTRACT

This thesis examined the effects of a 7-week sprint training programme upon integrated metabolic, ionic, and respiratory responses during maximal exercise. Two separate studies were conducted. **Study 1** examined the adaptations effected by sprint training in metabolic, ionic, and respiratory variables during a single bout of constant load exercise conducted (i) to exhaustion, and (ii) under conditions of identical work pre- and post-training, in untrained men. **Study 2** investigated firstly the acute metabolic, ionic, and respiratory responses to a single bout of maximal exercise in subjects with type 1 diabetes mellitus (T1D) and compared them with those of a non-diabetic group (ND). Secondly, Study 2 examined the effect of sprint training on metabolic control in the T1D group. Thirdly, Study 2 investigated the metabolic, ionic, and respiratory adaptations to sprint training during constant load maximal exercise under conditions of identical work pre- and post-training in the T1D group and compared them with those of the ND group.

In **Study 1**, untrained men cycled to exhaustion at 130% pre-training $\dot{V}O_{2peak}$ before (PreExh) and after training (PostExh), as well as performing another post-training test with identical work to PreExh (PostMatch). Muscle biopsies were taken at rest and immediately post-exercise in each test, and arterialised venous blood was sampled at rest and at various times during exercise and recovery. In separate tests, ventilation and gas exchange were assessed whilst exercising to exhaustion pre- (PreResp) and post-training (PostResp). Sprint training was conducted thrice-weekly with each session entailing four (Week 1) to ten (Weeks 6, 7), 30-s maximal cycling bouts.

After training, in PostExh, exercise time to exhaustion was extended 21% ($P<0.001$). Despite greater work being performed after training, net ATP degradation and IMP accumulation were reduced ($P<0.05$). The net degradation of PCr did not differ between PreExh and PostExh. In PostExh, whilst glycogen degradation and muscle lactate accumulation were unchanged in comparison to PreExh, the rise in muscle $[H^+]$ was reduced ($P<0.05$). Peak values for plasma catecholamines, $[Lac^-]$, and $[H^+]$ were higher ($P<0.05$), and the SID and $[A_{tot}]$ were lower ($P<0.01$), with peak plasma $[K^+]$ unchanged during exhausting exercise after training. Peak $\dot{V}_E$ was higher during PostResp than PreResp, however $\dot{V}O_2$, $\dot{V}CO_2$ and HR did not differ between tests.

In the post-training matched-work test (PostMatch), net ATP degradation and IMP accumulation were markedly reduced ($P<0.01$), whilst PCr degradation was unchanged in comparison to PreExh. Glycogen degradation, and muscle Lac^- and H^+ accumulation were also reduced ($P<0.05$) compared with PreExh. In plasma, peaks for $[K^+]$, [NAdr], $[Lac^-]$, $[H^+]$ and PCO_2 were all significantly lower after training in PostMatch.

Study 1 demonstrated that sprint training resulted in improved cellular energy balance, reduced cellular acidosis, and lower glycogenolysis during maximal exercise, suggesting that aerobic metabolism was enhanced, which may have allowed an increased time to fatigue. The inclusion of both exhausting and matched-work tests after training allowed clarification of some discordant findings in the sprint training literature.

In **Study 2**, untrained men and women with and without type 1 DM cycled to exhaustion at 130% $\dot{V}O_{2peak}$ prior to training (PreFB) and performed an identical matched-work test after training (PostMB). Both groups were postabsorptive and the T1D group delayed administration of the morning insulin dose prior to testing. Muscle biopsies were taken at rest and immediately post-exercise in each test, and arterialised venous blood was sampled at rest and at various times during exercise and recovery. In separate tests, ventilation and gas exchange were assessed whilst exercising to exhaustion pre- (PreFResp) and post-training (PostFResp), and when exercising for the same time as PreFResp after training (PostMResp). In another separate test, conducted only in the T1D group (T1D 'Resting Study'), the effect over time of delaying the morning insulin dose was examined at rest for selected metabolic and ionic variables.

In PreFB, time to exhaustion did not differ between T1D and ND groups. Whilst glycogen degradation, glycolytic intermediate accumulation, and the glycogenolytic rate was similar between groups, muscle Lac^- content was higher at exhaustion in the T1D group ($P<0.05$). Consistent with higher Lac^-, muscle anaerobic ATP production ($P<0.01$) was higher in the T1D group. PDH*a* at rest tended to be lower (24%) in the T1D group, however this difference did not attain statistical significance. High-

energy phosphate degradation during maximal exercise did not differ between groups. Maximal *in vitro* HK activity at rest did not differ between groups, however, HK activity was reduced after exercise in the ND group, but not the T1D group ($P<0.01$). Muscle [^{3}H]ouabain binding site content did not differ between groups prior to training. Plasma glucose concentration rose sharply with exercise, and continued to rise, unabated for the remainder of recovery in the T1D group, in contrast ($P<0.001$) to the transient rise evident in the ND group. Peak [NAdr] was higher in the T1D group ($P<0.05$) whilst plasma [Lac^-] did not differ. Plasma [K^+] and [Na^+] did not differ between groups during exercise and early recovery, however were respectively higher and lower ($P<0.05$) after 45-60 min recovery in the T1D group. The cardiorespiratory responses to maximal exercise were essentially similar in the two groups.

The percentage HbA_{1c} was lower ($P<0.05$) and daily insulin dose unchanged in the T1D group after training. In PostMB, muscle glycogen degradation ($P=0.052$), pyruvate ($P<0.05$) and lactate accumulation, the glycogenolytic and glycolytic rates, and net ATP degradation ($P<0.01$) during maximal matched-work exercise were all reduced in comparison to PreFB, with no difference between groups. Similarly, muscle anaerobic ATP production, and the rate of production, were both lower during matched-work exercise after training ($P<0.01$). After training, PDH*a* was lower at rest ($P<0.05$), with no difference between groups. Both HK ($P<0.001$) and CS ($P<0.01$) activities were increased at rest in both groups after training, however, similar to effects noted in the PreFB test, both activities fell with exercise in the ND group ($P<0.05$), but not the T1D group. [^{3}H]ouabain binding site content increased after training ($P<0.05$) with no difference between groups. In PostMB, whilst IRI was unchanged, the rise in plasma glucose in response to exercise tended to be lower (22-26%) in both groups, however did not attain statistical significance. Plasma [Lac^-] and [H^+] were lower after training in PostMB, with no difference between groups. Plasma [K^+] and [Na^+] were lower in PostMB in both groups ($P<0.05$), and the late rise in [K^+] in PreFB in the T1D group was not evident in PostMB. Mean [PG], P_{CO_2}, and Hct were higher, and [HCO_3^-] and [Cl^-] lower in the T1D group across all times in PreFB and PostMB ($P<0.05$). In PostFResp, time to exhaustion was extended ($P<0.001$) 64% in the T1D group and 33% in the ND group compared to PreFResp, tending to be higher in the T1D group ($P=0.06$). In PostMResp, the peak

values for $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$ were lower than in PreFResp, with the T1D group having a lower (P=0.06) peak $\dot{V}CO_2$ than the ND group. Mean $\dot{V}_E/\dot{V}CO_2$ and $\dot{V}_E/\dot{V}O_2$ were lower in the T1D group (P<0.05) than ND group across the three respiratory tests.

In the T1D **'Resting Study'**, selected metabolic and ionic variables showed little change over time, in marked contrast to the changes evident in the PreFB and PostMB exercise tests. Thus, interpretation of the responses during the exercise tests for the T1D group was not confounded by underlying (or supervening) metabolic deterioration consequent to delaying the morning insulin dosage.

It is concluded that the lower glycogen degradation found in Study 1, and the reduced rates of glycogenolysis, glycolysis and anaerobic ATP production, and increased CS activity found in Study 2, after training, when considered with the reduction in perturbation of other muscle metabolites evident in both studies, is consistent with enhanced oxidative metabolism as a consequence of sprint training. However, it cannot be excluded that enhanced muscle lactate transport may have contributed to the findings. It is also concluded that a short programme of sprint training may be undertaken with metabolic safety by subjects with type 1 DM in moderate metabolic control. Further, despite the 'fixed' insulin concentration prevailing during exercise and recovery in the T1D subjects, and hence some differences in acute responses to maximal exercise, that the metabolic and ionic adaptations and cardiorespiratory responses to sprint training are essentially similar in subjects with and without type 1 DM.

TABLE OF CONTENTS

VOLUME I

VOLUME II

LIST OF TABLES

LIST OF FIGURES

SUMMARY OF TEST TERMINOLOGY: STUDIES 1 AND 2

STUDY 1

Pre-training tests:

PreExh Invasive constant load test conducted to exhaustion at a power output calculated to elicit 130% $\dot{V}O_{2peak}$, in which blood and muscle samples were obtained

PreResp Non-invasive (respiratory) constant load test conducted to exhaustion at a power output calculated to elicit 130% $\dot{V}O_{2peak}$, in which expired gas was collected and ventilation assessed

Post-training tests:

PostMatch Invasive matched-work constant load test conducted for the same time as PreExh at a power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, in which blood and muscle samples were obtained

PostExh Invasive constant load test conducted to exhaustion at a power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, in which blood and muscle samples were obtained

PostResp Non-invasive (respiratory) constant load test conducted to exhaustion at a power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, in which expired gas was collected and ventilation assessed

N.B. Each of the above tests was conducted at the same power output

STUDY 2

Pre-training tests:

PreFB Invasive constant load test conducted to exhaustion at a power output calculated to elicit 130% $\dot{V}O_{2peak}$, in which blood and muscle samples were obtained

PreFResp Non-invasive (respiratory) constant load test conducted to exhaustion at a power output calculated to elicit 130% $\dot{V}O_{2peak}$, in which expired gas was collected and ventilation assessed

Post-training tests:

PostMB Invasive matched-work constant load test conducted for the same time as PreExh at a power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, in which blood and muscle samples were obtained

PostFB Invasive constant load test conducted to exhaustion at a power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, in which blood and muscle samples were obtained

PostMResp Non-invasive (respiratory) matched-work constant load test conducted for the same time as PreFResp at a power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, in which expired gas was collected and ventilation assessed

PostFResp Non-invasive (respiratory) constant load test conducted to exhaustion at a power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, in which expired gas was collected and ventilation assessed

N.B. Each of the above tests was conducted at the same power output

T1D 'Resting Study' A test conducted at rest for the same duration as the PreFB and PostMB tests, designed to examine the effect of delaying the morning insulin dose on metabolic and ionic control in the T1D group

ABBREVIATIONS

1. SUBJECT GROUPS

ND Non-diabetic group, Study 2

T1D Group with type 1 diabetes mellitus, Study 2

2. CARDIORESPIRATORY VARIABLES

HR Heart rate

$\dot{V}CO_2$ Carbon dioxide output

$\dot{V}E$ Expired ventilation

$\dot{V}E \cdot \dot{V}O_2^{-1}$ Ventilatory equivalent for oxygen

$\dot{V}E \cdot \dot{V}CO_2^{-1}$ Ventilatory equivalent for carbon dioxide

$\dot{V}O_2$ Oxygen uptake

RER Respiratory exchange ratio

Subscripts

peak Peak value obtained during incremental test to exhaustion

peak 130 Peak value obtained during tests conducted at 130% $\dot{V}O_{2peak}$

3. GLUCOREGULATION

Adr Adrenaline

BG Blood glucose

BGL Blood glucose level

CSII Continuous subcutaneous insulin infusion

DM Diabetes mellitus

GLUT4 Insulin-regulable glucose transporter

HbA_{1c} Glycosylated haemoglobin

IRG Immunoreactive glucagon

IRI Immunoreactive insulin

i.v. Intravenous

NAdr Noradrenaline

MCR Metabolic clearance rate

PG	Plasma glucose
R_a	Rate of appearance
R_d	Rate of disappearance
STZ	Streptozotocin
[ ]	Brackets denote concentration

4. ION REGULATION

A_{tot}	Total weak acids
Cl^-	Chloride
H^+	Hydrogen ion
HCO_3^-	Bicarbonate
K^+	Potassium
Lac^-	Lactate
Na^+	Sodium
SID	Strong ion difference, which in plasma = $([K^+] + [Na^+]) - ([Lac^-] + [Cl^-])$

5. BLOOD VARIABLES

Hct	Haematocrit
Hb	Haemoglobin
FFA	Free fatty acids
PPr^-	Plasma protein
PV	Plasma volume
Δ	Delta

Subscripts

a	arterial
a-v	arteriovenous
v	venous

6. MUSCLE METABOLISM AND ION REGULATION

ADP	Adenosine 5'-diphosphate
AMP	Adenosine 5'-monophosphate
ATP	Adenosine 5'-triphosphate
ATPase	Adenosine triphosphatase
$\beta_{in\ vitro}$	*In vitro* buffering capacity
$\beta_{in\ vivo}$	*In vivo* buffering capacity
Ca^{2+}	Calcium
CHO	Carbohydrate
CoA	Coenzyme A
Cr	Creatine
CS	Citrate synthase
DCA	Dichloroacetate
d.m.	dry mass
F 6-P	Fructose 6-phosphate
G 1-P	Glucose 1-phosphate
G 6-P	Glucose 6-phosphate
GS	Glycogen synthase
GP	Glycogen phosphorylase
[^{3}H]ouabain	tritiated ouabain
HAD	β-hydroxyacyl-CoA dehydrogenase
HCD	High carbohydrate diet
HK	Hexokinase
HMP	Hexose monophosphates
IMP	Inosine 5'-monophosphate
LCD	Low carbohydrate diet
LDH	Lactate dehydrogenase
NAD^+	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NH_4^+	Ammonia
PCr	Creatine phosphate
PDH	Pyruvate dehydrogenase
PDHP	Pyruvate dehydrogenase phosphatase

PDK	Pyruvate dehydrogenase kinase
PFK	6-phosphofructokinase
P_i	Inorganic phosphate
Pyr^-	Pyruvate
SDH	Succinate dehydrogenase
TCA	Tricarboxylic acid
TCr	Total creatine
w.w.	Wet weight

Italicised or subscripted fonts

a	Active form
b	Less active form
f	Free
t	Total

1. INTRODUCTION

Intense exercise results in a marked elevation in ATP utilization, provokes considerable metabolic and ionic perturbation in contracting skeletal muscle, and is characterised by a rapid onset and pronounced degree of muscular fatigue which is evidenced by a decline in power output (McCartney *et al.*, 1986; Sharp *et al.*, 1986; Kowalchuk *et al.*, 1988b; Nevill *et al.*, 1989; Spriet *et al.*, 1989; McKenna *et al.*, 1993; Putman *et al.*, 1995a; McKenna *et al.*, 1997a; Hargreaves *et al.*, 1998). Whilst performance during single or repeated bouts of maximal exercise is usually enhanced following a programme of sprint training (Sharp *et al.*, 1986; Boobis *et al.*, 1987; Bell & Wenger, 1988; Nevill *et al.*, 1989; Stathis *et al.*, 1994; McKenna *et al.*, 1997b; MacDougall *et al.*, 1998), the fundamental metabolic and ionic mechanisms enabling this adaptation remain controversial.

Improved performance during maximal exercise following sprint training has been linked with an enhanced glycolytic rate in the exercising skeletal muscle (Sharp *et al.*, 1986; Nevill *et al.*, 1989; Linossier *et al.*, 1993). Augmented muscle glycolysis is suggested by findings of higher phosphofructokinase (PFK) activity (Sharp *et al.*, 1986; Jacobs *et al.*, 1987; Nevill *et al.*, 1989; Linossier *et al.*, 1993; MacDougall *et al.*, 1998), and higher accumulation of muscle lactate (Sharp *et al.*, 1986; Boobis *et al.*, 1987; Nevill *et al.*, 1989; Linossier *et al.*, 1993), and glycolytic intermediates (Nevill *et al.*, 1989) following fatiguing maximal exercise. However, the glycolytic rate has also been demonstrated to be unchanged during maximal exercise following sprint training (Boobis *et al.*, 1983). A lack of change after sprint training has been reported for PFK (Pilegaard *et al.*, 1999) and glycogen phosphorylase (GP) activities (Sharp *et al.*, 1986; MacDougall *et al.*, 1998). Similarly, after sprint training, no change was evident during exhaustive exercise in glycogen degradation (Nevill *et al.*, 1989), glucose 6-phosphate (G 6-P) accumulation (Linossier *et al.*, 1993), muscle lactate (Lac^-) accumulation (Stathis *et al.*, 1994), or the arterio-venous blood Lac^- concentration difference across the exercising leg (McKenna *et*

al., 1997b; Pilegaard *et al.*, 1999). Thus, the effects of sprint training on glycolysis during maximal exercise are unclear.

Recent work has demonstrated a significant contribution of oxidative metabolism to ATP generation during single and repeated bouts of brief maximal exercise. In the final 15 s of a maximal 30-s cycle sprint, 60% of the ATP was produced oxidatively, and in the final 15 s of the third of three 30-s bouts, oxidative metabolism generated 80% of the ATP (Parolin *et al.*, 1999). Averaged over the duration of the first and third 30-s bouts, the contribution of oxidative metabolism to total ATP generation was 28-29% (Withers *et al.*, 1991; Putman *et al.*, 1995a), and 63%, respectively (Putman *et al.*, 1995a). Repeated bouts of maximal exercise, such as undertaken in a programme of sprint training, may therefore be anticipated to enhance oxidative ATP generation during an acute bout of maximal exercise. Although muscle oxidative enzyme activities are higher following sprint training (Jacobs *et al.*, 1987; Cadefau *et al.*, 1990; MacDougall *et al.*, 1998; Pilegaard *et al.*, 1999), oxygen uptake ($\dot{V}O_2$) during brief, intense exercise was unchanged in one study (Nevill *et al.*, 1989) and tended to be higher in another after training (McKenna *et al.*, 1997b).

Consequences of intense exercise include muscle adenine nucleotide degradation and muscle K^+ loss. The effect of sprint training on each of these also remains unresolved. For example, adenine nucleotide degradation during maximal exercise was reduced following training in one study (Stathis *et al.*, 1994), but unchanged in two others (Boobis *et al.*, 1987; Nevill *et al.*, 1989). Whilst sprint training increased muscle sodium-potassium ATPase (Na^+-K^+-ATPase) content (McKenna *et al.*, 1993), and increased net Na^+ and K^+ uptake by contracting muscle during intense exercise (McKenna *et al.*, 1997a), the expected training effect of reduced exercise-induced hyperkalaemia was not evident after sprint training (McKenna *et al.*, 1993; McKenna *et al.*, 1997a).

Thus, despite extensive investigation into the effects of sprint training upon muscle metabolism, and to a lesser extent, ionic regulation and respiratory responses, many important issues remain unresolved. Further, very few studies (Nevill *et al.*, 1989;

McKenna *et al.*, 1997a; McKenna *et al.*, 1997b) have adopted an integrative approach whereby each of respiratory, metabolic and ionic adaptations to sprint training has been examined. With one exception (Nevill *et al.*, 1989), each of the above studies has solely compared a pre-training test with a post-training test in which maximal exercise was conducted to exhaustion, and hence the latter generally entailed greater work. Some of the discordant findings evident in the literature may be resolved by replicating the work achieved during the pre-training exhausting test in a further post training test.

Thus, the first study of this thesis examined the effects of sprint training upon metabolic, ionic, and cardiorespiratory variables during maximal exercise when exercising to exhaustion at a constant workrate, and when pre- and post-training work was matched.

Whilst acute responses to maximal exercise and effects upon such with sprint training have been extensively studied in healthy men, and to a lesser extent in healthy women, there have been very few studies that have examined acute and chronic effects of maximal exercise in other groups, e.g. those with chronic conditions such as type 1 diabetes mellitus (DM). Based upon participation of those with type 1 DM in high-intensity sports and physical activities, a 1994 review (Wasserman & Zinman, 1994) highlighted a need for research into the effects of high intensity exercise. However, very few studies that have examined the effects of intense exercise upon those with type 1 DM have been performed, and the effect of sprint training is unknown.

The effect of high intensity submaximal exercise on plasma glucose concentration has been examined in subjects with type 1 DM on continuous subcutaneous insulin infusion (Mitchell *et al.*, 1988) or after insulin injection (Zander *et al.*, 1983). Two studies investigated the effect of exhausting exercise at 80-100% $\dot{V}O_{2peak}$ on the regulation and rates of appearance and disappearance of glucose in moderately-trained subjects with type 1 DM with a basal intravenous insulin infusion (Purdon *et al.*, 1993; Sigal *et al.*, 1994b). No studies have reported upon the effects of delaying the morning insulin dose (a common practice prior to undertaking endurance exercise) on glucoregulation during and after maximal exercise in subjects with type 1 DM. No sprint training studies have

included subjects with type 1 DM, and indeed, no studies have examined the effects of sprint training on glucoregulation in non-diabetic subjects.

Similarly, few studies have examined muscle metabolism in subjects with type 1 DM. Studies conducted at rest (see Table 2.15) have demonstrated that in postabsorptive (overnight fasted) subjects with type 1 DM who have delayed their morning insulin dose, muscle glycogen may be lower, muscle glucose higher, glycolytic intermediates similar, and muscle lactate and lactate dehydrogenase (LDH) activity higher than values reported at rest for non-diabetic subjects. Higher resting muscle lactate and LDH has been suggested to be related to a lower fraction of the active form of the enzyme pyruvate deydrogenase (PDH*a*) (Saltin *et al.*, 1979; Wallberg-Henriksson *et al.*, 1984). One study only has reported upon PDH*a* in postabsorptive patients with type 1 DM, with non-significantly lower values found after overnight euglycaemia (achieved with an i.v. insulin infusion).

In non-diabetic subjects, maximal exercise results in a considerable acceleration of glycogenolysis and glycolysis, with marked perturbation evident in the intracellular milieu. No studies have examined the effect of maximal exercise on muscle metabolism in subjects with type 1 DM.

Maximal exercise evokes a marked rise in plasma potassium concentration ($[K^+]$) that is rapidly reversed in recovery (Kowalchuk *et al.*, 1988b; Vøllestad *et al.*, 1994; McKenna *et al.*, 1997a). A number of reports have described higher plasma $[K^+]$ at rest in subjects with type 1 DM who had fasted overnight and delayed their morning insulin dose (Viberti, 1978; McNair *et al.*, 1982), yet skeletal muscle [^{3}H]ouabain binding site content may be higher than in non-diabetics (Schmidt *et al.*, 1994). Insulin has long been known to acutely reduce plasma $[K^+]$ (Briggs *et al.*, 1923-24; Harrop & Benedict, 1924), whereas acute reduction of basal insulin secretion results in progressive hyperkalaemia (DeFronzo *et al.*, 1978). Accordingly, in patients with type 1 DM, serum $[K^+]$ was found to increase curvilinearly with increasing blood glucose level (BGL) at rest (McNair *et al.*, 1982). Despite these differences in K^+ regulation evident at rest, no studies have reported

upon the effect of intense exercise and subsequent recovery on plasma [K^+] in those with type 1 DM. Such investigation would be of both scientific interest and clinical importance.

Additionally, sprint training has been demonstrated to enhance K^+ regulation during maximal exercise in non-diabetics (McKenna *et al.*, 1997a). Effects of such training on those with type 1 DM, in whom K^+ regulation may be altered, are unknown.

Thus, the second study of this thesis firstly investigated the metabolic, ionic, and cardiorespiratory responses to maximal exhausting exercise in a group of subjects with type 1 diabetes mellitus, and compared them with responses in a non-diabetic group. Secondly, the effects of sprint training upon the same variables were assessed and compared between the two groups.

2. REVIEW OF LITERATURE

The review of literature is organized such that effects of acute maximal exercise on a particular variable, effects of type 1 diabetes mellitus (DM) on the variable, and effects of sprint training on the variable are examined. The effect of maximal exercise in subjects with type 1 DM on many variables is largely unknown, and no sprint training studies have included those with type 1 DM: thus studies that have investigated high intensity submaximal exercise and modes of training other than sprinting have also been included.

The review is presented in five sections:

Section A - Type 1 DM, aspects of metabolic control, and exercise training

Section B - Cardiorespiratory responses

Section C – Glucoregulation

Section D – Ion regulation

Section E – Muscle metabolism

Brief summaries are included throughout the review and are presented in ***bold italics***.

Section A – Type I diabetes mellitus, aspects of metabolic control, and exercise training

2.1 Diabetes Mellitus (DM)

Currently, diabetes mellitus (DM) is one of six National Health Priority Areas (Australian Bureau of Statistics, 2000). Diabetes mellitus describes a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate, fat, and protein metabolism, resulting from defects in insulin secretion, insulin action, or both (Alberti *et al.*, 1998). A recent provisional report from the World Health Organization (WHO) recommended that disorders of glycaemia be classified under four categories according to aetiology and clinical stages (Alberti *et al.*, 1998). Type 1 DM, previously termed insulin-dependent diabetes mellitus, is characterised by β–cell destruction, usually leading to absolute insulin deficiency, and proclivity to ketoacidosis, and may be of autoimmune or non-autoimmune (idiopathic) origin. The causes of hyperglycaemia in type 2 DM, previously termed non-insulin-dependent diabetes mellitus, range from insulin resistance and a relative deficiency of insulin, to a predominantly secretory defect with or without insulin resistance (although almost always there is a major contribution from insulin resistance). Disorders of glycaemia such as those induced by genetic defects in β–cell function, genetic syndromes, drugs or chemicals, or endocrinopathies were grouped together as 'other specific types', and the final category was gestational DM. These recommendations for classification and diagnosis have been adopted to constitute the official position in Australasia (Colman *et al.*, 1999). This thesis will focus upon type 1 DM.

2.2 Type 1 diabetes mellitus

In 1995, approximately 0.4% of the Australian population had type 1 diabetes, with the incidence in males slightly higher than in females (Australian Bureau of Statistics, 1998). Type 1 DM is a chronic autoimmune (or, rarely, an idiopathic) disease, which causes β-cell destruction and leads to an absolute deficiency of insulin, hence necessitating administration of exogenous insulin to prevent hyperglycaemia and ketoacidosis. The genetic susceptibility to type 1 DM has a strong association with the major histocompatibilty region (HLA-DQ and/or DR class II molecules on chromosome 6), with disease risk possibly being modulated by the insulin gene on chromosome 11

and the cytotoxic T-lymphocyte antigen gene on chromosome 2 (Eisenbarth, 1986; Lernmark, 1999). The peak incidence of autoimmune type 1 DM occurs in childhood and adolescence, but may occur at any age (Alberti *et al.*, 1998). Thirty to fifty percent of identical twins are concordant for type 1 diabetes, thus genetic factors alone cannot account for the development of diabetes (Borg & Sherwin, 2000). In those with genetic susceptibility to the development of diabetes mellitus, an environmental event (e.g. viral, drug-induced) triggers an autoimmune response that results in the production of T-lymphocytes, anti-islet cell, anti-insulin, and anti-glutamic acid decarboxylase antibodies (Eisenbarth, 1986; Lernmark, 1999); a stage which may precede overt diabetes by up to 10 years (Eisenbarth, 1986). A stage of progressive loss of glucose-stimulated insulin secretion precedes the overt hyperglycaemia of diabetes, by which stage extensive β-cell destruction is evident, with total loss of β-cells after several years (Eisenbarth, 1986).

In idiopathic type 1 diabetes, a rare subtype, there is no HLA association and no evidence of β-cell autoimmunity, however β-cell destruction is evident (Borg & Sherwin, 2000).

2.2.1 Insulin resistance in type 1 DM

In patients with type 1 diabetes of 2 - 20 years' duration, insulin action was ~40% lower than in non-diabetic subjects, and was inversely related to the level of glycosylated haemoglobin, which exceeded 10% (Yki-Järvinen & Koivisto, 1986). Similarly, whole-body glucose clearance was reduced 60% during a hyperinsulinaemic euglycaemic clamp and was strongly inversely correlated with fasting plasma glucose concentration in subjects with type 1 DM of 10 years' duration (DeFronzo *et al.*, 1982). More specifically, femoral skeletal muscle glucose uptake was reduced 46% in moderately controlled (HbA_{1c} 8.5%) subjects with type 1 DM of only 5 years' duration (Nuutila *et al.*, 1993). These studies indicate that significant peripheral insulin resistance usually exists in subjects with type 1 DM of 2 – 20 years' duration and may be the consequence of chronic hyperglycaemia (Yki-Järvinen & Koivisto, 1986). The latter probably acts via the glucosamine pathway to impair glucose uptake.

During a sequential hyperglycaemic, then euglycaemic hyperinsulinaemic clamp, administered after a 24-hour period of hyperglycaemia (20 $mmol \cdot l^{-1}$) maintained by a

10% glucose infusion in subjects with type 1 DM, both forearm glucose uptake and total insulin-stimulated glucose disposal were reduced, compared to when the clamp was preceded by a 24-hour period of 'normoglycaemia' (7.1 $mmol \cdot l^{-1}$) with a saline infusion (Vuorinen-Markkola *et al.*, 1992). The reduction in total disposal was mostly accounted for by reduced non-oxidative glucose disposal, and was supported by lower muscle glucose uptake and less increase in muscle glycogen during hyperinsulinaemia; insulin-resistant effects that were suggested to be due to 'glucose toxicity' (Vuorinen-Markkola *et al.*, 1992). Acute insulin resistance, manifested by reduced insulin-mediated glucose disposal, was also induced in non-diabetic subjects by infusion of hypertonic mannitol (Bratusch-Marrain & DeFronzo, 1983), thus hyperosmolality may contribute to insulin resistance. In a recent review (Rossetti *et al.*, 1990), the pathogenetic consequences of chronic hyperglycaemia were identified as including microvascular complications (neuropathy, nephropathy, retinopathy), macrovascular disease, glucose toxicity which impaired carbohydrate metabolism, basement membrane thickening, and glycosylation of proteins.

2.2.2 Glycosylated haemoglobin (HbA_{1c}), postabsorptive and mean daily BGL, and daily insulin dosage

Glucose binds essentially irreversibly to certain proteins, e.g. haemoglobin, and slowly equilibrates over time. The degree of covalent modification (i.e. non-enzymatic glycation) of the β chain of haemoglobin A with D-glucose is a function of time and the extracellular glucose concentration (Stevens *et al.*, 1977; Mortensen & Christophersen, 1983; Brownlee, 1992). A fasting venous plasma glucose concentration <6.1 $mmol \cdot l^{-1}$ is considered to be 'normal', and one which is not associated with an increased risk of developing micro- and macrovascular complications (Alberti *et al.*, 1998). The percentage of total haemoglobin A_1 ($HbA_1 = HbA_{1a} + HbA_{1b} + HbA_{1c}$), and one of the minor fractions, haemoglobin A_{1c} (HbA_{1c}), that is glycosylated in non-diabetic subjects is usually in the range of 5-8% and 3–6%, respectively (Koenig *et al.*, 1976; Stevens *et al.*, 1977; Caro, 1994). HbA_1 is highly correlated ($r = 0.997$) with HbA_{1c} (Dunn *et al.*, 1979), and generally reflects the prevailing extracellular glucose level over a period of 3-12 weeks (Koenig *et al.*, 1976; Dunn *et al.*, 1979; Boden *et al.*, 1980; Alberti *et al.*, 1998). However, HbA_1 was demonstrated to rise within one week of heightened hyperglycaemia (Dunn *et al.*, 1979; Boden *et al.*, 1980), but to fall more slowly after

restitution of lower levels of glycaemia; hence the HbA_{1c} may be disproportionately representative of higher, rather than average, glucose concentrations (Boden *et al.*, 1980). The authors therefore concluded that changes in glycosylated haemoglobin are sufficiently prompt to allow detection of a rapidly deteriorating metabolic condition, however may be less sensitive in detecting an improving metabolic state (Boden *et al.*, 1980).

In the Diabetes Control and Complications Trial (DCCT) (DCCT Research Group, 1993; DCCT Research Group, 1995) in which 1,441 patients with type 1 DM were studied, the mean HbA_{1c} prior to commencing the trial was 8.8% in the primary prevention group and 8.9-9.0% in the secondary intervention group, with 50% of patients having an HbA_{1c} of between 7.8 and 10.1% (albeit those with HbA_{1c} below 6.5% were excluded from the trial, excellent metabolic control is not the clinical norm in type 1 DM). The degree of total glycaemic exposure, reflected by the HbA_{1c} and the duration of DM, was related to the risk of diabetic complications, with lower risk of retinopathy, nephropathy and neuropathy progression in patients with lower HbA_{1c}, i.e. better metabolic control (DCCT Research Group, 1995). An HbA_{1c} of 6% was associated with 50% less risk of retinopathy progression than an HbA_{1c} of 7%, and thus a goal of normoglycaemia was desirable, albeit difficult to achieve with only 5% of patients undergoing intensive therapy being able to maintain an average value around 6% (DCCT Research Group, 1993; DCCT Research Group, 1995). However, intensive therapy in the DCCT, initiated to reduce HbA_{1c}, resulted in a 3-fold higher risk of severe hypoglycaemia when compared to conventional treatment (DCCT Research Group, 1995). Therefore, goals recommended by the DCCT were to initiate treatment as soon as possible so as to reduce the level of chronic glycaemia (reflected by HbA_{1c}) to as close to normal as possible, without compromising the safety of the patient (DCCT Research Group, 1995). A 'therapeutic window' for HbA_{1c} of 7–7.5% has been suggested to be desirable to reduce the incidence of microvascular complications, whilst also minimising the incidence of severe hypoglycaemia (Dahl-Jørgensen *et al.*, 1994). A 2-yr study of patients with type 1 DM who were commenced on continuous subcutaneous insulin infusion, demonstrated significant reductions in total glycosylated haemoglobin (from 10.2 to 7.6%) and capillary basement membrane thickness (reduced 25% to be similar to non-diabetic values), but no change in either variable in a matched group maintained on conventional injection therapy (Raskin *et al.*, 1983). A significant correlation was found between the

level of glycosylated haemoglobin and basement membrane width, but not between duration of diabetes or age and basement membrane width (Raskin *et al.*, 1983).

A large cross-sectional study (the EURODIAB IDDM Complications Study) of patients with type 1 DM found that 25% of patients with a duration of diabetes of 5 years or less had microangiopathy (retinopathy and/or microalbuminuria), whereas 82% of those with diabetes for 14 years or more had microangiopathy (Karamanos *et al.*, 2000). Patients with a short duration of diabetes and microangiopathy had higher prevalence of cardiovascular disease, cigarette smoking and hypertension, but did not differ in degree of glycaemic control from those without microangiopathy (Karamanos *et al.*, 2000). The parallel evolution of micro- and macroangiopathy was suggested to reflect a generalized endothelial dysfunction. In contrast, patients with long-term diabetes and microangiopathy had significantly higher HbA_{1c}, cholesterol, triglycerides and higher systolic and diastolic blood pressures than those without microangiopathy (Karamanos *et al.*, 2000). In comparison to patients with diabetes of short duration and microangiopathy, patients with diabetes of long duration without microangiopathy had higher insulin doses, more frequent hypoglycaemia, better glycaemic control, lower blood pressure, a better lipid profile and better endothelial function (Karamanos *et al.*, 2000).

In a review of clinical, experimental and epidemiological evidence, it was concluded that hyperinsulinaemia could have a direct role in the development of atherosclerosis, and may also have an indirect role via its effects on dyslipoproteinaemia and hypertension (Stout, 1990). Due to the subcutaneous route of exogenous insulin administration in most patients with type 1 DM, relative hyperinsulinaemia exists overnight and between meals, and thus patients' tissues are exposed to higher levels of insulin than non-diabetics' (Stout, 1990). The attainment of good blood glucose control with the lowest possible insulin dose, and regular exercise, were strategies recommended to prevent or ameliorate the development of atherosclerotic lesions in type 1 DM (Stout, 1990).

2.2.3 Effects of exercise training on metabolic control in type 1 DM

No studies have examined the effect of sprint training on HbA_{1c}, glycaemia or insulin requirements either in subjects with or without type 1 DM. However, a number of

longitudinal and cross-sectional studies have examined the effects of submaximal exercise training on glycaemic control, glycaemia and insulin dosage in subjects with type 1 DM (Tables 2.1 and 2.2).

2.2.3.1 Effects of endurance training

Endurance training and general exercise of 15 to 60 min duration, whether continuous or intermittent, does not appear to improve metabolic control (Yki-Järvinen *et al.*, 1984; Zinman *et al.*, 1984; Mandroukas *et al.*, 1986; Wallberg-Henriksson *et al.*, 1986; Schneider *et al.*, 1992), however, may reduce daily insulin requirements (Table 2.1) (Costill *et al.*, 1979; Yki-Järvinen *et al.*, 1984; Schneider *et al.*, 1992). Carbohydrate intake may be increased with exercise training programmes (Zinman *et al.*, 1984), and coupled with reduced insulin requirements (which may suggest an improved sensitivity), may explain the lack of significant change in HbA_{1c} or fasting plasma glucose levels (Schneider *et al.*, 1992). However, one study (Costill *et al.*, 1979) did report a 17% reduction in fasting blood glucose level and a 23% reduction in daily insulin dose; the latter occurring within the first 2-3 weeks of endurance training. A mild cardiovascular conditioning programme, conducted at 60% maximum heart rate, reduced both HbA_{1c} and insulin dosage in subjects with type 1 or type 2 DM who had visual impairment from retinopathy (Table 2.1) (Bernbaum *et al.*, 1989). However, since that study included subjects with type 2 DM, in whom exercise training may improve HbA_{1c} (Schneider *et al.*, 1984; Trovati *et al.*, 1984; Zierath & Wallberg-Henriksson, 1992), and results were not presented separately for the two groups, the effect of exercise training in the type 1 DM subjects alone could not be determined.

In children and adolescents with type 1 DM who participated in thirteen, weekly 60 min exercise sessions (consisting of games, jogging, running, gymnastics) at an intensity that elevated HR to 150 $b \cdot min^{-1}$ for at least 45 min, training improved $\dot{V}O_{2peak}$, had no affect upon fasting blood glucose, but increased HbA_{1c} (Table 2.1) (Huttunen *et al.*, 1989). Those children who participated in fewer exercise sessions had poorer metabolic control after the training program, however, also began the program with significantly higher HbA_{1c}; whilst no deterioration of metabolic control was evident in matched subjects who did not participate in the training programme (Huttunen *et al.*, 1989). In adolescents with type 1 DM, twelve weeks of exercise training (during which subjects were

instructed not to alter insulin dose or diet) increased $\dot{V}O_{2max}$, did not alter HbA_{1c}, however did improve insulin sensitivity 23% (reflected by increased glucose utilization in a euglycaemic clamp); whereas a sedentary group showed no change (Table 2.1) (Landt *et al.*, 1985).

2.2.3.2 Effects of strength training or the addition of a strength training component

Twelve, 45 min sessions of combined aerobic and strength training significantly reduced HbA_{1c} from 7.56 to 6.85% in 7 subjects with type 1 DM (Jovanovic-Peterson *et al.*, 1989). Similarly, 12 weeks of thrice-weekly aerobic and weights circuit training significantly reduced HbA_{1c} in adolescents with well-controlled type 1 DM (with no change in insulin dosage), but did not affect HbA_{1c} in non-diabetic subjects (Mosher *et al.*, 1998) (Table 2.1). An 8-month exercise programme which entailed 15 min intermittent cycling at 70% of heart rate reserve and 15 min strength training performed 3 times per week, significantly reduced HbA_{1c} and reduced daily insulin dose in 8 out of 10 subjects with type 1 DM (Table 2.1) (Peterson *et al.*, 1979). In the same study, in 6 of 7 subjects from whom muscle biopsies were obtained, the 8-10 month exercise training reduced basement membrane width (by 33%) to values similar to those of non-diabetics (Peterson *et al.*, 1980). The other subject had neither an improvement in HbA_{1c} nor in width of the basement membrane (the latter actually increased) after training (Peterson *et al.*, 1980). Ten weeks of heavy resistance training reduced HbA_{1c} and fasting [BG] (mean of values measured before and after a training session), and tended to result in higher kilojoule intake, but did not affect daily insulin dose (Table 2.1) (Durak *et al.*, 1990). Although lean body mass was not reported in that study (Durak *et al.*, 1990), a strong inverse correlation between HbA_{1c} and muscle cross-sectional area was found after strength training in subjects with type 2 DM (Eriksson *et al.*, 1997).

2.2.3.3 Cross-sectional comparisons

A cross-sectional comparison between competitive athletes (mixed aerobic and anaerobic training) and sedentary subjects revealed fasting [BG] and insulin sensitivity to be similar, glycaemic control to be poorer, but insulin dose lower in athletes (Table 2.2) (Ebeling *et al.*, 1995). Kilojoule intake was not assessed, however it was suggested

Table 2.1: *Longitudinal studies examining training effects upon glycaemic control, glycaemia and insulin dose in diabetes mellitus*

Study	Training: *f*; duration	Training: duration, intensity	Subjects: *n*(M,F) DM/N	HbA_{1c} (%)		Fasting [PG] $mmol \cdot l^{-1}$		Mean daily BGL $mmol \cdot l^{-1}$		Insulin dose U/day	
				pre	post	pre	post	pre	post	pre	post
(Costill *et al.*, 1979)	5/week; 10 weeks	30 min 60-70%	(12,-)DM	-	-	13.2	11*	-	-	48	37
			(13,-) N			5.1	5.1				
(Peterson *et al.*, 1979)	3/week; 8 months	35min mixed ETx, STx	10(3,7) DM	10.3	7.6*	-	-	-	-	33.2	31.5
(Wallberg-Henriksson *et al.*, 1982)	2.4/week; 16 weeks	60min jog, run, games, gymnastics	(9,-) DM	10.4‡	11.3	12.5	12.8	-	-	49	49§
(Wallberg-Henriksson *et al.*, 1984)	3/week; 8 weeks	45min jogging or I/T running	(10,-) DM	9.8‡	10.8	-	-	~9.5	~9.5	44	44#
			(10,-) N	6.2‡	-	-	-	-	-	-	-
(Yki-Järvinen *et al.*, 1984)	4/week;	4x15min @ 70%	7(6,1) DM	8.6‡	8.9	6.6	7.3	5.8	6.3	35	33*
	6 weeks	No training	6(4,2) DM	8.4‡	9.2	4.7	6	10.5	6.8	34	34
		No training	25(19,6) N	-	-	5	5	-	-	-	-
(Zinman *et al.*, 1984)	3/week;	45min @ 60-85%	7(2,5) N	7.7	7.4	4.9	4.7	-	-	-	-
	12 weeks		13(7,6) DM	10.7	10.3	10.8	11.2			37.6	40.0
(Landt *et al.*, 1985)	3/week;	25min @160 $b \cdot min^{-1}$	9(3,6) DM	12	12	-	-	-	-	69	66
	12 weeks	No training	6(4,2) DM	12	12	-	-	-	-	62	62
(Mandroukas *et al.*, 1986)	3/week; 10 weeks	3x4min @ 80-90%, 23 min stat/dyn	9(6,3) DM†	10.6‡	10.2	9	9	-	-	37	36
(Wallberg-Henriksson *et al.*, 1986)	Daily; 5 months	15min @ 60-90%	(-,6) DM	10.4‡	~11	-	-	~8.7	~8.3	32	32
		No training	(-,7) DM	10.6‡	~11			~8.3	~8.8	43	43
(Bernbaum *et al.*, 1989)	3/week; 12 weeks	15-30 min @ 60% max HR	47(19,28) DM†	12.3	11.4*	12.1	11.9	-	-	48	41*
(Huttunen *et al.*, 1989)	1/week;	45min @150 $b \cdot min^{-1}$	17(10,7) DM	9.8	10.5*	13.4	14.0	-	-	-	-
	3 months	No training	17(10,7) DM	9.4	9.7	14.3	14.4	-	-	-	-

Table 2.1 (cont.): *Longitudinal studies examining training effects upon glycaemic control, glycaemia and insulin dose in diabetes mellitus*

Study	Training: *f*; duration	Training: duration, intensity	Subjects: *n*(M,F) DM/N	HbA_{1c} (%)		Fasting [PG] $mmol \cdot l^{-1}$		Mean daily BGL $mmol \cdot l^{-1}$		Insulin dose U/day	
				pre	post	pre	post	pre	post	pre	post
(Durak *et al.*, 1990)	3/week; 10 weeks	60min StrTx	(8,-) DM	6.9	5.8*	-	-	7.8	7.1*	46	42
(Schneider *et al.*, 1992)	3-4/week; 12 weeks	30-50 min @ 50-75% HRR	25-39 DM	~11.5	~11	11.6	11.6	-	-	-	↓18%
(Mosher *et al.*, 1998)	3/week; 12 weeks	45 min aerobic and weights circuit	(10,-) DM	7.72	6.76*	11.7	11.1	-	-	69	69
			(11,-) N	4.50	4.47	5.2	5.2	-	-	-	-

f, frequency; pre, pre-training; post, post-training; intensity given in % $\dot{V}O_{2max}$; (M,F), number of male, female subjects; DM, subjects with type 1 diabetes mellitus; N, non-diabetic subjects; ETx, endurance training; StrTx, strength training; *, significantly different pre- *vs* post-training; -, value not reported; stat/dyn, between 4 min cycles static and dynamic muscle power exercises were performed, so that total time of training was ~35 min; DM†, patient group consisted of both those with type 1 and type 2 diabetes mellitus; ‡ HbA_1 (HbA_{1a} + HbA_{1b} + HbA_{1c}) measured pre- and post-training; ~, approximation of value from a graph; §, no change, but 5 patients reduced insulin dose by 4 - 8 U on exercise days; #, 3 subjects reduced daily dose by 4U in the latter part of the training period.

Table 2.2: *Cross-sectional studies examining training effects upon glycaemic control, glycaemia and insulin dose in diabetes mellitus*

Study	Comparison	Training: mode; amount	Subjects: *n*(M,F) DM	HbA_{1c} (%)	Fasting [PG] ($mmol \cdot l^{-1}$)	Insulin dose ($U \cdot day^{-1}$)
(Selam *et al.*, 1992)	Exercisers	Sport/ walking; 2-4 hr/week	32 DM	8.3	-	40.8
	vs sedentary	No regular exercise	18 DM	7.9	-	40.2
(Ebeling *et al.*, 1995)	Athletes *vs*	Mixed ETx, ATx; 3-10hr/week	(11,-) DM	8.4	9.8	41
	sedentary	No regular exercise	(12,-) DM	7.2	9.5	53
(Veves *et al.*, 1997)	Exercisers	Running; 36-45 km/week	23(20,3) DM - AN	8.3	-	-
	vs sedentary		7(4,3) DM + AN	9.8	-	-
		No regular exercise	5(4,1) DM	8.9	-	-

(M,F), number of male, female subjects; N, non-diabetic subjects; DM, subjects with type 1 diabetes mellitus; -, value not reported; ETx, endurance training; ATx, anaerobic training; - AN/ + AN, subjects without or with autonomic neuropathy.

that impaired metabolic control may have been related to the difficulty in maintaining a regular diet when travelling and competing (Ebeling *et al.*, 1995). In contrast, subjects with type 1 DM, both with and without neuropathy who exercised regularly (running), had similar HbA_{1c} to sedentary subjects with type 1 DM (Table 2.2), despite HbA_{1c} being inversely related to $\dot{V}O_{2max}$ (Veves *et al.*, 1997). However, the small subject number in the exercising neuropathic, and sedentary groups may have precluded the finding of significant differences. Additionally, the duration of type 1 DM in the regular exercisers without neuropathy was ~50% less than in the other two groups, which may further cloud interpretation. Another cross-sectional study (Selam *et al.*, 1992) found no difference in HbA_{1c} between regular exercisers and sedentary patients (Table 2.2) who had had type 1 DM for ~16 years, with 21-31% of patients having an $HbA_{1c} \leq 6.8\%$. In a large cross-sectional study of 221 patients with type 1 DM of 14 years' duration, mean HbA_{1c} (8.7%) did not differ between those who exercised or those who were sedentary (Ligtenberg *et al.*, 1999). In both studies, daily insulin dose was inversely correlated with exercise energy expenditure/physical activity, however HbA_{1c} was not correlated with amount of exercise (Selam *et al.*, 1992; Ligtenberg *et al.*, 1999).

In summary, there have been no studies examining the effect of sprint training on metabolic control. Endurance exercise training, whether mild or moderate, when coupled with freely varying diet and daily insulin dosage, may result in mild deterioration in HbA_{1c} or fasting [BG] or have no effect, however may result in a reduction in daily insulin requirement. Similarly, those who are endurance-trained may have lower insulin requirements, but similar HbA_{1c} to sedentary subjects. Competitive athletes may similarly have lower insulin requirements, however may have poorer metabolic control than the sedentary. In contrast, strength training, or combined strength and endurance training, may improve HbA_{1c}. The reason for the difference in effect on metabolic control between endurance and strength training may be related to the increased lean body mass potentially effected by the latter, or the greater disturbance to the pattern of food and exercise in the former, however since very few studies have examined such in type 1 DM, conclusions are somewhat tenuous. Since chronic hyperglycaemia, reflected by increased HbA_{1c} and hyperinsulinaemia

are associated with micro- and macrovascular disease and other cellular complications, it is important to assess the effect of chronic exercise on glycaemic control and insulin dosage, since interventions that chronically alter glycaemia and/or insulinaemia may contribute significantly to morbidity and mortality from diabetes mellitus.

Section B – Cardiorespiratory responses

2.3 Cardiorespiratory responses during intense exercise and with sprint training

2.3.1 Ventilation ($\dot{V}_E$), oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and HR during maximal constant load exhausting exercise

Each of $\dot{V}_E$ (McKenna *et al.*, 1997b), $\dot{V}O_2$ (Putman *et al.*, 1995a; Bogdanis *et al.*, 1996; McKenna *et al.*, 1997b), and $\dot{V}CO_2$ (Putman *et al.*, 1995a; McKenna *et al.*, 1997b) was progressively increased during a 30-s or 60-s (Green *et al.*, 1987a) maximal exercise bout, with peak $\dot{V}_E$ (Kowalchuk *et al.*, 1988a; McKenna *et al.*, 1997b) and HR evident during exercise (McKenna *et al.*, 1997b). Peak $\dot{V}O_2$ (Kowalchuk *et al.*, 1988a; Putman *et al.*, 1995a) and $\dot{V}CO_2$ (Kowalchuk *et al.*, 1988a) were attained at the cessation, or ~8 – 15 s after the cessation of an exhaustive 30-s sprint (Putman *et al.*, 1995a; McKenna *et al.*, 1997b). $\dot{V}O_2$ was higher (Putman *et al.*, 1995a; Bogdanis *et al.*, 1996), and $\dot{V}CO_2$ was lower (Putman *et al.*, 1995a) at the end of the second or third bouts of 30 s maximal exercise when repeated intermittent cycling was undertaken.

2.3.2 The relative oxygen cost of hyperpnoea during maximal exercise

The relative cost of exercise hyperpnoea in healthy young subjects exercising at $\dot{V}O_{2peak}$ has been estimated to be 9-15% of $\dot{V}O_{2peak}$, is positively correlated to absolute $\dot{V}_E$ (Aaron *et al.*, 1992), and requires 14-16% of cardiac output ($\dot{Q}$) (Harms *et al.*, 1998). In addition, the greater the hyperventilation, reflected by an increasing ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$), the greater the percentage of whole body $\dot{V}O_2$ devoted to $\dot{V}_E$, i.e. the oxygen cost of ventilation at heavy work rates is increased, such that at near-maximum exercise (when absolute $\dot{V}_E$ averaged ~150 $l \cdot min^{-1}$), the percentage of the increase in whole body $\dot{V}O_2$ directed to ventilation averaged 39% (Aaron *et al.*, 1992). Due to the competition for blood flow at maximal $\dot{Q}$, a consequence of the significant relative cost of hyperpnoea in maximal exercise is vasoconstriction in the exercising muscles, which compromises muscle perfusion and

$\dot{V}O_2$ (Harms *et al.*, 1997; Harms *et al.*, 1998). The authors suggest that changes in a respiratory muscle chemoreflex, consequent to changes in respiratory muscle work, may mediate the leg muscle vasoconstriction by altering sympathetic activity (Harms *et al.*, 1997).

2.3.3 Effects of sprint training on performance and $\dot{V}O_{2\,peak}$

2.3.3.1 Performance

Sprint training usually, but not invariably, results in improved performance. Peak power was increased 4-26% in a single 30- to 60-s exhaustive bout of cycling or running (Katz *et al.*, 1984; Sharp *et al.*, 1986; Boobis *et al.*, 1987; Bell & Wenger, 1988; Nevill *et al.*, 1989; Linossier *et al.*, 1993; Andersen *et al.*, 1994; Stathis *et al.*, 1994; McKenna *et al.*, 1997b), and peak power averaged across repeated 30-s sprint bouts was increased 6-22% (McKenna *et al.*, 1993; MacDougall *et al.*, 1998) after 6-12 weeks of sprint training. Following 8 weeks of high-intensity intermittent, one-legged training, peak and mean power were increased 15-16% in the trained leg during a 30-s maximal test comprised of dynamic one-legged kicking (Pilegaard *et al.*, 1999). Time to exhaustion was increased in an open-ended test following 6-8 weeks of sprint or high intensity training (Cunningham & Faulkner, 1969; Sharp *et al.*, 1986; Pilegaard *et al.*, 1999). Peak running speed was increased 11% after 6 weeks of high intensity training (Cheetham & Williams, 1987), and mean running speed was increased 3-6% after 8 months of combined sprint and strength training in young athletes (Cadefau *et al.*, 1990). Interestingly, several studies failed to find an improvement in performance after 4-8 weeks of sprint training, despite the change detected in other variables, e.g. muscle enzyme profile, fibre type (Jacobs *et al.*, 1987; Jansson *et al.*, 1990; Esbjörnsson *et al.*, 1993; Allemeier *et al.*, 1994)

2.3.3.2 Peak oxygen uptake ($\dot{V}O_{2\,peak}$)

Sprint training, comprised of repeated exhaustive sprints, resulted in either no change, or a small increase in $\dot{V}O_{2peak}$ (Table 2.3). Training programmes that entailed repeated sprints of 20-50s duration generally resulted in an improvement in $\dot{V}O_{2peak}$, as opposed to studies that employed briefer (5-6s) sprint bouts. Thirty-second sprint bouts stimulate oxidative metabolism to a greater degree than very brief sprints (Green *et al.*, 1987a;

Medbø & Tabata, 1989; Withers *et al.*, 1991; Balsom *et al.*, 1992; Putman *et al.*, 1995a; Bogdanis *et al.*, 1996; McKenna *et al.*, 1997b; Parolin *et al.*, 1999), which may explain the lack of change in $\dot{V}O_{2peak}$ in training programmes using brief sprints. Another factor may be the duration of the recovery periods between sprints: longer recovery between briefer sprints may allow each sprint to be performed in a more anaerobic manner. According to the Fick equation, the mechanisms by which $\dot{V}O_{2peak}$ may be improved after sprint training include increases in cardiac output and/or oxygen extraction, however, as recently noted (McKenna *et al.*, 1997b), the effects of sprint training upon these variables remain to be clarified. $\dot{V}O_{2peak}$ is considered to be primarily limited by oxygen delivery (Ekblom *et al.*, 1968; Saltin & Rowell, 1980; Bassett & Howley, 2000; Richardson, 2000).

One study (Bell & Wenger, 1988) found an increased one-leg $\dot{V}O_{2peak}$ for the untrained leg following a 7-week one-legged sprint training programme, and therefore inferred that central changes in oxygen transport, i.e. blood flow or oxygen content, had occurred. Elite track sprinters had 10% higher muscle blood flow than sedentary subjects, however the difference was not significant (Leinonen, 1980). Sprint-trained rats exhibited higher leg blood flows at maximal exercise (Laughlin *et al.*, 1988), and possibly even higher blood flow in supramaximal exercise (Musch *et al.*, 1991) compared to sedentary rats. In man, a small increase (4%↑) in leg blood flow (LBF) at the end of exhausting incremental (n=2) or constant load (n=3) one-legged exercise (132% more work was accomplished after training in the latter) was measured in the trained leg following 8 weeks of high-intensity one-legged knee extensor training (Pilegaard *et al.*, 1999). However, the significance of the small change in LBF was not commented upon (although since neither lactate release nor the arteriovenous difference for lactate was altered after training, it may be inferred that LBF was also unchanged). Interpretation of their results (Pilegaard *et al.*, 1999) is somewhat difficult given that $\dot{V}O_{2peak}$ was not reported, the subjects were all habitually physically active prior to training, the n was small, and data from two different tests was combined. Blood flow was closely related to oxygen demand and increased linearly with power without leveling off in incremental one-legged exercise in physically trained subjects (Andersen & Saltin, 1985). However,

the distribution of blood flow and its limitations differ between one- and two-legged exercise in man, with one-legged exercise representing a state of 'hyperperfusion' (Rowell, 1993), and thus the effects of one-legged sprint training may differ from those of two-legged sprint training. To the author's knowledge, the effect of sprint training upon mean arterial pressure and peripheral resistance in exhausting incremental exercise has not been reported, however, endurance training does not alter arterial blood pressure at maximal exercise; any increase in maximal $\dot{Q}$ is accommodated by increased vascular conductance (Rowell, 1993).

A similar time course and magnitude of increase in $\dot{V}O_{2peak}$, leg bood flow and muscle capillarization has been shown with endurance training (Andersen & Henriksson, 1977; Saltin *et al.*, 1977; Klausen *et al.*, 1981; Saltin & Gollnick, 1983). The benefit of increased capillary density is related to the reduction of diffusion distances between capillary and muscle cell and the resultant enhancement of flow distribution (Andersen & Henriksson, 1977; Saltin & Rowell, 1980; Klausen *et al.*, 1981). Trained sprinters tended to have higher capillary diffusion capacity (13%) than sedentary subjects (Leinonen, 1980), or had a higher number of capillaries per fibre than recreational runners (Olesen *et al.*, 1994), however 8 weeks of training with high intensity one-legged knee extension repetitions did not alter muscle capillary density (Pilegaard *et al.*, 1999). A more protracted period of training may be required to induce significant change in capillarization.

2.3.4 Sprint training, cardiorespiratory variables and constant load exhausting exercise

After sprint training, during a 30-s exhaustive exercise bout in which mean power was increased 6%, $\dot{V}O_2$ was unchanged (Nevill *et al.*, 1989) or tended to be higher, as was peak $\dot{V}_E$ (11%), whereas peak HR was unchanged (McKenna *et al.*, 1997b). Peak values for $\dot{V}O_2$ and $\dot{V}CO_2$ occurred after exercise, with both tending to be 7-8% higher after sprint training (McKenna *et al.*, 1997b).

Table 2.3: *Effect of sprint training upon* $\dot{V}O_{2\,peak}$

Study	Training				$\dot{V}O_{2\,peak}$ ($l \cdot min^{-1}$)		
	f	Wks	#, duration of sprints	Rec.	Pre	Post	Δ
(Cunningham & Faulkner, 1969)	5	6	*x*, 200m or 4km continuous	jog 200m	3.75	4.06	8%↑*
(Thorstensson *et al.*, 1975)	3-4	8	20-40, 5s	25-55s	3.57	3.76	5%↑
(Saltin *et al.*, 1976)	4-5	4	20-30, 30-50s @ 150%	1-1.5	2.75	3.18	16%↑*
		one leg	Untrained leg		2.76	2.80	1%↑
			Both legs		3.34	3.64	9%↑*
(Katz *et al.*, 1984)	4	8	8, 30s	4	4.01	4.29	7%↑*
(Sharp *et al.*, 1986)	4	8	8, 30s	4	4.09	4.40	8%↑*
(Cheetham & Williams, 1987)	4-5	6	Mixed run, I/T, circuit	NR	3.06	3.18	4%↑*
(Bell & Wenger, 1988)	4	7	15-20, 20s @ 150%	1	3.36	3.72	11%↑*
		one leg	Untrained leg		3.36	3.57	6%↑*
			Both legs		3.96	4.17	5%↑*
(Nevill *et al.*, 1989)	3-4	8	2, 30s; 6-10, 6s; 2-5, 2min @ 110%	10; 54s; 5	3.47	3.53	2%↑
(Linossier *et al.*, 1993)	4	7	2 x 8-13, 5s	55s, 15 bw	3.26	3.28	1%↑
(Allemeier *et al.*, 1994)	2-3	6	3, 30s	20	3.7	4.2	14%↑
(Stathis *et al.*, 1994)	3	7	3-10, 30s	3-4	3.48	3.62	4%↑
(McKenna *et al.*, 1997b)	3	7	4–10, 30s	3-4	3.53	4.02	14%↑*
(MacDougall *et al.*, 1998)	3	7	4-10, 30s	2.5-4	3.73	4.01	8%↑*

$\dot{V}O_{2\,peak}$, peak oxygen consumption; *f*, frequency per week; wks, number of weeks of training; #, number of sprints; Rec., recovery period in minutes (unless specified as seconds); Pre, pre-training; Post, post-training; Δ, change in $\dot{V}O_{2\,peak}$; *x*, number of sprints individualised; I/T, intermittent; *, significant increase; @ 150% or 110%; exercise conducted at 150 and 110% of $\dot{V}O_{2\,peak}$, all other bouts were 'all out'; NR, not reported; bw, 15 min between sets.

Increased maximal $\dot{V}_E$ was suggested to be due partly to a greater $\dot{V}CO_2$ and partly to a greater neural drive and/or feedback, whilst the small increase in $\dot{V}O_2$ was suggested to be due to an increase in muscle blood flow, and when considered with the $\dot{V}CO_2$, to reflect a greater oxidative metabolism following sprint training (McKenna *et al.*, 1997b).

2.3.5 Sprint training, cardiorespiratory variables and constant load matched-work exercise

Two studies in which maximal work was matched before and after sprint training found either no change in $\dot{V}O_2$ during a 2-min run at 110% pre-training $\dot{V}O_{2peak}$ after 8 weeks of training (Nevill *et al.*, 1989), or a 3% lower $\dot{V}O_2$ averaged across 12-24, 60-s trials at 120% $\dot{V}O_{2peak}$ after 3 days of training (Green *et al.*, 1987a). However, with regard to the latter study, the authors note that significance was only achieved due to the very large number of measurements and trials (Green *et al.*, 1987a). $\dot{V}E$, $\dot{V}CO_2$, and HR were unchanged across all trials between the first and the third day, whereas the ventilatory equivalent for oxygen was increased (Green *et al.*, 1987a). In further contrast, following 6 weeks of mixed interval and high intensity continuous running, a maximal sprint at the same treadmill grade and speed resulted in a 48% higher net $\dot{V}O_2$ during exercise, whilst time to exhaustion was extended 23% (to 64 s) (Cunningham & Faulkner, 1969). $\dot{V}O_2$ and $\dot{V}E$ were similar in the first 30 s of exercise, however were higher between 30-45 s of the run after training, and it was suggested that oxidative phosphorylation (as well as glycolysis and creatine phosphate breakdown) and/or oxygen transport was increased (Cunningham & Faulkner, 1969). The lack of change in $\dot{V}O_2$ in the first 30 s of exercise after training was suggested to be due to limitations in oxygen transport and/or utilization (Cunningham & Faulkner, 1969). The $\dot{V}O_2$ during maximal matched-work exercise has thus been reported to be unchanged, higher or lower than before training, whilst $\dot{V}E$ was unchanged or higher. Therefore the effects of sprint training cardiorespiratory variables during maximal matched-work exercise are equivocal and suggest the need for further research.

2.3.6 Effects of sprint training on the on-transient for $\dot{V}O_2$ during matched-work exercise

Breathing hyperoxic gas when exercising at a constant load of 82% $\dot{V}O_{2peak}$ resulted in a faster on-transient for $\dot{V}O_2$ and a lower oxygen deficit than when breathing room air, suggesting that oxygen transport was the rate-limiting step for the increase in $\dot{V}O_2$ at the

start of heavy exercise (MacDonald *et al.*, 1997). If sprint training accelerated the on-transient for $\dot{V}O_2$, then a higher $\dot{V}O_2$ during exercise may result. However, the effect of sprint training upon the on-transient for $\dot{V}O_2$ (or $\dot{V}CO_2$) in intense exercise is not known. Four days of endurance training accelerated the on-transient for $\dot{V}O_2$ during constant load exercise at 60% $\dot{V}O_{2peak}$, with a further reduction after 30 days of training (Phillips *et al.*, 1995). The adaptation was suggested to be related to a more rapid increase in femoral blood flow (Phillips *et al.*, 1995).

2.3.7 Effects of sprint training on the oxygen cost of hyperpnoea

The effect of sprint training on the oxygen cost of hyperpnoea is unknown. However, a recent review (Harms & Dempsey, 1999) states that whole body exercise training has little effect on the respiratory muscles and no effect upon the airways, however may indirectly affect respiratory muscle work by reducing the ventilatory response to heavy exercise. Unloading of the respiratory muscles using a proportional-assist ventilator during exercise at $\dot{V}O_{2peak}$ reduced whole body $\dot{V}O_2$ and $\dot{Q}$ (via reduced SV) (Harms *et al.*, 1998) and increased exercising leg muscle $\dot{V}O_2$ and $\dot{Q}$ in trained men (Harms *et al.*, 1997). Since repeated bouts of maximal exercise impose heavy demands upon ventilation, and hence markedly increase respiratory muscle work, sprint training may provide a strong stimulus for adaptation. Peak $\dot{V}_E$ is reported to be similar or higher during exhausting exercise after sprint training (McKenna *et al.*, 1997b), and if higher, would be predicted (Aaron *et al.*, 1992) to entail an increased work of breathing. The affect of training on $\dot{V}_E$, and hence respiratory muscle work, when pre- and post-training work is matched during exercise is unknown. However, when competitive cyclists exercised at 90% $\dot{V}O_{2peak}$ with or without a proportional-assist ventilator, time to exhaustion was increased 13% during respiratory muscle unloading and was attributed to a blood flow redistribution from respiratory to exercising leg muscles (Wetter *et al.*, 1998).

2.4 Cardiorespiratory effects of sprint training in subjects with type 1 diabetes mellitus

No sprint training studies have included subjects with type 1 DM.

2.4.1 Effects of type 1 diabetes mellitus on cardiopulmonary factors that may limit improvements in $\dot{V}O_{2\,peak}$ and/or alter $\dot{V}O_2$ and cardiorespiratory variables during maximal exercise following sprint training

2.4.1.1 Cardiac factors

Cardiopulmonary function during exercise was assessed after a 7-year study in non-diabetics (mean age 31 yrs) and in subjects with long-standing type 1 DM (21 years' duration; mean age 39 yrs), who were assigned to hyperglycaemic (HbA_{1c} 8.8%) and normoglycaemic groups (HbA_{1c} 5.6%) (Niranjan *et al.*, 1997). Resting cardiac output ($\dot{Q}$) was lower in the DM groups due to lower SV (corrected for body surface area), and $\dot{V}O_{2max}$ was 15 and 34% lower in the normo- and hyperglycaemic DM groups, respectively, compared to non-diabetics, with significantly lower maximum heart rate (HR) and work load (39% lower) evident only in the hyperglycaemic group (Niranjan *et al.*, 1997), and a non-significant 17% reduction in maximum work load in the normoglycaemic group. A depressed maximal $\dot{Q}$ ($\dot{Q}_{max}$) was suggested to be a primary source of exercise limitation in diabetic subjects (Niranjan *et al.*, 1997). However, no baseline cardiopulmonary or fitness data were reported, and thus no conclusions can be made regarding the cause of the reduced $\dot{Q}_{max}$.

In young patients with type 1 DM and without overt heart disease, abnormalities were detected in early diastole after graded exercise and were correlated with diabetes duration (Jermendy *et al.*, 1990). The impaired left ventricular function was suggested to be related to myocardial collagen glycosylation and/or microangiopathy; however no abnormalities were found at rest or in systolic function after exercise, and exercise time to exhaustion was similar between non-diabetic and DM groups (Jermendy *et al.*, 1990). A study of 30 young normotensive patients with type 1 diabetes (HbA_{1c} 10%) demonstrated higher diastolic blood pressure, less reduction in peripheral vascular

resistance, and lower $\dot{Q}$ compared to matched non-diabetics during exercise at 80 W (49-56% maximum workload) and at $\dot{V}O_{2peak}$ (Matthys *et al.*, 1996). $\dot{V}O_2$ at 80 W did not differ between groups (implying that oxygen extraction must have been higher in the group with DM), however $\dot{V}O_{2peak}$ was lower in the patients (Matthys *et al.*, 1996). Although the two groups were well matched for age, weight, height, BMI, and gender, no exercise history was reported; however, considering the lower $\dot{Q}$ at a set absolute workload, factors other than differences in fitness must be involved. The authors suggested that less vasodilatation in the exercising muscles or endothelial insufficiency may have accounted for their findings (Matthys *et al.*, 1996). Subjects with type 1 DM had significantly lower capillary density than non-diabetics, particularly around type IIa muscle fibres (24% lower) (Saltin *et al.*, 1979); a factor that may be of significance in high intensity exercise.

2.4.1.2 Pulmonary factors and gas exchange

Incremental exercise to exhaustion resulted in lower $\dot{V}_E$ (27%), $\dot{V}O_{2peak}$, and maximal workload (both 20% lower) in subjects with type 1 DM of ~15 years' duration (HbA_{1c} 9.2%) than in non-diabetics, but no differences in arterial PO_2, PCO_2, or the alveolar-arterial PO_2 difference (Wanke *et al.*, 1991). Thus the authors concluded that gas transfer did not limit $\dot{V}O_{2peak}$. At the same absolute submaximal workloads HR was higher in the subjects with type 1 DM, however no other differences were found, even in a subgroup of patients in whom autonomic neuropathy was present (Wanke *et al.*, 1991). Exercise history was not assessed, however no subject had undertaken any exercise training, and it was speculated that reduced maximal stroke volume (SV) explained the lower $\dot{V}O_{2peak}$ in subjects without autonomic neuropathy, whilst an impaired HR response to exercise may have further contributed in the subjects with neuropathy (Wanke *et al.*, 1991).

Reduced elastic recoil at low to middle lung volumes, and similar or reduced diffusing capacity (due to reduced pulmonary capillary blood volume) was demonstrated in subjects with type 1 DM (including subjects with and without retinopathy) compared to non-diabetic subjects, and was suggested to be due to elastin and collagen abnormalities

(Schuyler *et al.*, 1976; Sandler *et al.*, 1987). Pulmonary dysfunction was correlated with duration of DM, but was not associated with the presence of other diabetic complications (Sandler *et al.*, 1987). Subjects maintained at near-normoglycaemia (HbA_{1c} 5.7%) by insulin pump therapy for 6 years had normal pulmonary function, whilst those on twice-daily insulin injection had chronic hyperglycaemia (HbA_{1c} ~9%) and reduced pulmonary diffusing capacity at rest (Ramirez *et al.*, 1991). Reduced diffusing capacity of the lung was also demonstrated during exercise in a chronically hyperglycaemic group, and an increased oxygen cost of breathing was evident in both a normo- and hyperglycaemic group during exercise: these differences were suggested to be due to a loss of elastic recoil after glycosylation of collagen and elastin from chronic hyperglycaemia, and/or to microangiopathy (Niranjan *et al.*, 1997). Since results for the normoglycaemic group were consistently normal, or less abnormal than those for the hyperglycaemic group, the authors suggested that enhanced glycaemic control may potentially retard or reverse cardiopulmonary abnormalities (Ramirez *et al.*, 1991; Niranjan *et al.*, 1997). Unfortunately no baseline cardiopulmonary data were reported in either study (Ramirez *et al.*, 1991; Niranjan *et al.*, 1997), and thus whilst the subjects were followed for 6-7 years on some variables (e.g. HbA_{1c}), conclusions regarding differences at rest or during exercise are limited to a cross-sectional comparison; which may have been influenced by differing fitness levels (exercise history was not reported), rather than, or as well as by glycaemia and possible end-organ damage.

2.4.1.3 Oxygen affinity of HbA_{1c}

Another factor of physiological relevance is that HbA_{1c} has a higher oxygen affinity at normal arterial oxygen saturation (i.e. shifts the oxyhaemoglobin dissociation curve to the left) than haemoglobin A, and the elevated levels of HbA_{1c} in diabetes thus tend to reduce the oxygen availability to areas of active oxygen uptake and may play a role in the pathogenesis of microangiopathy (Ditzel, 1976). A lower P_{50} (the PO_2 at 50% oxygen saturation), higher haemoglobin concentration ([Hb]), and higher concentration of 2,3-diphosphoglycerate (2,3-DPG; an end-product of red cell glycolysis which reduces the affinity of Hb for oxygen) was found in children with type 1 DM who had no overt signs of retinopathy or neuropathy (Ditzel, 1976). The last two findings may be early compensations for decreased oxygen availability (Ditzel, 1976). Acute exhausting

exercise did not change 2,3-DPG from resting values, and 2,3-DPG was unaltered by 8 weeks of sprint training in non-diabetic subjects (Katz *et al.*, 1984). The effect of sprint training on 2,3-DPG, [Hb] and P_{50} in subjects with type 1 DM is unknown.

2.4.2 Effects of endurance training in subjects with type 1 DM

Whilst no studies have examined the effects of sprint training on cardiorespiratory variables in subjects with type 1 DM, a number of studies have examined endurance training. Training studies of 6–20 weeks' duration, using either continuous or intermittent exercise conducted for 20-60 min, 2-4 times per week at lower intensities (~60-90% $\dot{V}O_{2peak}$) yielded 7-8% (Wallberg-Henriksson *et al.*, 1982; Yki-Järvinen *et al.*, 1984; Mandroukas *et al.*, 1986; Wallberg-Henriksson *et al.*, 1986), 11-13% (Costill *et al.*, 1979; Wallberg-Henriksson *et al.*, 1984), or 20% improvements in $\dot{V}O_{2peak}$ in adults with type 1 DM (Zinman *et al.*, 1984). (See Table 2.1 for exercise training details in the above studies.) Studies that included a non-diabetic group who also undertook exercise training reported similar improvements in $\dot{V}O_{2peak}$ to those with type 1 DM (Costill *et al.*, 1979; Wallberg-Henriksson *et al.*, 1984; Zinman *et al.*, 1984).

$\dot{V}O_{2peak}$ was 24% higher in competitive athletes with type 1 DM than in sedentary subjects with DM (Ebeling *et al.*, 1995). Similarly, in another cross-sectional study, $\dot{V}O_{2peak}$ did not differ between subjects with or without DM who exercised regularly (running 45–55 km per week at similar intensity); however in another group of type 1 DM subjects who had autonomic neuropathy and also exercised regularly at a similar intensity, $\dot{V}O_{2peak}$ was 24% lower, and was statistically comparable (albeit 10-15% higher) to values in sedentary subjects with or without DM (Veves *et al.*, 1997). Resting HR was higher in the group with neuropathy, similar to the two sedentary groups, and maximum HR was lower than in any other group (Veves *et al.*, 1997). Endothelial function in both the micro- and macrocirculation of the arm was reduced (essentially less vasodilatation) when all three groups with diabetes were considered together (Veves *et al.*, 1997). It was concluded that diabetes did not affect $\dot{V}O_{2peak}$ unless neuropathy was present, and that regular exercise training did not improve endothelial function (although the limitation of assessing the endothelium of the arm in subjects who trained by running

was acknowledged); despite a significant inverse correlation between HbA_{1c} and $\dot{V}O_{2peak}$ (Veves *et al.*, 1997). One complicating factor in that study (Veves *et al.*, 1997) was that although all subjects groups were matched for age, the duration of DM in the regularly exercising subjects (11 yr) was only half that of the sedentary (20 yr) and exercising neuropathic groups (22 yr).

Increases in $\dot{V}O_{2peak}$ in non-diabetic subjects have been shown to be correlated with increases in leg blood flow and muscle capillary density (as reviewed above). However, one cross-sectional study found that relatively fit subjects ($\dot{V}O_{2peak}$ ~51 $ml \cdot kg^{-1} \cdot min^{-1}$) with type 1 DM had 25% lower skeletal muscle capillary density (per mm^2 fibre area) (Saltin *et al.*, 1979), whereas another found 13% higher density in untrained subjects with type 1 DM (Wallberg-Henriksson *et al.*, 1984), in comparison to non-diabetic subjects who were matched for $\dot{V}O_{2peak}$. Increased density in untrained subjects was suggested to be a compensation for impaired capillary function (Wallberg-Henriksson *et al.*, 1984). Ten weeks of thrice-weekly training comprised of three, 4-min efforts at 80-90% $\dot{V}O_{2peak}$ (each separated by strength training activities) increased $\dot{V}O_{2peak}$, increased mean muscle fibre area, and non-significantly increased the number of capillaries per fibre (19%), but did not alter capillary density in subjects with type 1 (and several with type 2) DM (Mandroukas *et al.*, 1986). However, since the programme included a strength training component, the lack of increase in capillary density may reflect the training stimulus rather than an affect of diabetes *per se* (Mandroukas *et al.*, 1986). Similarly, 16 weeks of physical training (jogging, running, games, gymnastics) in subjects with type 1 DM increased the number of capillaries per muscle fibre, but did not alter capillary density since mean fibre cross-sectional area of the quadriceps also increased (Wallberg-Henriksson *et al.*, 1982). Following that study, the same authors demonstrated that 8 weeks of endurance training did not significantly alter gastrocnemius muscle fibre area, however resulted in a 14% increase in the number of capillaries per muscle fibre and an 8% increase in muscle capillary density in non-diabetic subjects, but no significant change in either variable in subjects with type 1 DM, despite a similar increase in $\dot{V}O_{2peak}$ (Wallberg-Henriksson *et al.*, 1984). Within the latter group, some of the subjects, those with the shortest duration of diabetes, did show

an increase in capillarization, leading the authors to suggest that microangiopathy (evidenced by basement membrane thickening) may have reduced new capillary formation in subjects with longer-term diabetes (Wallberg-Henriksson *et al.*, 1984).

In summary, sprint training usually results in improved performance and a small increase in $\dot{V}O_{2\,peak}$. $\dot{V}O_2$, $\dot{V}CO_2$, ***and*** $\dot{V}_E$ ***may be unchanged or higher during and immediately after exhausting constant load maximal exercise. The effects of sprint training upon*** $\dot{V}E, \dot{V}O_2$, $\dot{V}CO_2$, ***and HR during matched-work maximal exercise are equivocal.***

The effects of sprint training are unknown in subjects with type 1 DM. A number of cross-sectional lower intensity exercise studies that did include subjects with type 1 DM failed to match the non-diabetic group for exercise history, and thus differences in $\dot{V}O_{2\,peak}$ ***may have been due to different habitud physical activity levels rather than to diabetes per se. It is thus difficult to discriminate between the covert effects of type 1 diabetes, e.g microangiopathy, and differences in habitual physical activity level of subjects with or without type 1 DM when interpreting studies which report impaired*** $\dot{V}O_{2\,peak}$. ***One cross-sectional study that did match subjects with type 1 DM and non-diabetic subjects for habitual exercise level, found no difference in*** $\dot{V}O_{2\,peak}$ ***between groups, although did find a reduced*** $\dot{V}O_{2\,peak}$ ***in another group with neuropathy (even when matched for exercise training load and intensity), and endothelial dysfunction in the groups with DM. Additionally, microangiopathy and/or glycosylation of collagen and elastin was suggested to account for the reported pulmonary or cardiac dysfunction at rest or during exercise in normotensive subjects with type 1 DM. However, whilst the insidious nature of hyperglycaemia (and hyperinsulinaemia) in causing micro- and macro- tissue damage may play a role in determining responses to exercise in subjects with type 1 DM, it has been suggested that training status and exercise history are of considerably more import (Veves et al., 1997).***

Section C – Glucoregulation

2.5 Insulin and glucoregulation

Insulin, which has a half-life of 3–7 min, is a 2-chain polypeptide synthesised and secreted in a pulsatile fashion (Lefèbvre *et al.*, 1987) by the β-cells of the pancreas, and delivered via the portal vein to the liver, where it is metabolised and approximately 40-60% is eliminated (Samols & Ryder, 1961; Ferrannini *et al.*, 1983b; Hovorka & Jones, 1994). Thus, fasting, portal vein insulin concentration is approximately two to three times that of the peripheral veins (Blackard & Nelson, 1970; Horwitz *et al.*, 1975; Ferrannini *et al.*, 1983b). Higher plasma insulin concentrations increase the amount removed by splanchnic and peripheral tissues; with renal clearance accounting for the majority of extrasplanchinc insulin removal in the basal state (Ferrannini *et al.*, 1983b). C-peptide, which has a half-life of ~ 30 min, is co-secreted in approximately equimolar amounts with insulin from a common precursor (proinsulin) (Rubenstein *et al.*, 1969), and since it is eliminated by the kidney rather than by the liver, has been used to assess pre-hepatic insulin secretion. Due to hepatic extraction of insulin, and the longer half-life of C-peptide, the ratio of C-peptide to insulin always exceeds unity in peripheral blood (Horwitz *et al.*, 1975). Under conditions of steady-state, e.g. rest or submaximal exercise, insulin secretion is preferably assessed by examining the concentration of plasma C-peptide; however, when dynamic conditions prevail, e.g. intense exercise, the time-concentration profile of plasma insulin is preferable (Hovorka & Jones, 1994). Arterial insulin concentration is determined by the difference between appearance and disappearance of insulin, and reflects post-hepatic insulin delivery.

2.5.1 Physiological effects of insulin

Insulin is the major physiological anabolic hormone which acts (in opposition to the catabolic hormones, e.g. catecholamines, glucagon) to modulate cellular carbohydrate, fat, and protein metabolism by altering enzyme activity or intracellular location. Insulin acts upon the liver to inhibit glycogenolysis, gluconeogenesis and ketogenesis, and stimulate glycogen, fatty acid and triglyceride synthesis; upon the fat cell to inhibit lipolysis and enhance glucose uptake and fatty acid and triglyceride synthesis; upon the muscle cell to enhance glucose uptake, stimulate glycogen and protein

synthesis, and enhance the oxidation of pyruvate (Newsholme & Leech, 1983). Insulin acts upon the vasculature to induce vasodilation and increase blood flow (Richter *et al.*, 1989; Anderson *et al.*, 1991; Laakso *et al.*, 1992; Baron, 1994; Hespel *et al.*, 1995; Rosdahl *et al.*, 1998), but also to increase muscle sympathetic nervous activity and plasma noradrenaline concentration (Rowe *et al.*, 1981; Anderson *et al.*, 1991).

The binding of insulin to its tyrosine kinase receptor (IRK) results in autophosphorylation and increased IRK activity, which then initiates a number of diverse, complex cellular cascades within seconds (Cheatham & Kahn, 1995; Saltiel, 1996), and occurs in a dose- and time-dependent manner (Freidenberg *et al.*, 1994). Insulin dephosphorylates or phosphorylates many of the important rate-limiting enzymes in skeletal muscle, e.g. glycogen synthase and pyruvate dehydrogenase (Saltiel, 1996), and induces translocation of the GLUT4 intracellular glucose transporters (Gumà *et al.*, 1995). However, whilst the physiological effect of insulin occurs rapidly after the activation of IRK, there is a time delay from the rise in arterial insulin concentration to the increase in IRK activity (Freidenberg *et al.*, 1994). This rate-limiting step comprises the time taken for plasma insulin to reach the interstitial compartment (which is essentially in equilibrium with the receptor-bound insulin) (Freidenberg *et al.*, 1994), and is less prolonged in liver compared to muscle (Miles *et al.*, 1995). The difference in times for half-maximum response (after insulin infusion) in liver and muscle IRK activities versus portal and arterial insulin concentrations were ~9 and 28 min, respectively, in anaesthetized dogs (Miles *et al.*, 1995). In humans, intravenous (i.v.) glucose injection was followed by a rise in serum insulin, which peaked after 3 min, then slowly returned to basal levels over the next 100 min; in contrast, blood lactate remained at basal levels for 10 min, then slowly rose, with time to half-maximum concentration being between 20 to 30 min; kinetics consistent with the action of insulin in a remote compartment (interstitium) (Watanabe *et al.*, 1995). Arterialized venous insulin levels rose with a half-time ($t_{½}$) of ~5 min, whereas the $t_{½}$ for glucose disposal was 59 min, and for IRK activation was ~57 min, during a euglycaemic-hyperinsulinaemic clamp in humans, in which a steady-state insulin concentration of ~120 $\mu U \cdot ml^{-1}$, representing maximum physiological levels,

was used (Freidenberg *et al.*, 1994). The $t_{½}$ for glucoregulatory actions may be shortened after intense exercise (Wojtaszewski *et al.*, 1997) (see below).

Insulin-mediated vasodilation, characterised by an ED_{50} (dose to produce a half-maximal response) of ~40 $\mu U \cdot ml^{-1}$ and a $t_{½}$ of ~40–100 min, has been suggested to act as an amplifier of insulin actions in peripheral tissues (Laakso *et al.*, 1990; Baron, 1994); however, both the delay and the relatively minor (~2-fold) effect upon the vasculature (as compared to an ~10-fold effect upon glucose uptake) has caused others to question the physiological relevance, and to assert that insulin's vasodilatory action only occurs at pharmacological doses (Yki-Järvinen & Utriainen, 1998; Zierler, 1999).

2.5.2 Fasting insulin concentration in non-diabetics

The normal β-cell secretes ~30 U insulin per day (Ward, W. K. *et al.*, 1986). In healthy, non-diabetic subjects, fasting immunoreactive insulin (IRI) concentration ranged between 4–23 $\mu U \cdot ml^{-1}$ (24–138 $pmol \cdot l^{-1}$) (Yalow & Berson, 1960; Samols & Ryder, 1961; Cochran *et al.*, 1966; Sutton *et al.*, 1969; Pruett, 1970; Nakagawa *et al.*, 1973; Rennie *et al.*, 1974; Horwitz *et al.*, 1975; Galbo *et al.*, 1977; Green *et al.*, 1979; Hilsted *et al.*, 1981; Chisholm *et al.*, 1982; Ferrannini *et al.*, 1983b; Jenkins *et al.*, 1986; Kjær *et al.*, 1986; Richter *et al.*, 1988; Coyle *et al.*, 1991; Marliss *et al.*, 1991; Montain *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Clark & Hales, 1994; Sigal *et al.*, 1994a; Coggan *et al.*, 1995; Sigal *et al.*, 1996; Langfort *et al.*, 1997; Manzon *et al.*, 1998).

2.5.3 Fasting insulin concentration in subjects with type 1 DM – total vs free insulin

Patients with type 1 diabetes mellitus (DM) develop circulating antibodies to bovine-porcine insulin preparations within a few months of insulin treatment (Berson *et al.*, 1956; Berson & Yalow, 1964; Yki-Järvinen & Koivisto, 1986); thus the plasma or serum from these patients includes antibody-bound insulin, unbound or 'free' insulin, and free antibodies (Heding, 1972). Only free insulin is considered to be biologically active. The presence of endogenous antibodies to insulin results in an elevation of total IRI (Clark & Hales, 1994), and thus plasma or serum insulin, sampled from

subjects with type 1 DM, cannot be quantified by radioimmunoassay until the antibody-bound insulin is precipitated out by the addition of polyethylene glycol (PEG) (Nakagawa *et al.*, 1973). The advent of biosynthetic human insulin should theoretically have reduced the development of anti-insulin antibodies in patients with type 1 DM (Clark & Hales, 1994), however, these patients may also have antibodies, both to insulin (Fineberg *et al.*, 1983), and to circulating covalent insulin aggregates (Robbins *et al.*, 1987; Clark & Hales, 1994). Despite insulin-treated subjects exhibiting antibodies to insulin, an early report indicated that the reason that clinical insulin resistance was not a more common problem, was that the antibodies bind no more than 5–10 U of insulin per litre, which in a distribution space of ~7 litres, and with an approximate removal rate of 25% per day, equates to a wastage of only 17 U of insulin per day (Berson & Yalow, 1964). However, whilst the presence of antibodies to insulin may be of itself insufficient to result in significant insulin resistance, insulin resistance of a clinically significant degree is common in those with type 1 DM (DeFronzo *et al.*, 1982; Yki-Järvinen & Koivisto, 1986; Nuutila *et al.*, 1993).

Fasting, free insulin level, determined on patients with type 1 DM, using the PEG extraction technique prior to immunoassay, averaged between 4–12 $\mu U \cdot ml^{-1}$ (24–72 $pmol \cdot l^{-1}$) (Nakagawa *et al.*, 1973; Kuzuya *et al.*, 1977; Hilsted *et al.*, 1981; Meinders *et al.*, 1988; Vuorinen-Markkola *et al.*, 1992); was lower than (Nakagawa *et al.*, 1973; Hilsted *et al.*, 1981; DeFronzo *et al.*, 1982; Meinders *et al.*, 1988; Nuutila *et al.*, 1993), or similar to (Yki-Järvinen *et al.*, 1984; Yki-Järvinen & Koivisto, 1986; Kahn *et al.*, 1992; Sigal *et al.*, 1994b) levels in non-diabetic subjects; and did not correlate with insulin requirement, duration of insulin therapy, or total insulin levels (Nakagawa *et al.*, 1973; Kahn *et al.*, 1992). Since portal vein insulin is considerably higher than peripheral concentration (Blackard & Nelson, 1970; Horwitz *et al.*, 1975), similar or lower peripheral free insulin concentrations in subjects with type 1 DM compared to non-diabetics, may result in hepatic hypoinsulinaemia (Hanna *et al.*, 1980), and thus some degree of hyperglycaemia. Hyperglycaemia can acutely induce insulin resistance which is manifested by reduced whole-body and skeletal muscle glucose uptake and reduced glycogen synthesis in subjects with type 1 DM

(Vuorinen-Markkola *et al.*, 1992), however, it does not alter insulin removal (Ferrannini *et al.*, 1983b).

2.5.4 Effect of exercise on insulin concentration in non-diabetics

In moderate submaximal exercise, immunoreactive insulin (IRI) concentration is characteristically reduced in both continuous (Galbo *et al.*, 1977; Zinman *et al.*, 1979; Hilsted *et al.*, 1981; Chisholm *et al.*, 1982; Jenkins *et al.*, 1986; Hagberg *et al.*, 1988; Meinders *et al.*, 1988; Raguso *et al.*, 1995) and intermittent modes (Mæhlum *et al.*, 1977). The reduction in plasma insulin is necessary to allow a sufficient increment in hepatic glucose output and adipose tissue lipolysis. During a single bout of intense submaximal to maximal exercise in untrained subjects, IRI concentration either declines somewhat (Cochran *et al.*, 1966; Hartley *et al.*, 1972; Rennie *et al.*, 1974; Bloom *et al.*, 1976; Jakober *et al.*, 1983; Kjær *et al.*, 1986; Kjær *et al.*, 1990; Coggan *et al.*, 1995; Coggan *et al.*, 1997), or is not changed (Zander *et al.*, 1983; Mitchell *et al.*, 1988; Montain *et al.*, 1991). Recovery from intense submaximal to maximal exercise is characterised by marked increases in plasma IRI concentration, consequent to the sharp exercise-induced increase in plasma glucose concentration, with peak insulin concentration occurring approximately 5-20 min after the cessation of exercise, and a slow decline towards baseline values evident thereafter (Bloom *et al.*, 1976; Hermansen & Vaage, 1977; Kjær *et al.*, 1986; Mitchell *et al.*, 1988; Kjær *et al.*, 1990).

2.5.5 Effect of exercise on insulin concentration in type 1 DM

In submaximal exercise of 40 min duration, plasma free insulin concentration was unchanged in subjects with type 1 DM who had administered their last insulin dose 10 hours previously, and was lower prior to exercise than in non-diabetic controls (Hilsted *et al.*, 1981). However, when continuous i.v. or subcutaneous infusion of insulin was administered in subjects with type 1 DM, although plasma free insulin did not differ during non-exhaustive exercise conducted at ~75% $\dot{V}O_{2max}$ (Raguso *et al.*, 1995), or during exhaustive exercise at 80-100% $\dot{V}O_{2max}$ (Mitchell *et al.*, 1988; Purdon *et al.*, 1993), it was higher than in non-diabetic controls. One hour after breakfast and administration of morning insulin (dose reduced by 33%), a brief,

incremental cycle test to exhaustion produced no change in free IRI in subjects with type 1 DM (Hübinger *et al.*, 1985).

2.5.6 Exercise and the $t_{½}$ for insulin

A recent study in which an euglycaemic-hyperinsulinaemic clamp was applied after 3 hours recovery from 60 min of one-legged kicking at 75% maximum capacity, demonstrated increased arterial insulin concentration that occurred with a $t_{½}$ of <1 min (Wojtaszewski *et al.*, 1997). Increased glucose clearance occurred with a $t_{½}$ of ~20 min in the exercised leg, but 52 min in the non-exercised leg; and increased IRK activity occurred with a $t_{½}$ of 26 min which did not differ between legs (Wojtaszewski *et al.*, 1997). The effect following maximal exercise, in which marked changes in blood flow to the exercising muscles occur, has not been examined. However, these results imply that the $t_{½}$ for effects of insulin is accelerated after exercise, perhaps consequent both to enhanced insulin sensitivity and the high (but rapidly declining) blood flow immediately after exercise.

2.5.7 Insulin clearance and effects of exercise in subjects with and without type 1 DM

One study only has examined the effects of exercise on insulin clearance and included subjects both with and without type 1 DM (Tuominen *et al.*, 1997). In a hyperinsulinaemic euglycaemic clamp performed 12 hours after a marathon run, insulin clearance was augmented similarly in both groups in comparison to a similar test on a control day (Tuominen *et al.*, 1997).

In summary, plasma IRI usually declines or doesn't change during intense submaximal to maximal exercise in non-diabetics, but then rapidly increases in early recovery. Although few studies have reported plasma free IRI in untrained subjects with type 1 DM, during exercise ranging from sub- to maximal intensity and subsequent recovery, plasma free IRI does not change in subjects with type 1 DM; however, concentration may be higher or lower than non-diabetics depending on the timing and method of administration of the previous insulin dose. The most marked difference between subjects with and without type 1 DM will be in recovery when physiological increases in insulin do not occur in those with type 1 DM, and

thus peripheral effects vary accordingly. Further, exogenous administration of insulin, even if it results in similar peripheral concentrations to those of non-diabetics, will be associated with hepatic hypoinsulinaemia (Hanna et al., 1980), and hence hyperglycaemia. The effects of insulin in recovery from maximal exercise may be potentiated by enhanced insulin sensitivity and high, but rapidly declining blood flow.

2.5.8 Insulin binding, exercise, exercise training and blood flow

Whilst no data is available for the untrained, in moderately-trained subjects, skeletal muscle insulin binding is reduced 30–50% during 60 min exercise at ~75% $\dot{V}O_{2peak}$ (Bonen *et al.*, 1985). However, the relationship between insulin binding and insulin action (e.g. glucose metabolism) is not clear (Bonen *et al.*, 1985), since a maximal response is achieved when only a minority of receptor sites are occupied (Kahn, 1978). During exercise at <50% $\dot{V}O_{2peak}$ in humans, both insulin-mediated and insulin-independent actions occur and a synergistic relation exists between the effects of insulin and exercise (DeFronzo *et al.*, 1981a; Wasserman *et al.*, 1991), with increased blood flow perhaps mediating the synergism (DeFronzo *et al.*, 1981a). With regard to the latter, the time-course of glucose uptake by the exercising muscles closely parallels changes in blood flow (DeFronzo *et al.*, 1981a). During maximal exhaustive exercise, the role of insulin during exercise is probably of lesser significance, although is heightened in recovery (Calles *et al.*, 1983; Sigal *et al.*, 1994b; Sigal *et al.*, 1996).

A 10-week single-leg endurance training programme (6 days per week, 30 min cycling at 70% single-leg $\dot{V}O_{2peak}$) had no effect on insulin binding determined from muscle biopsies taken 16 hours after the final training session, leading the authors to conclude that insulin receptor function is unlikely to be affected by training (Dela *et al.*, 1993).

2.5.9 Effects of sprint training on plasma IRI during and after maximal exercise

No studies have examined the effect of sprint training on plasma IRI in subjects with or without type 1 DM. However a number of studies have reported effects in the moderately- to highly-endurance-trained or after endurance training.

2.5.9.1 Effect of continuous exercise to exhaustion on IRI in moderately endurance-trained subjects

Over, 30 years ago Pruett (1970) demonstrated a 58% reduction in plasma insulin when four moderately-trained men cycled to exhaustion (~29 min) at 87% $\dot{V}O_{2peak}$, then a sharp increase in IRI after 2 min recovery in two subjects. Exhaustive cycling (~12-14 min) at 85-100% $\dot{V}O_{2peak}$ did not change (Calles *et al.*, 1983; Marliss *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994b), or reduced plasma IRI (Sigal *et al.*, 1994a; Marliss *et al.*, 2000), and was followed early in recovery (4-10 min) by a rapid doubling of IRI, then a slow decline to resting values after 40-60 min. Moderately-trained women had a similar exercise-induced reduction when cycling to exhaustion at 88% $\dot{V}O_{2peak}$, but a higher and more sustained IRI response during recovery than similarly trained men (Marliss *et al.*, 2000).

2.5.9.2 Effect of intermittent maximal exercise on IRI in moderately endurance-trained subjects

Maximal arm cycling, intermittently superimposed upon submaximal leg cycling did not alter plasma IRI (Green *et al.*, 1979), whereas a single 30-s maximal cycling bout in moderately-trained men doubled basal IRI by 30 min recovery (Langfort *et al.*, 1997). The highest physiological increment in plasma IRI reported in the literature, a 3.7-fold elevation above resting levels, occurred after 17 min recovery from five intermittent maximal runs of 60 s duration (separated by 4 min rest periods) in five moderately-trained men and women (Hermansen *et al.*, 1970). However, included amongst the five subjects was one subject with a particularly marked IRI response, with the peak being 7-fold above basal values; interestingly, this disparity with the other four subjects was not reflected in the blood glucose results, perhaps suggesting a degree of peripheral insulin resistance in the subject. Although some of the above

studies were designed for another purpose (and included other groups or experiments), none included untrained subjects for comparison.

2.5.9.3 Effect of intense submaximal exercise on IRI in highly endurance-trained subjects

Highly-trained cyclists (Rennie *et al.*, 1974; Bloom *et al.*, 1976; Coggan *et al.*, 1995) and other endurance athletes (Montain *et al.*, 1991) had similar, or lower resting insulin concentration (Kjær *et al.*, 1986; Hagberg *et al.*, 1988) than sedentary subjects. Sutton *et al.* (Sutton *et al.*, 1969) reported no change in serum insulin concentration in either highly-trained or untrained subjects during and after ~30 min exhaustive cycling. Another relatively early study (Rennie *et al.*, 1974) compared the effects of 20 min of strenuous exercise in racing cyclists and sedentary men, and found a greater fall in plasma IRI in the cyclists, however, heart rate was higher in the cyclists after 10 min of exercise, thus a greater relative intensity rendered comparison between groups difficult. In studies conducted at the same relative intensity there was either no change (Bloom *et al.*, 1976; Hagberg *et al.*, 1988; Coyle *et al.*, 1991; Montain *et al.*, 1991) or a similar fall in plasma IRI (Coggan *et al.*, 1995) in highly-trained men during 8-120 min non-exhaustive cycling at 70-80% $\dot{V}O_{2max}$, and a lower IRI rise in recovery (Bloom *et al.*, 1976) in comparison to untrained men. Cycling to exhaustion (14-15 min) at ~85% $\dot{V}O_{2max}$ reduced plasma IRI by 15-20% in highly-trained men, and was followed by a rapid doubling of basal levels early in recovery then a slow decline to basal levels after 60 min (Sigal *et al.*, 1996; Manzon *et al.*, 1998); no untrained subjects were included for comparison.

2.5.9.4 Effect of maximal exercise on IRI in highly endurance-trained subjects

Three minutes of running at 100 % $\dot{V}O_{2max}$ resulted in similar reductions in plasma IRI concentration in elite endurance-trained and untrained subjects, but after running for a further 2 min at 110 % $\dot{V}O_{2max}$, a considerably higher post-exercise increase in IRI in the trained subjects was noted, which persisted for the 30 min recovery period (Kjær *et al.*, 1986).

2.5.10 Effect of intense exercise on plasma IRI in endurance-trained subjects with type 1 DM, or following endurance training

Fasting, free IRI did not differ between subjects with type 1 DM who were sedentary and subjects who had undertaken 6 weeks of cycle training, conducted for 60 min, four times per week at 70 % $\dot{V}O_{2peak}$ (Yki-Järvinen *et al.*, 1984), or between athletes and sedentary subjects with type 1 DM (Ebeling *et al.*, 1995). Three hours of running (and the subsequent 60 min recovery) produced no change in plasma free IRI in endurance-trained subjects with type 1 DM who had administered their last insulin dose 16-26 hours previously, but a significant fall in plasma IRI in non-diabetics (Meinders *et al.*, 1988). Four to twelve hours of arduous cross-country ski racing resulted in similar reductions in free IRI in endurance-trained subjects with type 1 DM and non-diabetic subjects (Sane *et al.*, 1988). The athletes with type 1 DM in that study (Sane *et al.*, 1988) reduced their morning short-acting insulin dose by 38-58%, and their intermediate-acting insulin dose by 28-38%; the latter would be expected to elevate plasma IRI after some hours, and therefore would have attenuated the reported fall in plasma free IRI. Due to the nature of these two studies (Meinders *et al.*, 1988; Sane *et al.*, 1988), work output and relative intensity could not be carefully matched in the subjects with type 1 DM and the non-diabetic subjects; in addition breakfast was consumed 1-2.5 hrs before exercise, with no attempt to ensure parity between groups. In a more controlled laboratory study in which both groups were postabsorptive, exhaustive exercise at 89-98% $\dot{V}O_{2max}$ did not alter plasma free IRI during exercise or recovery in subjects with type 1 DM who were being continuously infused with i.v. insulin at basal rates; however IRI was higher during exercise than in moderately-trained non-diabetics who also had no change in IRI during exercise, but evidenced the expected sharp rise in early recovery (Sigal *et al.*, 1994b).

In summary, no studies have examined the effect of sprint training on IRI during maximal exercise and recovery. In moderately- to highly-endurance-trained subjects attenuated or similar IRI responses compared with untrained subjects occur at rest and during non-exhaustive exercise. Whilst there are few studies upon which to base a conclusion regarding the effects of intense exhaustive exercise in trained versus untrained subjects, IRI responses during exercise and recovery appear similar. Similar to trained non-diabetics, these few studies indicate that

plasma free IRI is usually unchanged, but may fall during intense exercise in endurance-trained subjects with type 1 DM; however the mode and timing of insulin delivery affects the response.

2.6 Catecholamines

The catecholamines comprise noradrenaline, adrenaline and dopamine. Noradrenaline is the major neurotransmitter, being released at the sympathetic nerve terminals that supply for example, skeletal muscle fibres, the skeletal muscle vascular bed and the liver. Plasma noradrenaline concentration ([NAdr]) reflects the balance between the 'spillover' (which is proportional to neuronal firing rate) that escapes neuronal re-uptake and enzymatic degradation, and the clearance from the circulation (Esler *et al.*, 1984; Savard *et al.*, 1987). As a consequence of the uneven distribution of sympathetic outflow, local concentrations at target organs will be considerably higher than those measured systemically, and organ-specific differences in spillover and changes in clearance will affect plasma concentrations (Esler *et al.*, 1990; Rowell, 1993). For example, the usual 10–20% of noradrenaline that spills over into the circulation may be increased by enhanced washout resulting from the pronounced hyperaemia of maximal exercise (Esler *et al.*, 1990). Despite the limitations, plasma [NAdr] provides a valid qualitative index of overall sympathetic nervous activity (Rowell, 1993). Adrenaline is the main catecholamine secreted by the adrenal medulla, comprising approximately 80% of secretion (the other 20% being noradrenaline). Net extraction of adrenaline occurs across all organs except the brain and adrenal medulla, with the heart and hepatomesenteric circulation fractionally extracting ~60–80%, and the kidneys and skeletal muscle 35–55% (Esler *et al.*, 1990); and hence either arterial or arterialised venous blood samples are required to assess secretion (Kjær *et al.*, 1985; Kjær, 1989). The origin of plasma dopamine is uncertain and the threshold for its cardiovascular actions exceeds resting levels by 100-fold (Esler *et al.*, 1990).

Receptors for noradrenaline and adrenaline have been termed α- and β-adrenergic receptors (Ahlquist, 1948), and are further subdivided based on the differing potencies of agonist drug effects upon them. Noradrenaline has greater α stimulating effects than adrenaline, while the latter is a more potent activator of β-receptors (Williams,

1986). Both catecholamines have greater affinity for β_2 than β_1 receptors. Human skeletal muscle adrenergic receptors are mainly of the β_2 subtype (Liggett *et al.*, 1988), and are located on the external cell membrane (including t-tubules), closely distributed with the Na^+,K^+ATPase (Williams, 1986; Liggett *et al.*, 1988). Alpha receptors have been identified in animal muscle (Akaike, 1981) and are speculated to be present in human muscle, due to observations of α-adrenergic actions (Richter *et al.*, 1982).

2.6.1 Physiological thresholds for the glucoregulatory effects of the catecholamines

Physiological thresholds for glucoregulatory effects have been determined following infusion of adrenaline into fasted, rested, supine subjects at varying rates (0.1-5.0 $\mu g \cdot min^{-1}$), which produced plasma concentrations ranging from 0.13-5.57 $nmol \cdot l^{-1}$ (24-1,020 $pg \cdot ml^{-1}$) (Clutter *et al.*, 1980). The venous plasma adrenaline threshold that was associated with increases in plasma glycerol was 0.41–0.68 $nmol \cdot l^{-1}$ (75–125 $pg \cdot ml^{-1}$); increases in plasma glucose concentration ([PG]) and glucose production (R_a), reductions in glucose clearance, and increases in lactate and β-hydroxybutyrate was 0.82-1.09 $nmol \cdot l^{-1}$ (150–200 $pg \cdot ml^{-1}$); and suppression of insulin secretion was 2.18 $nmol \cdot l^{-1}$ (400 $pg \cdot ml^{-1}$) (Clutter *et al.*, 1980). Thus, the major glucoregulatory actions of the catecholamines are hyperglycaemic, glycolytic, and ketogenic, and occur at plasma adrenaline concentrations ([Adr]) of 4–5-fold basal levels (Clutter *et al.*, 1980). A similar study in which noradrenaline was infused to achieve plasma venous concentrations ranging between 1.63–12.71 $nmol \cdot l^{-1}$ (275–2,150 $pg \cdot ml^{-1}$), demonstrated the plasma noradrenaline threshold for increases in glucose, glycerol and β-hydroxybutyrate, and decreases in lactate and insulin to be between 10.6–12.7 $nmol \cdot l^{-1}$ (1,800-2,150 $pg \cdot ml^{-1}$) (Silverberg *et al.*, 1978). Thus, an approximately 8-fold elevation over basal levels was required in venous plasma noradrenaline for hormonal, as opposed to neurotransmitter, actions to be evident (Silverberg *et al.*, 1978). An early study demonstrated no difference between [Adr] and [NAdr] in blood obtained simultaneously from the antecubital vein and the femoral vein, either at rest or during supine exercise (Vendsalu, 1960). Infusion of adrenaline at rest increased plasma glycerol, and increased [PG] at arterial [Adr] of 1.34 and 2.30

$nmol \cdot l^{-1}$, respectively, but did not affect insulin, even at an arterial [Adr] of 6 $nmol \cdot l^{-1}$ (Freyschuss *et al.*, 1986). The lower thresholds reported with venous (Clutter *et al.*, 1980) versus arterial blood sampling (Freyschuss *et al.*, 1986) reflect the effect of tissue extraction of catecholamines.

2.6.2 Effects of the catecholamines on glycaemia during exercise

The extent of glucoregulatory effects of catecholamines in humans is less clear during exercise, and has been examined by infusing adrenaline, or antithetically, by imposing adrenergic blockade.

In endurance-trained subjects, infusion of adrenaline to physiological levels during 40 min cycling at 71% $\dot{V}O_{2peak}$ resulted in higher plasma glucose ([PG]) than in a saline trial (Febbraio *et al.*, 1998). Since glucose uptake during exercise is not affected by adrenaline infusion (Kjær *et al.*, 1993), higher [PG] suggests that hepatic glucose output was higher during the adrenaline infusion.

Blockade of the coeliac ganglion (the innervation of which includes the liver and adrenal medulla) during non-exhaustive exercise of moderate intensity (74% $\dot{V}O_{2max}$) in moderately-trained subjects did not affect the rate of appearance (R_a) for glucose, however concomitant infusion of high concentration adrenaline (of a magnitude that may occur in high intensity exercise) did increase R_a and resulted in greater increments in [PG] than with blockade alone (Kjær *et al.*, 1993). Whilst R_a was not assessed, during exercise at a similar intensity (but continued for twice the duration) in highly-trained cyclists, β-blockade abolished the rise in [PG] that was evident during a control trial (Hargreaves & Proietto, 1994). In comparison to exercise at 100% $\dot{V}O_{2max}$ without pharmacological intervention, partial β-blockade during maximum exercise reduced absolute intensity to 87% $\dot{V}O_{2max}$, increased R_d 42%, but did not reduce R_a (which remained well correlated with catecholamine concentrations), or attenuate hyperglycaemia during exercise (Sigal *et al.*, 1994a). Matched $\dot{V}O_2$ exercise (without β-blockade) resulted in lower peak catecholamine concentrations, lower R_a and R_d, and less hyperglycaemia (Sigal *et al.*, 1994a). Based upon these findings, the authors suggested that significant α-adrenergic stimulation of

R_a occurs during intense exercise in humans, and that β-adrenergic stimulation normally acts to restrain increases in R_d during exercise and early recovery (Sigal *et al.*, 1994a).

Combined α- and β-blockade would be required to abolish or markedly attenuate the effects of the catecholamines during intense exercise, and thus determine the effect of intense exercise upon R_a relatively independent of the effects of catecholamines. One such study was performed on dogs running for 20 min at 85% maximum heart rate with or without hepatic dual blockade (Coker *et al.*, 1997). Unfortunately the intensity of exercise was insufficient to elevate [PG] either during exercise or in recovery (only 10 min sample reported), and whilst R_d was not reported, the [PG] results were supported by the relatively small rise (~2.5-fold) in R_a during exercise. A study conducted for 60 min at lower intensity (55–60% $\dot{V}O_{2max}$), in which untrained subjects exercised during (i) α- and β-blockade, (ii) an islet cell clamp (insulin and glucagon concentrations clamped), and (iii) α- and β-blockade plus an islet cell clamp, demonstrated that the euglycaemia that prevailed during control exercise was disrupted, with hypoglycaemia developing during blockade and during blockade plus islet clamp (Hoelzer *et al.*, 1986). These results suggested that the catecholamines play a primary glucoregulatory role (Hoelzer *et al.*, 1986); one which is probably further exaggerated during intense exercise (Calles *et al.*, 1983; Mitchell *et al.*, 1988; Marliss *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994a; Sigal *et al.*, 1996; Manzon *et al.*, 1998; Marliss *et al.*, 2000). Considering the above, the hyperglycaemic role of the catecholamines appears to be supported during intense exercise.

During exercise, catecholamines, amongst other factors (e.g. Ca^{2+}, inorganic phosphate) are implicated in initiating and maintaining glycogenolytic/ glycolytic flux in skeletal muscle.

2.6.3 Effects of the catecholamines on lipolysis and ketogenesis during exercise

The lipolytic and ketogenic effects of catecholamines are modulated according to exercise intensity. Combined α- and β-adrenergic blockade during exercise at 55-

60% $\dot{V}O_{2max}$ abolished the rise in FFA and glycerol and attenuated the rise in β-hydroxybutyrate (Hoelzer *et al.*, 1986). Similarly, plasma FFA and glycerol were lower during exercise at 74% $\dot{V}O_{2max}$ with coeliac ganglion blockade than without, and were returned towards non-blockade values by the addition of infused adrenaline (Kjær *et al.*, 1993).

In contrast to mild to moderate exercise, intense exercise is associated with reduction in FFA concentration, but elevation in plasma glycerol (Jones *et al.*, 1980; McCartney *et al.*, 1986). β-blockade abolished the rise in glycerol, and depressed resting FFA concentration and maintained the reduction throughout exhaustive exercise at 87% $\dot{V}O_{2max}$, in contrast to the exercise-induced reduction in FFA concentration and elevation in glycerol evident without blockade (Sigal *et al.*, 1994a). Thus, during intense exercise, muscle lipolysis is stimulated, but adipose tissue lipolysis inhibited (Jones *et al.*, 1980), despite high catecholamine concentrations.

2.6.4 Glucoregulatory effects of adrenergic blockade during exercise in subjects with type 1 DM

No studies have examined the glucoregulatory effect of blockade during maximal exercise in subjects with type 1 DM. Propranolol infusion during mild exercise (supine cycling at 40 % $\dot{V}O_{2max}$) prevented the rise in hepatic glucose output and increased glucose utilization in postabsorptive subjects with type 1 DM (on baseline insulin infusion), thus causing hypoglycaemia, but had no effect in non-diabetic subjects (Simonson *et al.*, 1984). In contrast, when exercise was performed with an infusion of phentolamine (α-adrenergic blocker), hepatic glucose production was enhanced in the subjects with type 1 DM, but was not affected in the non-diabetics, leading the authors to suggest that glucose homeostasis during exercise is more dependent upon adrenergic regulation in subjects with type 1 DM than in non-diabetics (Simonson *et al.*, 1984).

2.6.5 Effects of exercise on catecholamine concentrations

Increases in arterial [Adr] and [NAdr] are exponential in relation to exercise intensity (Vendsalu, 1960; Häggendal *et al.*, 1970; Kjær, 1989; Mazzeo & Marshall, 1989; Bahr *et al.*, 1991; Kjær *et al.*, 1991; Romijn *et al.*, 1993a), and increase in proportion to exercise duration (Galbo *et al.*, 1975; Calles *et al.*, 1983; Kraemer *et al.*, 1991). Noradrenaline clearance was reduced 11% from rest after 20 min of moderate intensity exercise (67% $\dot{V}O_{2peak}$) in untrained subjects (Leuenberger *et al.*, 1993), and adrenaline clearance was reduced 22% when exercising to exhaustion at 76% $\dot{V}O_{2max}$ (Kjær *et al.*, 1985), with an inverse relationship between [Adr] and relative exercise intensity (Kjær, 1989). However, the marked increases in catecholamine concentrations in moderate to intense exercise are primarily attributable to increased secretion, with only a small contribution from reduced clearance (Kjær *et al.*, 1985). Following the peaks at 0-1 min post-exercise, adrenaline and noradrenaline have half-times of ~1.5-3 min (Vendsalu, 1960; Hagberg *et al.*, 1979; Kjær *et al.*, 1986); therefore rapid sampling after exercise is crucial to obtain maxima.

2.6.6 Effects of intense submaximal to maximal exercise on catecholamine concentrations in untrained non-diabetics

Thirty min cycling at 80% $\dot{V}O_{2peak}$ in untrained subjects resulted in 5- and 10-fold increments to ~1.5 and 22.5 nmol·l^{-1}, in arterialized venous plasma [Adr] and [NAdr], respectively (Coggan *et al.*, 1995; Coggan *et al.*, 1997). Five min non-exhaustive running at 100, then 110% $\dot{V}O_{2max}$ increased plasma [Adr] and [NAdr] approximately 7-fold to ~3.6 and 21 nmol·l^{-1}, respectively, in untrained subjects (Kjær *et al.*, 1986). Arterialized venous adrenaline peaked immediately after exercise, then decayed with a $t_{½}$ of 1.7 min, whilst peak [NAdr] occurred at 1 min recovery and decayed similarly (Kjær *et al.*, 1986). A mixed group of untrained and trained subjects cycling at 110-140% $\dot{V}O_{2peak}$ attained peak arterial [NAdr] of 38.5 nmol·l^{-1} (Häggendal *et al.*, 1970).

2.6.7 Effects of intense submaximal to maximal exercise on catecholamine concentrations in untrained subjects with type 1 DM

There was no difference in peak values (4- to 9-fold increases) or rates of decay of the arterialized venous catecholamine concentrations in fasted subjects with type 1 DM

who were on continuous subcutaneous insulin infusion (CSII) or i.v. insulin infusion at basal levels, and were either normo- or hyperglycaemic, compared with non-diabetics of a similar fitness level, after cycling to exhaustion (Mitchell *et al.*, 1988), or for 30 min at 77-80% $\dot{V}O_{2max}$ (Raguso *et al.*, 1995). Venous plasma [Adr] and [NAdr] increased ~14-fold to ~4 and ~27 nmol·l^{-1} with exhaustive exercise at 98% $\dot{V}O_{2max}$ in untrained subjects with type 1 DM (on continuous basal i.v. insulin infusion), and did not differ from the catecholamine response in moderately-trained non-diabetics (Purdon *et al.*, 1993). In contrast, arterial plasma [Adr] and [NAdr] increased to a significantly greater degree in response to exhaustive incremental exercise in non-diabetics than in fasted subjects with type 1 DM who had their last insulin dose 16 hr previously (Jakober *et al.*, 1983). However, whether or not an endpoint of exhaustion was reached in the type 1 DM subjects is questionable based upon the metabolic and cardiorespiratory results; a further complicating factor was the approximately 20% higher $\dot{V}O_{2peak}$ in the non-diabetic subjects (albeit both groups were untrained). Antecubital venous [NAdr] and [Adr] increased to 7.5 and 0.9 nmol·l^{-1}, respectively following a brief, exhaustive, incremental exercise test in subjects with type 1 DM who had reduced their insulin dose and were 60 min postprandial (Hübinger *et al.*, 1985); no control group was studied. Ten min non-exhaustive cycling exercise (achieved HR of ~170 b·min^{-1}) resulted in 27–54% higher plasma [NAdr], but similar (non-significantly higher) [Adr], in untrained children with well-controlled type 1 DM (of 5–13 years duration) versus untrained non-diabetic children (Tulassay *et al.*, 1992). Exercise induced greater metabolic acidosis in the children with type 1 DM (who had their usual insulin dose and breakfast several hours prior to exercise): a finding suggested to be related to inhibition of muscle pyruvate oxidation; and a significant negative correlation was found between peak [NAdr] and blood pH (Tulassay *et al.*, 1992).

2.6.8 Effect of acid-base status on catecholamines during exercise

Acidosis induced during moderate exercise either by inhaling 4-5% CO_2, or by acetazolamide administration, resulted in ~2-fold higher [NAdr] and higher (although non-significant) [Adr] (Ehrsam *et al.*, 1982; Goldsmith *et al.*, 1990); whilst alkalosis induced by hyperventilation had no affect (Goldsmith *et al.*, 1990). In contrast,

metabolic alkalosis reduced the rise in [NAdr] and [Adr] during exhaustive exercise at 125% $\dot{V}O_{2max}$ in trained runners (Bouissou *et al.*, 1988). It was suggested that acid-base status (and the associated chemo-reception) was an important stimulus to sympathetic activity (Bouissou *et al.*, 1988; Goldsmith *et al.*, 1990): a notion that may be supported by the relationship between pH and muscle sympathetic nervous activity (Victor *et al.*, 1988; Seals & Victor, 1991).

2.6.9 Effects of sprint training on the catecholamine response to exercise in non-diabetics

Venous plasma [Adr] and [NAdr] were increased 3- (1.5 nmol·l^{-1}) and 5-fold (10.4 nmol·l^{-1}), respectively, 3 min after a maximal 30-s sprint (Nevill *et al.*, 1989). Eight weeks of sprint training had no affect on [Adr], but associated with the greater work performed during the 30-s sprint after training, [NAdr] was 20% higher (Brooks *et al.*, 1987; Nevill *et al.*, 1989). In the same study (Nevill *et al.*, 1989), there was no difference in the catecholamine responses to a 2 min run conducted at the same absolute speed (110% pre-training $\dot{V}O_{2max}$) before and after training, although concentrations were 13–20% lower. However, since catecholamine samples were taken 3 min after completing each exercise test, peak concentrations would have been missed, thus the effect of sprint training remains unclear.

The potential for a sprint training programme to alter catecholamine responses during exercise of either the same absolute or relative intensity may be illustrated by four studies that employed vigorous endurance training programmes. Untrained subjects cycled (5-min bouts at 100% $\dot{V}O_{2peak}$) or ran (40 min continuous) 6 days per week for 9 weeks (Winder *et al.*, 1979). Training reduced the peak [NAdr] and [Adr] (obtained after 90 min cycling at 58% pre-training $\dot{V}O_{2peak}$) by ~55%; an effect evident after only 3 weeks of training (Winder *et al.*, 1979). Following 8 weeks of vigorous running or cycling training, plasma venous [NAdr] peaked at 6.5 nmol·l^{-1}, 60 s after a 5 min cycle at 78% $\dot{V}O_{2max}$ (Hagberg *et al.*, 1979). Although pre-training results were not reported, after 8 weeks of detraining the venous noradrenaline response to the same absolute load was 2-fold higher (12.4 nmol·l^{-1}) (Hagberg *et al.*, 1979). Following high intensity training (30 min cycling sessions at 80% maximum heart

rate) for 20 weeks in sedentary men, venous plasma [NAdr] was halved (4.1 $nmol \cdot l^{-1}$) at the same absolute workload, but not different (although 26% higher) at the same relative intensity (Péronnet *et al.*, 1981). Seven weeks of physical training that included both aerobic and anaerobic activities resulted in lower venous [NAdr], but similar [Adr] during 5 min cycling at 93% $\dot{V}O_{2peak}$; however, whilst the workload used after training was 8% higher, it represented a slightly lower relative intensity (Hartley *et al.*, 1972).

It is apparent that most studies that have examined catecholamine responses to intense exercise have been conducted using moderately- or highly-trained subjects, rather than untrained subjects or employing a training programme. In addition, most studies have sampled blood from an antecubital or a dorsal hand vein, with few either arterializing the venous sample or sampling arterial blood. Table 2.4 summarises plasma catecholamine responses to intense submaximal to maximal exercise in trained subjects, and includes several lower intensity studies for comparison.

Highly-trained men had similar venous [Adr], but 2-fold higher [NAdr] than untrained men after 60 min exercise at 70 % $\dot{V}O_{2max}$ (Hagberg *et al.*, 1988). Conversely, elite endurance athletes had 2-fold higher arterialized venous [Adr] after a 2 min run at 110 % $\dot{V}O_{2max}$, but similar [NAdr] to untrained subjects (Kjær *et al.*, 1986); and athletes secreted more adrenaline in response to non-exercise stimuli than untrained subjects, leading the authors to conclude that endurance training increases medullary secretory capacity (Kjær & Galbo, 1988). In contrast, highly-trained cyclists had lower venous [Adr] and [NAdr] after 8 min cycling at 75 % maximum workload than untrained subjects (Bloom *et al.*, 1976). In further contrast, 10 x 30-s runs at 90 % $\dot{V}O_{2max}$ increased venous plasma [Adr] and [NAdr] by 15-fold and 24-fold, respectively, with no difference between highly-trained and untrained subjects (Deuster *et al.*, 1989). Thus, whether or not trained subjects consistently display an effect of training (compared to untrained subjects) in the catecholamine response to intense exercise of the same relative intensity remains unclear. However, when the same absolute work load was performed in trained and sedentary subjects, both [Adr] and [NAdr] were lower in the former group at every common work load in an incremental test to

Table 2.4 *Peak catecholamine concentrations following moderate to maximal exercise in moderately- and highly-trained subjects*

Study	Subjects: *n*, gdr, tr. status (Mod, High)	Exercise test: Ex/ NEx; duration; intensity (% $\dot{V}O_{2peak}$)	Blood: Art.; Artz.; Ven.	Peak [Adr] $nmol \cdot l^{-1}$	Peak [NAdr] $nmol \cdot l^{-1}$
(Kjær *et al.*, 1993)	8M; Mod	NEx; 20 min; 74%	Art.	4.3	~11
(Kjær *et al.*, 1991)	7M; Mod	NEx; 20 min; 82%	Art.	~6	~28
(Calles *et al.*, 1983)	8M, 3F; Mod	Ex; 12 min; 85%	Ven.	4.7	22.2
(Marliss *et al.*, 2000)	12F; Mod	Ex; 14 min; 88%	Ven.	4.6	33.3
(Sigal *et al.*, 1994b)	6M; Mod	Ex; 13 min; 98%	Ven.	~7	~37
(Marliss *et al.*, 1991)	6M; Mod	Ex; 13 min; ~100%	Ven.	~2.9	~27
(Marliss *et al.*, 1992)	18M; Mod	Ex; 7-12 min; ~100%	Ven.	~2.9	~27
(Sigal *et al.*, 1994a)	6M; Mod	Ex; 13 min; 100%	Ven.	7.1	36.3
(Purdon *et al.*, 1993)	6M; Mod	Ex; 13 min; 103%	Ven.	~7.8	~33
(Allsop *et al.*, 1990)	7M, 3F; ?Mod	Ex; 30 s; 'all out'	Ven.	10.2	37.1
(Langfort *et al.*, 1997)	8M; Mod	Ex; 30 s; 'all out'	Ven.	~1.2	~8
(Brooks *et al.*, 1990)	9M, 9F; ?Mod	?Ex; 10 x 6 s (1:5)	Ven.	~5	~30
(Gaitanos *et al.*, 1993)	8M; ?Mod	?Ex; 10 x 6 s (1:5)	Ven.	5.1	22.3
(Hagberg *et al.*, 1988)	11M; High ET	NEx; 60 min; 70%	Ven.	1.5	13.5
(Sigal *et al.*, 1996)	7M; High	Ex; 14 min; 84%	Ven.	5.7	36.8
(Romijn *et al.*, 1993a)	5?; High ET	NEx; 30 min; 85%	Artz.	3.4	30.1

Table 2.4 (cont.): *Peak catecholamine concentrations following moderate to maximal exercise in moderately- and highly-trained subjects*

Study	Subjects: *n*, gdr; tr. status (Mod, High)	Exercise test: Ex/ NEx; duration; intensity (% $\dot{V}O_{2peak}$)	Blood: Art.; Artz.; Ven.	Peak [Adr] $nmol \cdot l^{-1}$	Peak [NAdr] $nmol \cdot l^{-1}$
(Manzon *et al.*, 1998)	12M; High	Ex; 15 min; 87%	Ven.	~6.3	~38
(Marliss *et al.*, 2000)	16M; High	Ex; 14 min; 88%	Ven.	5.3	33.6
(Kjær *et al.*, 1986)	8M; High ET	NEx; 2 min; 110%	Artz.	8.7	22
(Cheetham *et al.*, 1986)	8F; High	Ex; 30 s; 'all out'	Ven.	1.3	11.7
(Lehmann *et al.*, 1981)	9M; High ET	Ex; Incr.	Ven.	5.2	24.8
(Mazzeo & Marshall, 1989)	12M; High ET	Ex; Incr.	Ven.	14.3	51.5
(Brooks *et al.*, 1988)	10M; High	Ex; 30 s; 'all out'	Artz.	~1.5	~13
(Deuster *et al.*, 1989)	7M; HighET	?Ex; 10 x 30 s (1:1); 90%	Ven.	4.2	28.9
(Näveri *et al.*, 1985)	9M; High S/ET or ET	Ex; 3 x 300 m; max. speed	Ven.	~8.5	~30

gdr, gender; tr. Status, training status; Mod, moderately-trained; High, Highly-trained; Note: In assessing metabolic etc responses to exercise, subjects have been classified as untrained (below the 85th percentile in normative age-based tables generated by the Institute for Aerobics Research, Dallas, TX, published in ACSM's Guidelines for Exercise Testing and Prescription (Kenney, 1995)), moderately trained (85th to 95th percentile), or highly trained (99th percentile), based upon reported $\dot{V}O_{2peak}$.Ex, test conducted to exhaustion; NEx, non-exhaustive test; Art., arterial; Artz., arterialized venous; Ven., venous; [Adr], adrenaline concentration; [NAdr], noradrenaline concentration; M, male subjects; F, female subjects; ~, indicates estimation from figure; ?, indicates not specified; ET, endurance trained; (1:5), (1:1), work: rest ratio in intermittent exercise; Incr., incremental test; S/ET, sprint/ endurance trained.

exhaustion, whilst catecholamine concentrations at exhaustion were similar (Lehmann *et al.*, 1981).

Cycling or running to exhaustion at intensities ranging from ~80% $\dot{V}O_{2peak}$ to maximal 6–30-s efforts induced substantial elevations in venous or arterialized venous plasma [Adr] and [NAdr], with no apparent difference between moderately- and highly-trained subjects (Table 2.4). Peak venous [Adr] generally ranged between 3–10 nmol·l^{-1}, which considerably exceeded the venous and arterial plasma thresholds for glucoregulatory actions (Clutter *et al.*, 1980; Freyschuss *et al.*, 1986). Peak [NAdr] generally ranged between 22–38 nmol·l^{-1}, which also markedly exceeded the glucoregulatory threshold values (Silverberg *et al.*, 1978). Lower concentrations were evident at lower intensities (one with arterial sampling) (Hagberg *et al.*, 1988; Kjær *et al.*, 1993), and in studies in which the first post-exercise blood sample was not obtained until 3 min of recovery (Cheetham *et al.*, 1986; Brooks *et al.*, 1988; Langfort *et al.*, 1997). Illustrative of the latter, a single, maximal 30-s treadmill sprint increased plasma venous [Adr] and [NAdr] 4-fold in trained women (Cheetham *et al.*, 1986). An identical 30-s sprint protocol, but more rapid post-exercise sampling (used by the same research group), yielded 30-fold elevations (Allsop *et al.*, 1990). Perhaps the highest catecholamine concentrations reported occurred in highly-trained runners immediately after an exhaustive incremental test, with peak venous [Adr] and [NAdr] of 14.3 and 51.5 nmol·l^{-1}, respectively (Mazzeo & Marshall, 1989). Untrained subjects were not included for comparison in the majority of these studies, however, the purpose of some studies was for another comparison (Kjær *et al.*, 1991; Kjær *et al.*, 1993; Purdon *et al.*, 1993; Sigal *et al.*, 1994a; Sigal *et al.*, 1994b; Sigal *et al.*, 1996; Manzon *et al.*, 1998; Marliss *et al.*, 2000).

2.6.10 Effects of exercise training on the catecholamine response to exercise in subjects with type 1 DM

There are no longitudinal studies that have examined the catecholamine response to high intensity exercise and training in subjects with type 1 DM.

Peak venous [Adr] was ~2-fold higher (2.2 nmol·l^{-1}) after 3 hours running at ~11 km·hr^{-1} in marathon runners with type 1 DM, who had their last insulin dose 16–26

hours previously, than in matched non-diabetic runners (Meinders *et al.*, 1988). Noradrenaline was also markedly higher in two of the five subjects with type 1 DM, which elevated the group peak to 7.7 $nmol \cdot l^{-1}$, vastly higher than in the non-diabetics (0.9 $nmol \cdot l^{-1}$) (Meinders *et al.*, 1988), although still relatively low in comparison to values reported for more intense exercise. Moderately-trained subjects with type 1 DM who were on i.v. insulin infusion sufficient to permit either eu- or hyperglycaemia, had similar peak venous [NAdr] (~30 $nmol \cdot l^{-1}$), but ~2-fold lower peak [Adr] (~3.5 $nmol \cdot l^{-1}$) than a non-diabetic group when exercising to exhaustion (12-13 min) at 89–98% $\dot{V}O_{2max}$; however, the overall catecholamine response during exercise and recovery did not differ between groups (Sigal *et al.*, 1994b).

In summary, catecholamine concentrations achieved during intense exercise markedly exceed the threshold for glucoregulatory actions. Trained subjects generally display similar or higher catecholamine concentrations than sedentary subjects during exercise conducted at the same relative intensity (in which considerably greater work was performed by the trained). However during performance of the same absolute submaximal workload, attenuated catecholamine responses are evident in subjects who have undertaken an endurance training programme. There is little information regarding the effects of sprint training upon catecholamine concentrations during, and shortly after, maximal exercise. No sprint training studies have examined subjects with type 1 DM. Based upon the very few cross-sectional studies which have examined subjects with type 1 DM during high intensity exercise, catecholamine responses appear to be similar when a basal infusion of insulin is administered, but may be higher after insulin withdrawal; the latter consistent with the suggestion that glucose homeostasis during exercise is more dependent upon adrenergic regulation in subjects with type 1 DM than in non-diabetics (Simonson et al., 1984).

2.7 Glucagon

Glucagon is a single chain polypeptide secreted by the α cells of the pancreas in response to adrenergic stimulation, reductions in blood glucose levels, and increases in blood amino acid levels and a number of the gastrointestinal hormones (Alford & Chisholm, 1979). The α cell is relatively insensitive to small reductions in blood

glucose (Alford & Chisholm, 1979), and fasting plasma glucose and glucagon levels are not correlated (Alford *et al.*, 1977); but responds sharply to hypoglycaemia, and is inhibited by hyperglycaemia (Alford & Chisholm, 1979) and insulin (Raskin *et al.*, 1975; Lewis *et al.*, 1996). In humans glucagon acts upon the liver, although in pharmacological concentrations it may have other actions, e.g. stimulate insulin secretion, increase myocardial contractility (Alford & Chisholm, 1979). At the liver it increases cyclic AMP and stimulates glycogenolysis, gluconeogenesis, and ketogenesis (Exton *et al.*, 1971; Ui *et al.*, 1973) and increases glucose output (Sherwin *et al.*, 1977); thus protecting against hypoglycaemia (Alford *et al.*, 1974; Chisholm & Alford, 1977). Glycogenolysis is more sensitive and stimulated to a greater degree by glucagon than either gluconeogenesis or ketogenesis (Exton *et al.*, 1971). Recent work (Carlson *et al.*, 1991), which casts a different light upon interpretation of results from earlier studies, suggests that glucagon may also act on adipose tissue as a lipolytic hormone in humans. Clearance of glucagon is primarily effected by the liver, thus portal concentrations exceed those of the periphery; whilst the kidney is the most important extra-hepatic site of glucagon degradation (Alford & Chisholm, 1979). Postabsorptive glucagon concentrations at rest range from 24 $ng \cdot l^{-1}$ (6.9 $pmol \cdot l^{-1}$) (Alford *et al.*, 1977) to 240 $ng \cdot l^{-1}$ (69.0 $pmol \cdot l^{-1}$) (Raguso *et al.*, 1995).

2.7.1 Glucagon secretion and type 1 DM

The usual mechanism of inhibition of α-cell glucagon secretion by hyperglycaemia may be attenuated in subjects with type 1 DM, and hyperglucagonaemia, secondary to insulinopaenia, may contribute to resting hyperglycaemia and aggravate ketogenesis (Chisholm & Alford, 1977). Following insulin therapy for 2–6 months, even in ketotic subjects with type 1 DM, glucagon was restored to normal levels and the α-cell responded normally to an acute glucose load; and it was concluded that insulin exerted a long-term permissive effect (Larkins *et al.*, 1978). However, insulin also has an acute effect in either directly or indirectly suppressing the α-cell, and a higher insulin dose may be required for the same suppression of glucagon in subjects with type 1 DM (Raskin *et al.*, 1975).

2.7.2 Effects of exercise on glucagon concentration in non-diabetics

Plasma immunoreactive glucagon (IRG) was not altered significantly during or after cycling to exhaustion (7–14 min) at 80% $\dot{V}O_{2peak}$ (Mitchell *et al.*, 1988), or in non-exhaustive cycling or running at 70-80% $\dot{V}O_{2peak}$ (Hagberg *et al.*, 1988; Coggan *et al.*, 1995; Raguso *et al.*, 1995; Coggan *et al.*, 1997), although tended to rise (~40 and 60%, respectively) after 8 min non-exhaustive cycling at 75% maximal work capacity and after 5 min recovery in untrained subjects (Bloom *et al.*, 1976). In brief, non-exhaustive running or cycling at 100% (3 min), then 110% $\dot{V}O_{2peak}$ (2 min), plasma IRG fell with exercise, and returned to basal values in recovery (Kjær *et al.*, 1986; Kjær *et al.*, 1990).

2.7.3 Effects of exercise on glucagon concentration in subjects with type 1 DM

In untrained subjects with type 1 DM in whom i.v. or subcutaneous insulin infusion sufficient to maintain euglycaemia at rest was continued during exercise, plasma IRG during non-exhausting (Raguso *et al.*, 1995) and exhausting exercise (Mitchell *et al.*, 1988) at 75-98% $\dot{V}O_{2peak}$ and subsequent recovery did not change significantly, or rose modestly, with a rapid return to baseline in early recovery (Purdon *et al.*, 1993), and did not differ from that of non-diabetic subjects. Similarly, in subjects in whom the morning insulin dose was reduced 66% and administered 60 min prior to exhaustive incremental exercise, plasma IRG was unchanged during exercise or recovery (Hübinger *et al.*, 1985); no non-diabetic subjects were studied.

2.7.4 Glucagon-to-insulin ratio

However, even if glucagon levels do not increase, if insulin falls during exercise, the relative effect of glucagon on the liver will be greater, especially since catecholamine concentrations rise considerably during intense exercise and act synergistically with glucagon to acutely increase hepatic glucose output (HGO) (Eigler *et al.*, 1979). A recent study employed an islet cell clamp to manipulate the glucagon-to-insulin ratio during intense exhausting exercise in highly-trained humans (Sigal *et al.*, 1996). HGO, i.e. the appearance of glucose (R_a), did not differ between clamped (unchanged glucagon-to-insulin ratio) and control conditions (increased ratio), although plasma

glucose increased more rapidly in control conditions without the clamp (Sigal *et al.*, 1996), probably due to the tendency for a lower rate of glucose disappearance (R_d). It has been concluded that glucagon plays a relatively minor role in contributing to the appearance of glucose during brief, intense submaximal or maximal exercise and recovery in humans (Marliss *et al.*, 1991; Purdon *et al.*, 1993; Sigal *et al.*, 1996; Manzon *et al.*, 1998). The suppressive effects of hyperglycaemia and the associated hyperinsulinaemia on plasma glucagon (Raskin *et al.*, 1975) may be expected to further diminish its effect during early recovery from intense exercise. In addition, since most of the energy supply for a single bout of maximal exercise is derived from the degradation of muscle glycogen (Spriet *et al.*, 1989), the effect of glucagon on the liver is likely to be of little consequence during exercise, however, in recovery, especially in subjects with type 1 DM, glucagon may assume greater significance (since plasma IRI does not rise), however this has not been investigated.

2.7.5 Effects of exercise training on plasma IRG in non-diabetic subjects

No sprint training studies have reported plasma IRG concentrations.

In moderately-trained subjects, basal plasma IRG was not altered either during exercise or in recovery from exhausting cycling (12-14 min) at 85-88% $\dot{V}O_{2peak}$ (Calles *et al.*, 1983; Marliss *et al.*, 2000), although a small increase was evident in female subjects at exhaustion (Marliss *et al.*, 2000). Similarly, small increases (or a tendency to increase during exercise and early recovery) were found in plasma IRG during cycling to exhaustion (12 - 13 min) at 89-100% $\dot{V}O_{2peak}$, with peak concentrations after 4 min recovery, then a decline to resting values by 20-40 min (Marliss *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994a; Sigal *et al.*, 1994b).

Plasma IRG rose slightly during 8 min of exercise at 75% of maximum workload, and after 5 min recovery in racing cyclists, similar to the response in untrained subjects, but concentrations were considerably lower overall in the cyclists (Bloom *et al.*, 1976). Plasma IRG did not change in highly-trained subjects during or after cycling to exhaustion (14-15 min) at 84-87% $\dot{V}O_{2peak}$ (Sigal *et al.*, 1996; Manzon *et al.*, 1998), or during or after 30 min exhausting cycling (Sutton *et al.*, 1969), and did not

differ from the response in untrained subjects (Sutton *et al.*, 1969). Highly-trained subjects had similar resting IRG concentrations, and a similar reduction in IRG during brief (5 min), non-exhaustive exercise at 100%, then 110% $\dot{V}O_{2peak}$, compared with untrained subjects, but had markedly higher IRG concentration in early recovery (Kjær *et al.*, 1986). In contrast, plasma glucagon was increased 55% following 15 min exhaustive running in endurance-trained runners; and rose 41% after 3 x 300m sprints in a mixed group of sprinters and endurance runners (Näveri *et al.*, 1985).

2.7.6 Effects of exercise training on IRG in subjects with type 1 DM

In moderately-trained subjects with type 1 DM, plasma IRG rose during exhaustive exercise at 89-98% $\dot{V}O_{2peak}$, peaked after 4 min recovery, and returned to resting levels by 30 min, and did not differ from the response of non-diabetic subjects (Sigal *et al.*, 1994b). Plasma IRG rose progressively throughout 3 hr running, to peak 50% above resting concentration in highly-trained long-distance runners with type 1 DM, but did not change in similarly trained non-diabetics (Meinders *et al.*, 1988). Whilst the subjects with type 1 DM had their last insulin dose 16-26 hr before exercise, and both groups consumed an unregulated breakfast 2.5 hr before the run, counter-regulatory hormones did not differ between groups before the run, and the authors concluded that glucagon rose to prevent hypoglycaemia (in the absence of physiological decreases in insulin during exercise) (Meinders *et al.*, 1988).

In summary, there appears to be little change or a small rise and rapid return to basal values in plasma IRG during intense exercise and recovery, whether in trained or untrained subjects, or in subjects with type 1 DM (providing the latter group are not insulinopaenic prior to exercise). Haemoconcentration induced by intense, exhaustive exercise would be expected to result in a small increase in plasma IRG concentration, followed by a return to baseline concentrations, providing that rates of secretion and clearance were not altered disproportionately during and after exercise, and thus may account for much of the rise reported in several studies. In addition, the plasma IRG assay has large inter-assay coefficients of variation (Alford et al., 1977), which may explain the different observations at similar exercise intensities. The relative effect of glucagon on the liver may be increased during exercise as a consequence of reduced insulin secretion, although

in maximal exercise this is likely to be of minor significance. However, glucagon may assume a greater role in recovery, especially in subjects with type 1 DM in whom insulin concentration does not increase in response to increased plasma glucose concentration.

2.8 Free fatty acids, glycerol, ketone bodies, and lactate

Adipose tissue lipolysis involves the hydrolysis of triglycerides to free fatty acids (FFAs) and glycerol, and is regulated by the activity of hormone-sensitive lipase (HSL), itself activated by the catecholamines and de-activated by insulin (Yeaman, 1990; Rasmussen & Wolfe, 1999). Due to the minimal activity of glycerol kinase in adipose tissue (Newsholme & Leech, 1983), glycerol cannot be recycled within the cell (and thus has been used as an index of lipolysis) and is released into the blood, where it circulates to the liver and serves as a gluconeogenic precursor (Jahoor *et al.*, 1990). Circulating FFA can be oxidized and subsequently used as an energy substrate for gluconeogenesis by the liver (Ruderman *et al.*, 1969) or as a substrate for oxidation in skeletal muscle (Havel *et al.*, 1967; Felig & Wahren, 1975; Wahren *et al.*, 1975; Oscai *et al.*, 1990; Romijn *et al.*, 1995). Intramuscular (and/or perhaps intermuscular) triglyceride (IMTG) hydrolysis releases glycerol into the circulation (Hagström-Toft *et al.*, 1997), however FFAs are utilized within the muscle (Essén, 1978; Jones *et al.*, 1980; Oscai *et al.*, 1990). Synthesis of the ketone bodies (acetoacetate, β-hydroxybutyrate) normally only occurs in the liver from the metabolism of fatty acids (Newsholme & Leech, 1983), however utilization occurs in many tissues (Krebs, 1961).

In terms of glucoregulation, lactate serves as an hepatic and renal gluconeogenic (Krebs, 1964) and a skeletal muscle glyconeogenic (Hermansen & Vaage, 1977; Åstrand *et al.*, 1986) precursor, and is formed from the reduction of pyruvate with NADH in a near-equilibrium reaction. Lactate also serves to inhibit lipolysis.

2.8.1 Effects of hyperinsulinaemia and/or catecholamine stimulation on lipolysis at rest

Microdialysis of adipose and muscle tissue at rest returned values of ~0.23 and ~0.10 $mmol \cdot l^{-1}$ for glycerol, whilst arterialized venous plasma glycerol concentration was

~0.06 mmol·l^{-1}, with a basal insulin concentration of ~8 mU·l^{-1} (Hagström-Toft *et al.*, 1997). Hyperinsulinaemic euglycaemia reduced lipolysis in both adipose and skeletal muscle tissue, whereas catecholamine stimulation (in response to hypoglycaemia) increased glycerol concentration in all three compartments, with greater absolute production in adipose tissue (Hagström-Toft *et al.*, 1997).

Delivery of phentolamine (α-adrenergic blocker) via a microdialysis probe to adipose tissue at rest rapidly increased lipolysis (indicated by glycerol levels), whereas propranolol (β-adrenergic blocker) had no effect (Arner *et al.*, 1990). Low-dose infusion of adrenaline and noradrenaline, which produced arterialized venous plasma concentrations of 1.17 and 5.87 nmol·l^{-1}, respectively, increased mean plasma FFA by 55–75% and ketone bodies by ~3-fold (Krentz *et al.*, 1996). High-dose infusions produced plasma concentrations of 4.9 (adrenaline) and 15.1 nmol·l^{-1} (noradrenaline) (which occur commonly during intense exercise - see Table 2.4), and increased plasma FFA 2.5–3.5-fold, however had no further effect upon ketone body concentration (Krentz *et al.*, 1996).

2.8.2 Effect of FFA on hepatic glucose output

HGO is increased by increased FFA and is controlled directly and indirectly by insulin, with the indirect effect usually dominant (Levine & Fritz, 1956; Lewis *et al.*, 1996; Mittleman *et al.*, 1997). The direct effect of insulin, i.e. effect upon liver via direct portal vein delivery, is likely to account for much of the early suppression of HGO, whereas the indirect effect assumes greater significance thereafter (Lewis *et al.*, 1998). The indirect effect, which accounts for ~80% of HGO suppression (Mittleman *et al.*, 1997), is partially mediated by an insulin-induced reduction in lipolysis which decreases plasma FFA, and hence suppresses hepatic gluconeogenesis and HGO (Rebrin *et al.*, 1995; Lewis *et al.*, 1996; Lewis *et al.*, 1997), and partially by α-cell suppression by insulin which reduces glucagon (Lewis *et al.*, 1998). Elevating FFA and glycerol concentrations during hyperglycaemia with relative insulinopaenia (and hyperglucagonaemia) augmented HGO in man (Ferrannini *et al.*, 1983a), and represents a similar scenario to insulin deficiency in type 1 DM.

2.8.3 Effects of hyperglycaemia on lipolysis

Induction of hyperglycaemia (10 mmol·l^{-1}) during a pancreatic-pituitary clamp (which prevented changes in glucoregulatory hormones), had no significant effect upon plasma FFA concentration, but reduced both glycerol and FFA R_a by 30–34% indicating suppressed lipolysis; and increased carbohydrate and reduced fat oxidation (paralleling availability) in resting humans (Carlson *et al.*, 1991). The mechanism of lipolytic suppression by physiological hyperglycaemia was suggested to be mediated via the adipocyte insulin receptor, both through binding and post-binding effects (Carlson *et al.*, 1991).

2.8.4 Effects of lactate on lipolysis

Hyperlactataemia (10 mmol·l^{-1} lithium lactate) increased the sensitivity of lipolysis (indicated by glycerol release) to insulin *in vitro*, with a half-maximal effect observed in rat adipocytes at insulin concentrations of ~5-10 μU·ml^{-1}; conditions likely to be evident during intense exercise and early recovery, which may facilitate the hepatic removal of lactate and contribute to an increase in blood glucose concentration (Green & Newsholme, 1979). Combined arterial hyperlactataemia (9.1 mmol·l^{-1}) and hyperglycaemia (~9 mmol·l^{-1}) may have contributed to the reduced plasma FFA and glycerol concentrations observed after 60 min portal insulin deficiency in dogs (Sindelar *et al.*, 1998). Hyperketonaemia (5 mmol·l^{-1} β-hydroxybutyrate) also inhibited lipolysis (Green & Newsholme, 1979); however 5 mmol·l^{-1} β-hydroxybutyrate concentrations usually only occur in non-diabetic subjects after prolonged starvation (Newsholme & Leech, 1983), or in patients with type 1 DM without residual β–cell function after protracted insulin withdrawal (Madsbad *et al.*, 1979).

2.8.5 Lipolysis at rest in type 1 DM

Subjects with type 1 DM who had received their last insulin injections 24-26 hr previously had elevated arterial [PG] (~14 mmol·l^{-1}), 2-fold higher FFA (~1 mmol·l^{-1}) and glycerol concentrations (0.09 mmol·l^{-1}), and 7- to 21-fold higher ketone body concentrations (0.3–0.8 mmol·l^{-1}) at rest than non-diabetics (Wahren *et al.*, 1984). The splanchnic fractional extraction of FFA did not differ between groups, but the combination of augmented net lipolysis, which increased the splanchnic inflow of

FFA, and higher intrahepatic conversion of FFA to ketones, gave rise to greater ketogenesis at rest in the group with type 1 DM (Wahren *et al.*, 1984). Even when sufficient insulin to ensure normoglycaemia was infused at rest, 1½ to 2-fold higher FFA (0.18 $mmol \cdot l^{-1}$) and glycerol (0.16 $mmol \cdot l^{-1}$), and ~4-fold higher β–hydroxybutyrate concentrations (0.4 $mmol \cdot l^{-1}$) were evident in subjects with type 1 DM compared to non-diabetics; perhaps due to a depletion of hepatic fructose 2,6-bisphosphate, which favours ketogenesis (Berk *et al.*, 1985).

Using the pancreatic clamp technique (without glucagon replacement) to determine dose-response curves for the antilipolytic effect of insulin, it was found that poorly controlled (HbA_{1c} 13.3%) patients with type 1 DM required more free insulin to suppress FFA flux (i.e. reduced insulin sensitivity with normal responsiveness) than non-diabetics (Jensen *et al.*, 1989). Half-maximal effect on lipolysis occurred at insulin concentrations of <2 and ~5 $mU \cdot l^{-1}$ in the non-diabetic and type 1 DM groups, respectively, demonstrating the marked sensitivity of the antilipolytic effect of insulin (Jensen *et al.*, 1989). Even in moderately controlled (HbA_{1c} 8.6%) subjects with type 1 DM, infusion of adrenaline (which achieved a plasma concentration of 2.57 $nmol \cdot l^{-1}$) during euglycaemic, hyperinsulinaemia (46 $mU \cdot l^{-1}$) induced lipolysis and restored plasma FFA and glycerol concentrations to baseline levels (having fallen below such with the hyperinsulinaemia); whereas in non-diabetic subjects lipolysis remained suppressed (Cohen *et al.*, 1996).

2.8.6 Effect of exercise on FFA, glycerol, ketone body, and lactate concentrations

In contrast to the predominance of α_2-mediated control of lipolysis at rest, during submaximal exercise β-adrenergic stimulation assumes ascendancy and promotes FFA and glycerol release from adipose tissue (Arner *et al.*, 1990), an effect that is enhanced by a greater β-adrenoreceptor responsiveness during submaximal exercise (Wahrenberg *et al.*, 1987). However, this effect is modulated by the prevailing insulin, glucose and lactate concentrations, all of which are dependent upon exercise intensity.

2.8.6.1 Mild intensity exercise in non-diabetics

Exercise at a mild intensity, i.e. 40-45% $\dot{V}O_{2peak}$, is associated with increased arterial FFA and glycerol concentrations as a consequence of enhanced lipolysis (Boyd *et al.*, 1974; Wahren *et al.*, 1984), and only a small rise in arterial [Lac^-] (Boyd *et al.*, 1974). After 60 min exercise at 40% $\dot{V}O_{2peak}$, during which FFA and glycerol increased 26% and ~2.5-fold, respectively, and [Lac^-] remained below 1 mmol·l^{-1}, infusion of lactate (which achieved arterial [Lac^-] of 8.8 mmol·l^{-1}; characteristic of a much higher exercise intensity) reduced lipolysis during a further 30 min exercise in untrained men (Boyd *et al.*, 1974). At a similarly low exercise intensity (45% $\dot{V}O_{2peak}$) arterial adrenaline and noradrenaline concentrations reached ~1.38 and 4.3 nmol·l^{-1}, respectively, after 60 min exercise (Wahren *et al.*, 1984); the former just exceeding the threshold at which plasma glycerol increased with adrenaline infusion (Freyschuss *et al.*, 1986), and the latter considerably below the threshold even in venous blood (Silverberg *et al.*, 1978). Thus, although the stimulatory effect of adrenaline only just exceeded the lipolytic threshold (however, in intense exercise concentrations of both catecholamines considerably exceed their respective thresholds), lactate appears to have a potent antilipolytic effect during exercise in untrained humans, despite a coincident reduction in insulin.

2.8.6.2 Maximal intermittent exercise in non-diabetics

Blood [Lac^-] rose to ~2.5, 4, and 5 mmol·l^{-1}, whilst glycerol and glucose concentrations did not change after single 10-, 30-, and 90-s 'all out' cycling bouts (performed on different days), respectively, in untrained men (Boulay *et al.*, 1995). Lactate continued to rise, peaking after 5 min recovery, and glycerol rose sharply to peak at 5–20 min recovery, with the greatest rises evident after the 90-s bout (after which blood glucose was also increased) (Boulay *et al.*, 1995). Similarly, plasma glycerol increased after the first of four 30-s maximal cycling bouts (separated by 4 min rest), and continued to rise linearly with each subsequent bout, reaching 5.5- to 6-fold higher values than at rest, whilst plasma FFA did not change and [Lac^-] increased to ~23 mmol·l^{-1} (McCartney *et al.*, 1986).

2.8.7 Effects of intense submaximal exercise in subjects with type 1 DM

No studies have reported FFA and glycerol responses in subjects with type 1 DM during and after maximal exercise. Glycerol rose with 10 min exercise at 80-85 % $\dot{V}O_{2peak}$ in untrained subjects with and without type 1 DM, and peaked at ~3.5 mmol·l^{-1} (Zander *et al.*, 1983), with no difference evident whether 4U of insulin was taken either 180 or 60 min prior to exercise. However, when the same individual subjects with type 1 DM were examined under each insulin protocol, peak glycerol concentrations after exercise were 3-fold higher when insulin was taken 180 min before exercise (when subjects were considered to be hypoinsulinaemic) (Zander *et al.*, 1983). FFA concentrations were higher at rest in the hypoinsulinaemic group, and although FFA fell with exercise, it remained ~3-fold higher than concentrations in the non-diabetics and in the subjects with type 1 DM who had insulin 60 min prior to exercise (considered hyperinsulinaemic) (Zander *et al.*, 1983).

The ~40% fall in FFA concentration that was observed in untrained non-diabetic subjects with intense exhaustive exercise at 80% $\dot{V}O_{2peak}$ did not occur in subjects with type 1 DM on continuous subcutaneous infusion of insulin (at baseline levels sufficient to achieve euglycaemia at rest, and hence higher IRI than non-diabetic subjects), although the fall in β-hydroxybutyrate, and rise in [Lac$^-$] (to ~6 mmol·l^{-1}) and catecholamines was similar (Mitchell *et al.*, 1988). In contrast, both untrained subjects with type 1 DM on constant i.v. insulin infusion and non-diabetics who were studied during 30 min exercise at 78% $\dot{V}O_{2peak}$ demonstrated increased plasma glycerol (to ~0.15 mmol·l^{-1}) and reduced FFA (to ~0.2 mmol·l^{-1}), with rates of appearance of FFA and glycerol being similar during exercise, but the latter higher in subjects with type 1 DM during recovery (Raguso *et al.*, 1995) (possibly due to the tracer/tracee ratio increasing, whilst that for the non-diabetics remained stable).

2.8.8 Effects of sprint training on lipolysis

In the only study to examine the effect of sprint training on fat metabolism, a 3-day programme comprised of up to twenty four 60-s exercise bouts (1:4, work:rest) at 120 % $\dot{V}O_{2peak}$ did not alter the fall (or belated rise after 24 bouts) in plasma FFA, or rises in blood [Lac$^-$] (Table 2.5) or glycerol (Green *et al.*, 1987b). No other human sprint

training studies have reported FFA, glycerol, or ketone body concentrations during or after intense exercise.

The plasma lactate response to exhausting exercise after sprint training, has received considerably more attention. Table 2.5 summarises the effects of sprint training on blood or plasma [Lac^-] during and after maximal exercise. All studies examined subjects exercising to exhaustion pre- and post-training, except Nevill *et al.* (1989) and Brooks *et al.* (1987) who included a matched-work test conducted at 110% pre-training $\dot{V}O_{2peak}$, and Green *et al.* (1987b), discussed above. Lactates were either similar or higher when exercising to exhaustion after training, when in most cases more work was performed. Higher [Lac^-] may possibly be anticipated to further suppress adipose tissue lipolysis, however has not been examined.

In the matched-work tests [Lac^-] did not differ significantly. However, the metabolic perturbation during exercise may have been insufficient to allow discrimination in two studies (Brooks *et al.*, 1987; Nevill *et al.*, 1989), and in the other (Green *et al.*, 1987b), the [Lac^-] represented the mean over a number of exercise bouts, and thus may have disguised differences. Thus, the effects of sprint training on plasma [Lac^-] during matched-work exercise remain unclear, and little is known of the relationship between lactate regulation, lipolysis, and glucoregulation during such exercise after sprint training.

2.8.9 Effects on lipolysis at rest and during intense exercise in endurance-trained subjects

Postabsorptive endurance-trained cyclists had similar IRI and catecholamine concentrations, but higher rates of release of FFA and glycerol, and a 4-fold higher rate of triglyceride-fatty acid recycling at rest than untrained subjects, which the authors suggested may enhance the potential for early fat oxidation in exercise (Romijn *et al.*, 1993b).

Early studies demonstrated that running at a self-selected pace for 90 min resulted in considerably higher FFA concentration and post-exercise ketosis in untrained subjects than competitive runners (Corbett *et al.*, 1969; Johnson *et al.*, 1969). Since glycerol

Table 2.5 *Lactate concentrations following maximal exercise before and after a sprint training programme, or in sprinters.*

Study	Subjects: *n*, gender	Duration	Pre/ Post training	Exercise test: Ex/ M/ IT; duration; intensity (% $\dot{V}O_{2peak}$)	Blood: Art.; Artz.; C.; Ven.; P/WB	Performance	End Ex [Lac$^-$] mmol·l^{-1}	Peak [Lac$^-$] mmol·l^{-1}	Peak time (min) E/ R
(Sharp *et al.*, 1986)	8M	8/52	Pre	Ex; Incr.	Ven. WB		~6.5	~11.5	5R
			Post			improved	~10*	~13.5*	4R
(Brooks *et al.*, 1987)	8	8/52	Pre	Ex; 30s; 'all out'	Ven. WB		-	12.1	3R
			Post			improved	-	13.9*	3R
			Pre	NEx, M; 2min;110%	Ven. WB		-	7.9	3R
			Post			-	-	7.2	3R
(Cheetham & Williams, 1987)	12F	6/52	Pre†	Ex; 30s; 'all out'	C.		-	15.4	5R
			Post			improved	-	14.7	5R
(Jacobs *et al.*, 1987)	7M, 4F	6/52	Post	Ex; 30s; 'all out'	C. WB		-	~11	4R
			Pre			no change	-	~12.5*	4R
(Green *et al.*, 1987b)	6M	3/7	Pre†	M, IT(1:4); 12-24x60s; 120%	Artz. WB		5.74‡	~8	E
			Post			-	5.39‡	~8	E
(Bell & Wenger, 1988)	8M, 1F	7/52	Pre	Ex; 60s, one leg	Ven. WB	improved	-	8.4	4R
			Post				-	7.8	4R
(Nevill *et al.*, 1989)	4M, 4F	8/52	Pre	Ex; 30s; 'all out'	C. WB		-	13	5R
			Post			improved	-	13.7	5R
			Pre	M; 2min; 110%	C. WB		-	8.1	5R
			Post			-	-	6.8	5R
(Stathis *et al.*, 1994)	6M, 2F	7/52	Pre	Ex; 30s; 'all out'	Ven. P		~12.5	~15	5R
			Post			improved	~16*	~20*	5R

Table 2.5 (cont.): *Lactate concentrations following maximal exercise before and after a sprint training programme, or in sprinters*

Study	Subjects: *n*, gender	Duration	Pre/ Post training	Exercise test: Ex/ M/ IT; duration; intensity (% $\dot{V}O_{2peak}$)	Blood: Art.; Artz.; C.; Ven.; P/WB	Performance	End Ex [Lac^-] $mmol \cdot l^{-1}$	Peak [Lac^-] $mmol \cdot l^{-1}$	Peak time (min) E/ R
(McKenna *et al.*, 1997b)	6M	7/52	Pre	Ex; 30s; 'all out'	Art. WB		5.1	16.2	5R
			Post			improved	5.0	16.4	5R
(Pilegaard *et al.*, 1999)	5M	8/52	UTx	Ex; one leg incr. or 78W constant	Art. P		~8	~8	End E
			Tx			improved	~10.5*	~10.5*	End E
(Costill *et al.*, 1983)	6M	3ST,3ET		Ex; 54-105s;125 %	Ven. WB		~8.5ST ~7ET	~14ST ~9.5ET	5-10R
(Hermansen *et al.*, 1984)	8	4ST,4ET		Ex, IT; 5x35-60s	Art. WB		24.1 ST 18.5 ET	24.1 ST 18.5 ET	End E
(Medbø & Sejersted, 1985)	12M	6ST,6ET		Ex; 55-57s; 'all out'	Art. WB		13.6ST 9.8ET	16.7ST 12.5ET	6R 3R
(Näveri *et al.*, 1985)	9M	ST		Ex; 3x300m	Ven. WB		16.7	16.7	End E
(Hirvonen *et al.*, 1987)	7M	ST		Ex; 40/60/80/100m	Ven. WB			4.5/5.7/ 7.2/8.2	?

Pre, pre-training; Post, post-training; Ex, test conducted to exhaustion; NEx, non-exhaustive exercise; M, matched-work test; IT, intermittent, numbers in brackets after IT indicate work: rest ratio; Art., arterial; Artz., arterialized venous; C., capillary; Ven., venous; P, plasma; WB, whole blood; [Lac^-], lactate concentration; End E/ R, end of exercise/ or in recovery; M, male subjects; F, female subjects; *x*/52, number of training weeks; *x*/7, number of training days; ST, sprint-trained subjects; ET, endurance-trained subjects; UTx, untrained leg; Tx; trained leg; † Subjects already moderately-trained according to $\dot{V}O_{2peak}$; ‡, mean [Lac^-] throughout IT exercise; * significant difference after training.

concentrations were similar, it was suggested that the athletes were able to oxidize fatty acids more effectively than the untrained (Johnson *et al.*, 1969). However, interpretation of these early studies is difficult given that the relative intensity (indicated by HR) was lower, and the absolute intensity (running speed) much higher in the athletic group. Exhausting exercise at 84-100% $\dot{V}O_{2peak}$ markedly elevated catecholamine concentrations, increased blood [Lac$^-$] to 11-13 mmol·l^{-1}, reduced FFA ~45-50%, increased blood glycerol ~1.5-fold, and reduced IRI ~20% in moderately- to highly-trained men (Sigal *et al.*, 1994a; Sigal *et al.*, 1996). Lactate fell slowly in recovery, remaining above resting levels until ~120 min, whilst glycerol continued to rise and peaked in early recovery, IRI rebounded and peaked well above resting levels at ~5 min recovery, and FFA concentration rose, but remained below resting levels until 120 min recovery (Sigal *et al.*, 1994a; Sigal *et al.*, 1996). FFA concentration was higher at rest, throughout exhaustive exercise at 88% $\dot{V}O_{2peak}$, and in recovery in trained women compared to trained men; whilst catecholamines (Table 2.4) and [Lac$^-$] (Table 2.5) did not differ and plasma IRI differed only in recovery, being considerably higher in the women (Marliss *et al.*, 2000). The authors suggested the FFA elevation in women may be oestrogen related (Marliss *et al.*, 2000).

2.8.9.1 Effects of lactate on lipolysis in the endurance-trained

In an early study, treadmill walking at a set speed and gradient which exhausted sedentary subjects in 24 min, was easily continued for 66 min with only mild fatigue in trained subjects (mountain climbers, rower, cyclist) (Cobb & Johnson, 1963). After 10-15 min of exercise, arterial FFA had fallen similarly by 39 and 33 %, but arterial [Lac$^-$] had risen to 6.4 (10-fold rise) and 1.3 mmol·l^{-1} (2-fold rise) in the sedentary and trained subjects, respectively, with a significant negative correlation ($r = -0.48$) between the change in FFA and lactate; from which it was concluded that increased lactate directly inhibited lipolysis (Cobb & Johnson, 1963). Exercise at the same absolute work load (necessitating a 6- to 7-fold increase above resting $\dot{V}O_2$) reduced both FFA turnover and plasma FFA concentration in untrained dogs, but increased both in trained dogs, leading the authors to suggest that lactate interfered with FFA release (Issekutz *et al.*, 1965). In later experiments, the relationship between FFA R_a and [Lac$^-$] was found to be exponential, with the rise in FFA R_a above resting levels being prevented when plasma [Lac$^-$] exceeded 6.25–7.29 mmol·l^{-1} (Issekutz *et al.*,

1975). A positive linear correlation was found between glycerol R_a and [Lac^-], and was suggested to be due to an increased formation of α-glycerophosphate which may enhance intracellular re-esterification of fatty acids (Issekutz *et al.*, 1975).

In a study of moderately-trained men, undertaken to examine the effect of high [Lac^-] on FFA concentrations during exercise, leg cycling at 60% $\dot{V}O_{2peak}$ was replaced (after 60 min) by four intermittent, 60-s bouts of maximal arm cranking (separated by 5 min), after and between which leg cycling continued (Green *et al.*, 1979). FFA levels rose with leg cycling, but were suppressed virtually to basal levels with maximal arm work which increased blood [Lac^-] from ~1.5 to 7.5 mmol·l^{-1} (and did not alter serum insulin); after which FFA slowly rose again for the remainder of leg cycling (Green *et al.*, 1979). A study in which arm cranking was superimposed upon leg cycling, thus raising the workload from 60 to 82% $\dot{V}O_{2peak}$, also demonstrated a sharp rise in arterial blood [Lac^-] to ~6.5 mmol·l^{-1}, a significant fall in FFA, a continued rise in glycerol, and a fall in insulin (Kjær *et al.*, 1991). Exercise at 70% $\dot{V}O_{2peak}$ continued for 40 min in moderately fit men, resulted in a significant fall (~40% below resting levels) in FFA influx into arterial blood, associated with high plasma [Lac^-] (~10 mmol·l^{-1}) (Jones *et al.*, 1980). The authors (Jones *et al.*, 1980) suggested that a lactate-induced increase in sensitivity of adipose lipolysis to insulin (Green & Newsholme, 1979) would enhance the antilipolytic effect of lactate on FFA. In the same study (Jones *et al.*, 1980), plasma glucose concentration increased progressively during exercise to peak at ~8 mmol·l^{-1} (and was similarly elevated in the Green *et al.* (Green *et al.*, 1979) and Kjær *et al.* (Kjær *et al.*, 1991) studies), and based upon the effects of hyperglycaemia at rest (Carlson *et al.*, 1991), may also have contributed to the suppression of FFA R_a. Despite the fall in FFA R_a, plasma glycerol concentration rose progressively during exercise to peak at 0.23 mmol·l^{-1} (~4-fold increase) at the end of exercise (Jones *et al.*, 1980). Thus it was concluded that heavy exercise inhibited adipose tissue lipolysis and stimulated intramuscular lipolysis; the latter involving immediate muscle oxidation of FFA, and release of glycerol into the circulation (Jones *et al.*, 1980).

Table 2.6 summarizes [Lac$^-$] data from studies in which trained subjects have been examined. Immediately after continuous exhaustive exercise of 30 s to 15 min duration, [Lac$^-$] varied between ~7 to 13 mmol·l^{-1}. Peak [Lac$^-$] occurred immediately after exercise for longer duration exercise (~7-15 min), and in early recovery (~3-9 min) for more intense exercise of 30 s to 5 min duration. Peak plasma [Lac$^-$] occurred earlier in recovery in women than men, but no other differences were noted during exercise or recovery (Brooks *et al.*, 1990). Intermittent, intense exercise bouts generally produced higher [Lac$^-$] after exercise and in recovery. Cycling to exhaustion in 2 min, or five maximal 40- to 60-s runs in a group mostly composed of trained subjects resulted in one individual attaining a peak blood [Lac$^-$] of 32.1 mmol·l^{-1} (Osnes & Hermansen, 1972). All these values either approximate or considerably exceed the concentrations that suppressed FFA release during exercise.

2.8.10 Effects of type 1 DM on lipolysis during intense exercise

No longitudinal studies have examined the effect of high intensity training on fat metabolism during and after maximal exercise in subjects with type 1 DM.

In an early study, moderately-trained subjects with type 1 DM who were postabsorptive and had administered their last insulin dose 16 hours previously, and non-diabetics, exercised intermittently on different days to exhaustion at 20, 50, 70, and 90% $\dot{V}O_{2peak}$ (Pruett & Mæhlum, 1973). Resting FFA concentrations were higher in the type 1 DM group, however there was no significant difference in the rise in FFA during exercise at 20 and 50% $\dot{V}O_{2peak}$, or the tendency to reduce plasma FFA at 90% $\dot{V}O_{2peak}$, or in blood lactate concentrations (Pruett & Mæhlum, 1973). Exhausting exercise (~13 min) at 91-98% $\dot{V}O_{2peak}$ increased blood [Lac$^-$] to ~13 mmol·l^{-1}, reduced FFA ~45%, and markedly elevated catecholamines in moderately-trained non-diabetics and well-controlled subjects with type 1 DM (either with continuous i.v. infusion to attain resting euglycaemia or hyperglycaemia), with no difference between groups (Sigal *et al.*, 1994b). Plasma FFA rebounded to baseline levels by 4 min recovery in all groups (Sigal *et al.*, 1994b), but then fell to reach a nadir at ~75 min recovery (after which a slow rise to resting values was evident) in the non-diabetic and type 1 DM group which commenced exercise with euglycaemia

Table 2.6 *Lactate concentrations following intense submaximal to maximal exercise in trained subjects.*

Study	Subjects: *n*, gender; training status (Mod, High)	Exercise test: Ex/ NEx; duration; intensity (% $\dot{V}O_{2peak}$)	Blood: Art.; Artz.; C; Ven.	Ex [Lac^-] $mmol \cdot l^{-1}$	Peak [Lac^-] $mmol \cdot l^{-1}$	Peak time (min), End E/ R
(Kjær *et al.*, 1991)	7M; Mod	NEx; 20min; 82%	Art.			
(Calles *et al.*, 1983)	8M, 3F; Mod	Ex; 12min; 85%	Ven.			
(Marliss *et al.*, 2000)	12F; Mod	Ex; 14min; 88%	Ven.	~10	~10	End E – 2R
(Sigal *et al.*, 1994b)	6M; Mod	Ex; 13min; 98%	Ven.			
(Marliss *et al.*, 1991)	6M; Mod	Ex; 13min; ~100%	Ven.	13.2	13.2	End E
(Marliss *et al.*, 1992)	18M; Mod	Ex; 7-12min; ~100%	Ven.	13.2	13.2	End E
(Sigal *et al.*, 1994a)	6M; Mod	Ex; 13min; 100%	Ven.	~13	~13	End E
(Purdon *et al.*, 1993)	6M; Mod	Ex; 13min; 103%	Ven.			
(Åstrand *et al.*, 1986)	5M, 2F; Mod	Ex; 5min; maximal	Art.	12.1	15.2	4R
(Karlsson & Saltin, 1970)	3M; Mod	Ex; 2-3min; maximal	C.WB	6.7	13.4	R
(Kowalchuk *et al.*, 1988b)	3M; ?	Ex; 30s; 'all out'	Art. P	10	14	3.5R
(Greenhaff *et al.*, 1994)	5M, 1F; ?Mod	Ex; 30s; 'all out'	Ven. WB	12.9	17.1	10R
(Allsop *et al.*, 1990)	7M, 3F; ?Mod	Ex; 30s; 'all out'	Ven.			
(Langfort *et al.*, 1997)	8M; Mod	Ex; 30s; 'all out'	Ven.	-	~10.5	3R
(Esbjörnsson-Liljedahl *et al.*, 1999)	20M, 19F; ?Mod	Ex; 30s; 'all out'	Ven. WB	-	~12,Γ ~9.5,E	9R 9R
(Hargreaves *et al.*, 1998)	6M; Mod	Ex; 3 x 30s; 'all out'	Ven. P	16.9	19	4R
(Lindinger *et al.*, 1992)	5M; ?	Ex; 4 x 30s; 'all out'	Art. P	21	21	End E
(Brooks *et al.*, 1990)	9M, 9F; ?Mod	?Ex; 10x6s (1:5)	Ven.	~13	~14	3-5R
(Gaitanos *et al.*, 1993)	8M; ?Mod	?Ex; 10x6s (1:5)	Ven.	12.6	12.6	End E

Table 2.6 (cont.): *Lactate concentrations following intense submaximal to maximal exercise in trained subjects*

Study	Subjects: *n*, gender; training status (Mod, High)	Exercise test: Ex/ NEx; duration; intensity (% $\dot{V}O_{2peak}$)	Blood: Art.; Artz.; C; Ven.	Ex [Lac^-] $mmol \cdot l^{-1}$	Peak [Lac^-] $mmol \cdot l^{-1}$	Peak time (min), End E/ R
(Sigal *et al.*, 1996)	7M; High	Ex; 14min; 84%	Ven.	11	11	End E
(Romijn *et al.*, 1993a)	5?; High	NEx; 30min; 85%	Artz.			
(Manzon *et al.*, 1998)	12M; High	Ex; 15min; 87%	Ven. WB	~11.5	~11.5	End E
(Marliss *et al.*, 2000)	16M; High	Ex; 14min; 88%	Ven.	~11	~11	End E – 2R
(Karlsson *et al.*, 1971)	13M; High	Ex; 2-3min; 100%	C. WB	-	14.6	End E – 8R
(Kjær *et al.*, 1986)	8M; High	NEx; 2min; 110%	Artz.			
(Cheetham *et al.*, 1986)	8F; High	Ex; 30s; 'all out'	C. WB	-	11.6	3R
(Mazzeo & Marshall, 1989)	12M; High	Ex; Incr.	Ven.	8.3	-	-
(Brooks *et al.*, 1988)	10M; High	Ex; 30s; 'all out'	Artz.	11.1	15.8	5R
(Withers *et al.*, 1991)	6M; High	Ex; 30/ 60/ 90s; 'all out'	Ven. WB	~8/11/13.5	12.6/16.4/18.9	7.5R
(Deuster *et al.*, 1989)	7M; High	?Ex; 10x30s (1:1); 90%	Ven.			
(Balsom *et al.*, 1992)	7M; High	Ex; 40x15m/ 20x30m/15x40m	C. WB	~6.5/13/15	~6.5/14/17	End E/2R/4R

Mod, moderately-trained; High, Highly-trained; Ex, test conducted to exhaustion; NEx, non-exhaustive test; Art., arterial; Artz., arterialized venous; C, capillary; Ven., venous; [Lac^-] lactate concentration; M, male subjects; F, female subjects; ET, endurance trained; (1:5), (1:1), work: rest ratio in intermittent exercise; Incr., incremental test; S/ET, sprint/ endurance trained; m, metres.

(then had their insulin infusion doubled in recovery to mimic the non-diabetic group response), but not in the hyperglycaemic group; thus highlighting the role of insulin in control of lipolysis in recovery.

In summary, significant lipolysis occurs during intense exercise. Plasma glycerol concentration is increased in recovery from a single 10-s maximal exercise bout, and FFA release and concentration is typically reduced during intense submaximal exercise. Increased exercise intensity is associated with increased IMTG hydrolysis and reduced FFA release from adipose tissue. Adrenaline and noradrenaline increase markedly with intense exercise and promote lipolysis in adipose tissue and muscle. Insulin is either unchanged or reduced during intense exercise; the latter acting to reduce lipolytic inhibition (although lipolytic sensitivity to insulin may be enhanced by high [Lac^-]). Counteracting the effects of reduced insulin and increased catecholamines is a considerable rise in [Lac^-], and to a lesser extent glucose, both of which act to suppress FFA release from adipose tissue. In recovery FFA concentration rebounds towards resting levels, then may be slowly reduced again over the next 60 min. The rapid rise after intense submaximal exercise may be related to enhanced adipose tissue blood flow, and the subsequent suppression may be attributed to the combined effects of very high [Lac^-], hyperglycaemia, and hyperinsulinaemia (in response to hyperglycaemia), coupled with rapidly falling catecholamine concentrations. If relatively insulinopaenic, subjects with type 1 DM may have higher resting concentrations of FFA, glycerol and ketone bodies than non-diabetics, intense exercise may or may not suppress FFA concentrations, and differences will be more marked in recovery from intense exercise, unless an insulin bolus is administered. Endurance training reduces adipose tissue lipolysis but enhances non-plasma FFA oxidation (presumably from an intramuscular source) when exercising at the same absolute work load. However the effects of sprint training on lipolysis during and after maximal exercise remain virtually unknown, and have never been investigated in subjects with type 1 DM.

2.9 Plasma glucose and the integrated glucoregulatory response to intense exercise and training

2.9.1 Effect of glucose on the liver

The effect of glucose on the liver is more important than that of insulin (Hers, 1976). Glucose inhibits the activity of phosphorylase *a*, the active form of glycogen phosphorylase; the relative proportions of the active and inactive (*b* form) forms are respectively controlled by phosphorylation and dephosphorylation and determine the rate of hepatic glycogenolysis (Hers, 1976). Thus when arterial or portal venous glucose concentrations are high, phosphorylase *b* dominates, glycogenolysis is inhibited, and glucose uptake and glycogen synthesis are stimulated. Although arterial plasma glucose concentration ([PG]) is not tightly controlled, varying with dietary, hormonal and activity status (Zierler, 1999), perturbations to [PG] elicit brisk counter-regulatory responses.

2.9.2 Hepatic blood supply and exercise

Glucose arising from intestinal absorption is delivered to the liver via the portal vein, in which the confluence of the venous drainage of the gastrointestinal organs, spleen and pancreas occurs, and from which arises 70% of the total hepatic blood supply (Rowell, 1993). Most endogenously produced glucose is released by the liver (there is also renal glucose production) into the hepatic veins and thence into the inferior vena cava. Hepatic blood flow varies inversely with heart rate (Rowell, 1993) and hence is reduced during exercise in an intensity-dependent manner (Rowell *et al.*, 1964; Wahren *et al.*, 1971; Kjær *et al.*, 1993), with 57% reduction at 74% $\dot{V}O_{2peak}$, a 62% reduction evident shortly after commencing a constant heavy work load (inducing a 10-fold increase in $\dot{V}O_2$) (Wahren *et al.*, 1971), and up to an 84% reduction at near maximal exercise (Rowell *et al.*, 1964). Even after 4 min recovery from exhausting maximal exercise, estimated splanchnic blood flow was 56% below resting values (Åstrand *et al.*, 1986). Hepatic clearance is inversely related to relative exercise intensity, and is primarily affected by hepatic blood flow, not by a change in the extraction ratio (Rowell *et al.*, 1964). Despite the considerable reduction in hepatic blood flow during exercise, splanchnic $\dot{V}O_2$ remains stable as a consequence of a marked widening of the hepatic arteriovenous oxygen difference (Rowell *et al.*,

1964). High-dose infusion of noradrenaline, which produced arterialized venous plasma concentrations of 15.1 nmol·l^{-1}, reduced resting hepatic portal blood flow in man by 18%, whereas adrenaline had no effect (Krentz *et al.*, 1996). However, local [NAdr] at target organs will be considerably higher than systemic concentration and organ-specific changes in clearance will contribute to local effects (Esler *et al.*, 1990; Rowell, 1993); thus during intense exercise the liver, which has a rich sympathetic innervation (Rowell, 1993), will be exposed to very high [NAdr] which will contribute to the marked reduction in hepatic blood flow. Direct neural innervation of the liver also leads to very rapid activation (within ~30 s) of glycogenolytic enzymes, as recently reviewed (Shimazu, 1996).

2.9.3 Cellular glucose uptake

In steady-state conditions, glucose concentration in arterial blood is 0.09 mmol·l^{-1} (0.017 mg·ml^{-1}) higher than in arterialized venous blood, and 0.17 mmol·l^{-1} (0.031 mg·ml^{-1}) higher than in venous blood (McGuire *et al.*, 1976). Venous [PG] varies according to the rate of arterial blood flow and tissue glucose uptake (Zierler, 1999). Glucose uptake is affected by exercise, and by both glucose and insulin concentrations. Cellular glucose uptake occurs by a process of facilitated diffusion which involves transport across the sarcolemma by the glucose-specific transporters (GLUTs). In muscle and adipose tissue the predominant GLUT is the GLUT-4 protein (Klip & Pâquet, 1990). In humans, distinct intracellular pools of GLUT-4 transporters respond to stimulation by insulin (Gumà *et al.*, 1995) or exercise (Kristiansen *et al.*, 1996) by translocating to the plasma and t-tubule membranes, where they effect an increase in glucose uptake (see sections 2.16.1, 2.16.2). In addition, noradrenaline may increase the activity of glucose transporters already in the plasma membrane, independently of changes in either insulin or blood flow (Shimazu, 1996).

2.9.4 Effects of intense exercise on plasma glucose concentration in non-diabetics

Postabsorptive arterial blood plasma glucose concentration averaged 5 mmol·l^{-1} in non-diabetic subjects (Zierler, 1999). In untrained subjects, blood glucose ([BG]) or [PG] was not altered by cycling for 30 min at 80% $\dot{V}O_{2peak}$ (Coggan *et al.*, 1995;

Coggan *et al.*, 1997), 5 min at 97-98% $\dot{V}O_{2peak}$ (Hartley *et al.*, 1972; Katz *et al.*, 1986), or running incrementally at 60, 100, then 110% $\dot{V}O_{2peak}$ (Kjær *et al.*, 1986; Kjær *et al.*, 1990); however a significant rise occurred in recovery (Katz *et al.*, 1986; Kjær *et al.*, 1986; Kjær *et al.*, 1990). Arterial [BG] was increased by 1.2 $mmol \cdot l^{-1}$ (from 7.3 $mmol \cdot l^{-1}$; light breakfast preceded test) immediately after exhaustive intermittent exercise (3 x 60-s cycle bouts separated by 4 min rest), and further increased after 5 min recovery to peak at 8.9 $mmol \cdot l^{-1}$ (Hermansen & Vaage, 1977).

2.9.5 Effects of insulin and insulin deficiency on plasma glucose concentration during exercise

Whilst insulin and exercise activate independent pools of glucose transporters, and may do so simultaneously (Coderre *et al.*, 1995) with additive effects (Gao *et al.*, 1994), insulin deficiency reduces maximal glucose transport (Wallberg-Henriksson & Holloszy, 1985). Moderate to severe resting hyperglycaemia, indicative of insulinopaenia, has long been known to worsen with exercise. Fasting blood glucose concentrations in excess of 16.7 $mmol \cdot l^{-1}$ were exacerbated by 30 min rowing in 8 patients with type 1 DM (Richardson, 1934), and although the positive brachial arterial-femoral venous glucose difference was increased or unchanged during 45 min mild exercise in two subjects with type 1 DM who had resting [BG] of ~9 and 16 $mmol \cdot l^{-1}$, respectively, arterial [BG] rose mildly (Sanders *et al.*, 1964). However, in the former study, only 0.1 unit of insulin given before exercise effected a reversal in the hyperglycaemia (Richardson, 1934). Even prior to that study, endurance exercise had been reported to markedly enhance the action of insulin, having both an immediate, and a delayed effect of lowering blood glucose concentration (Lawrence, 1926), although as the following illustrates, the direction of change in [BG] or [PG] is not only dependent upon prevailing insulin levels, but also upon exercise intensity.

2.9.6 Effect of intense exercise on plasma glucose in subjects with type 1 DM

In subjects with type 1 DM, cycling at ~75% $\dot{V}O_{2peak}$ with a continuous i.v. infusion of short-acting insulin (Raguso *et al.*, 1995); and exhaustive exercise at 80-85% $\dot{V}O_{2peak}$ (Zander *et al.*, 1983) and brief, incremental exercise to exhaustion (Hübinger

et al., 1985) both conducted ~60 min after subcutaneous injection of insulin, failed to induce any significant change in [BG], either during exercise or in recovery (Table 2.7). The insulin regimens raised free insulin levels to 2 (Raguso *et al.*, 1995) to 4 times non-diabetic levels (Hübinger *et al.*, 1985), however the glucose R_a in the former was similar to control subjects, and did not differ from glucose R_d (Raguso *et al.*, 1995). Raguso *et al.* (1995) did not employ an exhaustive exercise test, and reported correspondingly low plasma [Adr] and [NAdr], and Hübinger et al. (1985) only demonstrated modest catecholamine concentrations, which may also have contributed to the lack of significant change in [PG] during exercise and recovery. In one study (Jakober *et al.*, 1983) [PG] appeared to increase during exercise and remain stable in recovery in subjects with type 1 DM (Table 2.7), however the values were not commented upon. Additionally, although the incremental test was reported to be conducted to exhaustion, and no subject had autonomic dysfunction (Jakober *et al.*, 1983), the cardiorespiratory and metabolic values appear to belie that an exhaustion end-point was attained.

[PG] rose during exhausting cycling at 80% $\dot{V}O_{2peak}$, and continued to rise during recovery to peak 3 $mmol \cdot l^{-1}$ above resting levels in untrained subjects with type 1 DM who were receiving CSII at basal levels sufficient to maintain euglycaemia at rest (free IRI was 3–4 times above non-diabetic levels during exercise) (Mitchell *et al.*, 1988) (Table 2.7). The progressive rise in [PG] during recovery was exaggerated in a type 1 DM group that was similarly on basal CSII, but commenced exercise with mild hyperglycaemia (Mitchell *et al.*, 1988). In untrained subjects with type 1 DM who exercised (whilst on basal i.v. insulin infusion: free IRI ~2-3 times greater than non-diabetics during exercise) at a work load that demanded ~100% $\dot{V}O_{2peak}$ at exhaustion, [PG] increased by ~2.2 $mmol \cdot l^{-1}$ during exercise, peaked at 7.9 $mmol \cdot l^{-1}$ in early recovery, then varied little throughout the remainder of recovery (Purdon *et al.*, 1993) (Table 2.7). R_a and R_d increments and decrements were identical in type 1 DM and non-diabetic subjects (the latter group was moderately trained), however, recovery hyperglycaemia was due to lower metabolic clearance (MCR; R_d/[PG]) in *the subjects with type 1 DM, which implied that normal post-exercise metabolic* clearance requires hyperinsulinaemia (Purdon *et al.*, 1993).

Table 2.7 *Free immunoreactive insulin and glycaemia in untrained and trained subjects with type 1 DM when exercising at intense submaximal to maximal intensities.*

Study	*n*/ gender, UTR/ TR	Baseline [PG] ($mmol \cdot l^{-1}$)	Fasted /Fed	Insulin delivery	†Exercise intensity/ duration	Free IRI ($\mu U \cdot ml^{-1}$) in ex/rec	Delta PG exercise ($mmol \cdot l^{-1}$)	Delta PG recovery ($mmol \cdot l^{-1}$)
(Hilsted *et al.*, 1981)	8M, UTR	15	Fasted	Delayed morning dose	100 W, ~20 min	4.5/ same	+1 - 2	NR
(Jakober *et al.*, 1983)	6, UTR	10	Fasted	Delayed morning dose	Incremental, 12 min	NR	+2(?)	No change
(Zander *et al.*, 1983)	11, UTR	12.6	Fasted	4U SC, 3 hr pre-ex	80-85% $\dot{V}O_{2max}$, 10 min	NR	No change?	+3.5
	15, UTR	13.4	Fasted	4U SC, 1hr pre-ex				No change
(Hübinger *et al.*, 1985)	4M, 5F, UTR	8.5	Fed	2/3 usual SC before b/fast, 1 hr pre-ex	Incremental, 2–4 min	36/ same	No change	No change
(Mitchell *et al.*, 1988)	2M, 4F UTR	4.8	Fasted	Basal rate CSII	80% $\dot{V}O_{2peak}$, 10–13 min		+0.8	+2.2
	3M, 1F	8.3	Fasted	Basal rate CSII			+0.9	+3.1

Table 2.7 (cont.): *Free immunoreactive insulin and glycaemia in untrained and trained subjects with type 1 DM when exercising at intense submaximal to maximal intensities.*

Study	*n*/ gender, UTR/ TR	Baseline [PG] ($mmol \cdot l^{-1}$)	Fasted /Fed	Insulin delivery	†Exercise intensity/ duration	Free IRI ($\mu U \cdot ml^{-1}$) in ex/rec	Delta PG exercise ($mmol \cdot l^{-1}$)	Delta PG recovery ($mmol \cdot l^{-1}$)
(Purdon *et al.*, 1993)	6M, UTR	4.8	Fasted	i.v. infusion	80-100% $\dot{V}O_{2peak}$, 12 min	19/ same	+2	+1
(Sigal *et al.*, 1994b)	6M,UTR	4.8	Fasted	i.v. infusion	89-98% $\dot{V}O_{2peak}$, 13 min	15/12	+2.2	#
	7M, TR	9	Fasted	i.v. infusion		12/ same	+1.5	#
	6M, TR	4.8	Fasted	2 x i.v. exhn‡		13/ 17	+3.2	- 4
(Raguso *et al.*, 1995)	4M, UTR	7	Fasted	i.v. infusion	77% $\dot{V}O_{2peak}$, 30 min*	11/ same	No change	No change

n, number of subjects; UTR, untrained; TR, trained; [PG], plasma glucose concentration; IRI, immunoreactive insulin concentration (Plasma glucose and insulin concentrations are generally approximations from graphs); † All exercise bouts conducted to exhaustion, except *. Delta PG exercise, change in PG from rest to exhaustion; Delta PG recovery, change in PG from exhaustion to end of recovery; M, male; ~, approximately; NR, not reported; + or – signs indicate a positive or negative change in PG; ?, question mark indicates that the value was not commented upon, and has therefore been estimated; U, units of insulin; SC, subcutaneous; b/fast, breakfast; pre-ex, prior to exercise; F, female; CSII, continuous subcutaneous insulin infusion; i.v., intravenous. ‡ i.v. insulin infusion rate was doubled at exhaustion, and continued until PG had returned to pre-exercise levels; # Peak PG was attained in early recovery, after which no significant change in concentration occurred.

2.9.7 Effects of sprint training on glucoregulation in non-diabetics

Table 2.8 summarises the very few longitudinal studies that have examined the effect of sprint training on glucoregulation during and after maximal exercise, or compared trained with sedentary subjects. Only two sprint training studies have examined [BG] during intense exercise. Numerous 60-s cycling bouts at 120% $\dot{V}O_{2peak}$ did not raise [BG], however on the third consecutive day of training using the same absolute work load, [BG] was 16% lower overall (Green *et al.*, 1987b) (Table 2.8). Whilst the authors (Green *et al.*, 1987b) conceded that the reduced [BG] may have been due to the training-induced hypervolaemia, they suggest that was unlikely given the relatively tight regulation of [BG]. Insulin and catecholamine concentrations were not measured so their roles in this adaptation are indeterminate. In support of Green *et al.* (1987), another study reported 15% lower fasting [PG] in trained endurance athletes compared to sedentary subjects (LeBlanc *et al.*, 1977). Eight weeks of sprint training did not affect peak [BG] during recovery from an 'all out' 30-s treadmill sprint in which more work was performed after training and 'peak' noradrenaline concentration was 20% higher (Nevill *et al.*, 1989). However following a 2-min run conducted at the same absolute work load, the rise in [BG] in early, but not later recovery was attenuated after training, although catecholamine concentrations did not differ (Nevill *et al.*, 1989) (Table 2.8). However, the interpretation of the blood glucose responses in relation to catecholamine concentrations is confounded by the fact that post-exercise blood samples were obtained at 3 min recovery, and consequently the peak [NAdr] and [Adr] would have been missed.

In cross-sectional studies, [PG] was either the same or higher in trained subjects during exercise at the same relative intensity and in recovery than in sedentary subjects, with varied catecholamine responses. In endurance-trained cyclists, [PG] rose during high-intensity exercise as a consequence of the rise in R_a exceeding the rise in R_d, however, in untrained subjects, a higher rise in R_d which matched the rise in R_a, resulted in no change in [PG] during exercise at the same relative intensity (Coggan *et al.*, 1995). Neither the fall in insulin, nor the rise in catecholamines differed between the two groups, and the mechanism accounting for the difference remains unclear (Coggan *et al.*, 1995). In contrast to sedentary subjects, in elite athletes R_a exceeded R_d in maximal exercise, thus [PG] rose, and the peak [PG] in

recovery was considerably higher (Table 2.8), due to 65% higher R_a (Kjær *et al.*, 1986). Whilst the tracer estimates were obtained under highly non-steady-state conditions, and may therefore be inaccurate (Marliss *et al.*, 1992; Coggan *et al.*, 1995), a peak [PG] of 9.28 $mmol \cdot l^{-1}$, that was 35% higher in trained than in sedentary subjects, was consistent with the two-fold higher peak [Adr] (Kjær *et al.*, 1986) and a greater R_a.

2.9.8 Effects of sprint training on glucoregulation in subjects with type 1 DM

No sprint training studies have included subjects with type 1 DM. The glucoregulatory response during and after maximal exercise following sprint training therefore remains unknown.

2.9.9 Glucoregulation during and after intense exercise in endurance-trained non-diabetic subjects

In trained subjects, postabsorptive glucose concentration averaged 4.72 $mmol \cdot l^{-1}$ and ranged between 3.9–5.6 $mmol \cdot l^{-1}$ (Table 2.9).

During exercise at 80–100% $\dot{V}O_{2peak}$, glucose R_a was consistently higher than R_d, and hence glucose concentration increased, peaking either at the end of exercise or in early recovery at a mean value of 7.32 $mmol \cdot l^{-1}$ (Table 2.9). Neither the R_a for glucose nor the [PG] at exhaustion differed significantly between exercise at ~80% $\dot{V}O_{2peak}$ and ~100% $\dot{V}O_{2peak}$ in moderately-trained cyclists (Williams *et al.*, 1995), for whom hormonal concentrations were not reported. Combined arm and leg exercise at 82% $\dot{V}O_{2peak}$ increased plasma catecholamines 3 to 4-fold more than leg exercise alone (55% $\dot{V}O_{2peak}$), increased R_a a further 3-fold and arterial [BG] 57%, and reduced arterial IRI, whilst having no affect upon glucagon (Kjær *et al.*, 1991). The dissociation of hepatic glucose production and feedback from [BG] was suggested to be due to increased sympathoadrenal activity (Kjær *et al.*, 1991).

Table 2.8 *Difference in glucoregulatory responses to intense exercise and recovery in trained versus untrained subjects*

Study	Subjects *n* TR, *n* UTR Training period	Duration; Intensity of exercise (% $\dot{V}O_{2peak}$)	Response of trained in comparison to untrained subjects (or in comparison to pre-training response in LTS)					
			Exercise			Recovery		
			[PG]	Insulin	Cats	[PG]	Insulin	Cats
(Bloom *et al.*, 1976)	6 TR cyclists, 6 UTR	7 min; 75%	Higher	Higher	Lower	Higher	Lower	Lower
(Kjær *et al.*, 1986)	8 TR runners/ skier, 8 UTR	5 min; 100-110%	Higher	Same	Higher	Higher	Higher	Same
(Deuster *et al.*, 1989)	7 TR runners, 7 UTR	5 min*; I/T 90%	Same	NR	Same	Same	NR	Same
(Coggan *et al.*, 1995)	8(5,3) TR cyclists, 8(5,3) UTR	30 min; 80%	Higher	Same	Same	NR	NR	NR
(Hartley *et al.*, 1972)	7 UTR, LTS 7 weeks	5 min; 93-98%	Same	Higher	Lower	Same	Same	Same
(Green *et al.*, 1987b)	6 fit, LTS 3 days	12-24 min*; I/T 120% pre	Lower	NR	NR	NR	NR	NR
(Nevill *et al.*, 1989)	8(4,4) UTR, LTS 8 weeks	30s; 'all out'	NR	NR	NR	Same	NR	Higher
		2 min; 110% pre	NR	NR	NR	Lower	NR	Same

TR, trained; UTR, untrained; LTS, longitudinal training study; [PG], plasma glucose concentration; Cats, catecholamine concentrations; *, total time of exercise reported in Table; I/T, intermittent; (5,3), (4,4); (number of male, female subjects); pre, pre-training; NR, not reported.

2.9.9.1 Effect of intense submaximal exercise on glucoregulation in trained non-diabetics

At exercise intensities greater than 85% $\dot{V}O_{2peak}$, peak IRI, which invariably occurred in recovery, was usually double that at rest (Table 2.9). The ratio of immunoreactive glucagon to IRI (IRG/IRI) rose, or occasionally did not change during intense submaximal to maximal exercise (Table 2.9). Despite 2-fold higher insulin concentrations in highly-trained subjects during intense exhausting submaximal exercise with an islet cell clamp, during which IRG/IRI was unchanged, the glucose R_a was similar to control exercise conditions (in which IRG/IRI increased) (Table 2.9) and was closely correlated with [NAdr] and [Adr], although increments in plasma glucose concentration during exercise were initially slower since the glucose R_d tended to be higher early in exercise with the clamp (Sigal *et al.*, 1996). These results lead the authors to conclude that neither the absolute concentrations of glucagon or insulin nor their molar ratio was the major determinant of the increment in R_a during intense exercise (Sigal *et al.*, 1996). A later study similarly concluded that since changes in IRG/IRI were small and out of phase with the changes in R_a, that a major glucoregulatory role during intense exercise is unlikely (Marliss *et al.*, 2000). Plasma insulin did not change during exhausting submaximal to maximal exercise, the IRG/IRI ratio was either unchanged or rose with exercise (Table 2.9), and the marked increases in [Adr] (14-fold) and [NAdr] (14 to 18-fold) (Table 2.4) were positively correlated with R_a throughout intense exercise and recovery, leading the authors to propose that the catecholamines are the major regulators of R_a, and also serve to constrain rises in R_d, whilst the doubling of insulin in recovery acts primarily to increase R_d (Marliss *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994b).

Kjær et al. (1993) have argued against the primacy of the catecholamines in determining glucose R_a, based upon a lack of difference in glucose R_a with and without partial blockade of the coeliac ganglion during an islet cell clamp. However since exercise was conducted firstly for 20 min at 41% $\dot{V}O_{2max}$, then for another 20 min at 74% $\dot{V}O_{2max}$ (Kjær *et al.*, 1993), the mild intensity may have influenced responses at the moderate intensity of exercise, especially since with the islet clamp insulin levels were relatively high during mild exercise. Additionally, noradrenaline

only reached moderate concentrations and appeared to fall slightly as exercise at 74% $\dot{V}O_{2max}$ progressed (when the concentration that the liver is exposed to in intense exercise may be considerably higher than that measured systemically); the rise in R_a (~3-fold) was lower than in intense exercise (see Table 2.9), as was the rise in [Lac^-]; and an infusion of adrenaline increased both R_a and [PG].

A recent study examined glucoregulation in trained men and women during and after intense submaximal exercise (Marliss *et al.*, 2000) (Table 2.9). R_a was similar in men and women during exercise (Table 2.4) and early recovery was highly correlated with both [NAdr] ($r = 0.92$) and [Adr] ($r = 0.85$), with no gender differences evident in the catecholamine response, however, hyperglycaemia was greater in women in recovery, and induced greater hyperinsulinaemia (Marliss *et al.*, 2000). Metabolic clearance and R_d were lower in women both during exercise and in early recovery, however, when corrected to fat free mass, R_d was the same or greater than in men (Marliss *et al.*, 2000).

2.9.9.2 Effect of maximal exercise on glucoregulation in trained non-diabetics

In trained subjects, a single, exhaustive 30-s bout of exercise increased [BG] or [PG] by 1.65 $mmol \cdot l^{-1}$, with peak concentration occurring in early recovery (Table 2.9); however, one study (Langfort *et al.*, 1997) found no change (or a reduction) in [BG] in recovery from a 30-s sprint, which was curious given the ~2-fold rise in insulin. Intermittent maximal exercise resulted in a greater glucose increment, which peaked slightly later in recovery (5–10 min) and averaged 3.06 $mmol \cdot l^{-1}$ (Table 2.9). Two of these studies examined plasma IRI concentration in relation to the exercise-induced hyperglycaemia, with one demonstrating no change during or after non-exhaustive exercise (Green *et al.*, 1979), and the other reporting an ~4-fold increase 17 min after five exhaustive 60-s bouts (Hermansen *et al.*, 1970), although as previously reviewed (see section 2.5.9.2), this value was affected by one subject with considerably higher IRI. Three, 300m runs increased [BG] by ~3.2 $mmol \cdot l^{-1}$ in a mixed group of sprint- and endurance-trained runners, and catecholamines were elevated 8 to 12-fold, leading the authors to conclude that the sympathoadrenal system is of major importance for liver glucose production (Näveri *et al.*, 1985).

Table 2.9 *Glucose kinetics, IRG-to-IRI ratio, and insulin concentration during intense submaximal to maximal exercise and recovery in trained non-diabetic subjects.*

Study	Exercise test: Ex/ NEx; duration; intensity (% $\dot{V}O_{2peak}$)	Rest [Gluc] mmol·l^{-1}, IRI mU·l^{-1}	Ex Gluc R_a	Ex Gluc R_d	Ex IRG/ IRI	Peak [Gluc] mmol·l^{-1}	Peak IRI mU·l^{-1}	Peak time Gluc, IRI
(Coggan *et al.*, 1995)	NEx; 30 min; 80%‡	~4.5, 4.3	↑4.4x	↑3.4x	↑	5.8	↓	End E, -
(Kjær *et al.*, 1991)	NEx; 20min; 82%†	5.1, ~5.5	↑~2.4x	↑~1.9x	↑	8	~6.8	5R, R
(Sigal *et al.*, 1996)	Ex; 14min; 84%	C,5.1, 15.5	↑~6.7x	↑~6x	↑	7.7	~22	4R, 4R
		IC, 4.5, 15.6	↑~5.9x	↑~4x	↔	7.4	~24	
(Calles *et al.*, 1983)	Ex; 12min; 85%	4.7, 16	↑5x	↑2.6x	↑	6.7	29	5R, 5R
(Romijn *et al.*, 1993a)	NEx; 30min; 85%	-, -	↑~5.8x	-	-	8.2	-	End E, -
(Pruett, 1970)	Ex; 29min; 87%	4.4, 15	-	-	-	~5.3	~17	2R, 2R
(Manzon *et al.*, 1998)	Ex; 15min; 87%	C,5.0, ~15	↑7x	↑4.7x	↑	~7.8	~27	2R, ~5R
		G,5.9, ~30	↑5x	↑2.6x	↑	~8.8	~30	~10R, ~5R
(Marliss *et al.*, 2000)	Ex; 14min; 88%	M4.9, ~14	↑~6.5x	↑~5.2x	↑	7.2	~22	4R, ~5R
		F4.7, ~14	↑~6.5x	↑~4.8x	↑	8.7	~27	4R, ~20R
(Sigal *et al.*, 1994b)	Ex; 13min; 98%	~4.9, ~10	↑~7.5x	↑4x	↑	~7.2	~20	2R, 4R
(Marliss *et al.*, 1991)	Ex; 13min; ~100%	~4.9, ~11	↑7x	↑3x	↔	7.0	~22	EndE, ~5R

Table 2.9 (cont.): *Glucose kinetics, IRG-to-IRI ratio, and insulin concentration during intense submaximal to maximal exercise and recovery in trained non-diabetic subjects*

Study	Exercise test: Ex/ NEx; duration; intensity (% $\dot{V}O_{2peak}$)	Rest [Gluc] $mmol \cdot l^{-1}$, IRI $mU \cdot l^{-1}$	Ex Gluc R_a	Ex Gluc R_d	Ex IRG/ IRI	Peak [Gluc] $mmol \cdot l^{-1}$	Peak IRI $mU \cdot l^{-1}$	Peak time Gluc, IRI
(Marliss *et al.*, 1992)	Ex; 7-12min; ~100%	~4.8, ~14	↑~7x	↑~3.5x	↑	~6.9	~30	4R, 4R
(Sigal *et al.*, 1994a)	Ex; 13min; 100%	~4.8, ~10	↑8.4x	↑~3.8x	↑	7.2	~21	2R, ~2R
(Purdon *et al.*, 1993)	Ex; 13min; 103%	4.8, ~11	↑7x	↑4x	↑	7.2	~22	2R, 4R
(Williams *et al.*, 1995)	Ex; 28min; 80%	-, -	↑~3.8x§	-	-	6.2	-	End E, -
	Ex; 6.5min; 100%	-, -	↑~4.3x§	-	-	6.4	-	End E, -
(Kjær *et al.*, 1986)	NEx; 3min; 100%†	~5.6, ~8	↑~1.2x (↑5x,1R)	↓~15% (↑2x,1R)	↔	9.3	~23	5R, R
(Åstrand *et al.*, 1986)	Ex; 5min; 100%	4.23, -	-	-	-	7.4	-	4R, -
(Cheetham *et al.*, 1986)	Ex; 30s; 'all out'	4.4, -	-	-	-	6.1	-	5R, -
(Cheetham & Williams, 1987)	Ex; 30s; 'all out'	4.7, -	-	-	-	6.2	-	5R, -
(Brooks *et al.*, 1988)	Ex; 30s; 'all out'	4.3, -	-	-	-	6.1	-	5R, -
(Allsop *et al.*, 1990)	Ex; 30s; 'all out'	4.4, -	-	-	-	6.0	-	5R, -
(Langfort *et al.*, 1997)	Ex; 30s; 'all out'	~5.8, ~22	-	-	-	↔(~5)	~38	-, 30R

Table 2.9 (cont.): *Glucose kinetics, IRG-to-IRI ratio, and insulin concentration during intense submaximal to maximal exercise and recovery in trained non-diabetic subjects*

Study	Exercise test: Ex/ NEx; duration; intensity (% $\dot{V}O_{2peak}$)	Rest [Gluc] $mmol \cdot l^{-1}$, IRI $mU \cdot l^{-1}$	Ex Gluc R_a	Ex Gluc R_d	Ex IRG/ IRI	Peak [Gluc] $mmol \cdot l^{-1}$	Peak IRI $mU \cdot l^{-1}$	Peak time Gluc, IRI
(Deuster *et al.*, 1989)	?Ex; 10x30s (1:1); 90%	~5.3, -	-	-	-	~7.1	-	~10R, -
(Näveri *et al.*, 1985)	Ex; 3x300m‡	~5, -	-	-	-	~8.2	-	End E, -
(Green *et al.*, 1979)	NEx; 4x60s(1:4)arms#	4.5, ~22	-	-	-	8.2	↔	~5R, -
(Hermansen *et al.*, 1970)	Ex; 5x60s (1:4)	4.6, 13	-	-	-	9.5	48	7R, 17R
(Brooks *et al.*, 1990)	?Ex; 10x6s (1:5)	-, -	-	-	-	6.8	-	5R, -
(Gaitanos *et al.*, 1993)	?Ex; 10x6s (1:5)	3.9, -	-	-	-	5.6	-	5R, -

Ex, test conducted to exhaustion; NEx, non-exhaustive test; [Gluc], glucose concentration; IRI, immunoreactive insulin concentration; Gluc R_a (R_d), rate of appearance (disappearance) of glucose; IRG/IRI, ratio of immunoreactive glucagon to IRI; ‡, no data reported for recovery; ~, indicates approximation from a graph; x; times (multiplier); End E, end of exercise; 5R, 5th minute of recovery; †, preceded by submaximal exercise; C, control subjects; IC; islet cell clamp; G, glucose infusion; M, male subjects; F, female subjects; §, resting values not reported, calculated assuming resting value of 10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; ?, uncertain of end-point; m, metres; (1:1, 4, 5), work: rest ratio in intermittent exercise; #, preceded by leg work at 60% $\dot{V}O_{2peak}$.

2.9.9.3 Effect of intense exercise on glucoregulation during recovery in trained non-diabetics

Plasma glucose concentration rose with exhaustive exercise at 85% $\dot{V}O_{2peak}$, peaked 41% above baseline after 5 min recovery, then slowly fell, but remained above resting levels during a further 25 min recovery (Calles *et al.*, 1983) (Table 2.9). Whilst the rate of appearance (R_a) of glucose was elevated 5-fold at exhaustion through to 5 min recovery, R_d fell 33%, with the result that R_a markedly exceeded R_d; then from 20 to 30 min recovery R_d was greater than R_a, the latter having returned to baseline (Calles *et al.*, 1983). The return of R_a towards baseline in early recovery is probably attributable to the falling catecholamine concentrations, rather than to the physiological hyperinsulinaemia that follows the exercise-induced hyperglycaemia (Purdon *et al.*, 1993). R_a fell identically whether the normal post-exercise hyperinsulinaemia ensued, or whether hyperinsulinaemia was doubled via an i.v. bolus (Purdon *et al.*, 1993).

2.9.9.4 Effect of hyperglycaemia at rest during intense exercise in non-diabetics

In non-diabetic subjects, resting hyperglycaemia somewhat reduced the rise in R_a and enhanced R_d during intense exercise (Manzon *et al.*, 1998). Despite complete suppression of endogenous R_a by exogenous glucose infusion prior to exercise, mild hyperglycaemia, and the accompanying hyperinsulinaemia, endogenous R_a and [PG] increased to 60 and 67 % of normal, respectively, during intense exercise to exhaustion (Manzon *et al.*, 1998) (Table 2.9). Total (exogenous plus endogenous) R_a was 31 % higher in the hyperglycaemic group, however, R_d and metabolic clearance were also higher (Manzon *et al.*, 1998), thus contributing to the somewhat attenuated rise in [PG]. Noradrenaline and [Adr] (which did not differ between hyperglycaemic and control groups) increased 12 to 15-fold during exercise (Table 2.4), and were significantly correlated with endogenous R_a. Plasma glucagon was suppressed, and plasma IRI, despite falling during exercise, remained approximately double that of the control group, thus the glucagon-to-insulin ratio rose a little during exercise (Table 2.9), however was lower than in controls and probably contributed little to the R_a (Manzon *et al.*, 1998).

2.9.10 Effects of intense exercise on glucoregulation in trained subjects with type 1 DM

When fit subjects with type 1 DM exercised to exhaustion at 80-100% $\dot{V}O_{2peak}$, whilst being intravenously infused with baseline levels of insulin (free IRI ~2-3 times greater than non-diabetics during exercise), plasma glucose increased by 3.8 $mmol \cdot l^{-1}$ during exercise and early recovery, but then remained stable, albeit markedly elevated for the remainder of recovery (Sigal *et al.*, 1994b) (Table 2.7). R_a increments and decrements were identical in subjects with type 1 DM and non-diabetics, however, hyperglycaemia supervened in recovery in the subjects with type 1 DM due to lower MCR (Sigal *et al.*, 1994b). Doubling the i.v. insulin infusion in recovery in subjects with type 1 DM increased MCR to non-diabetic levels and progressively reduced recovery hyperglycaemia (Sigal *et al.*, 1994b), and administration of an i.v. bolus of insulin at exhaustion in non-diabetic subjects resulted in hypoglycaemia (Purdon *et al.*, 1993), thus demonstrating the role of insulin in enhancing MCR.

Resting hyperglycaemia in subjects with type 1 DM receiving i.v. insulin at a basal rate also attenuated the rise in [PG] and R_a during exhausting exercise at 89-98% $\dot{V}O_{2max}$ (Sigal *et al.*, 1994b) (Table 2.7), however, in contrast to hyperglycaemic non-diabetics (Manzon *et al.*, 1998), and in comparison to normoglycaemic non-diabetics, MCR was lower throughout the experiment (Sigal *et al.*, 1994b).

In summary, studies in which sedentary and trained subjects exercised intensely at the same relative intensity (not to exhaustion), with the consequence that the latter accomplished more work, found that the increment in [PG] during exercise and recovery was either similar or greater in those who were trained. The magnitude of the rise in [PG] or [BG] depends upon both the intensity and mode (continuous versus intermittent) of exercise. In subjects with type 1 DM, pre-existing insulinopaenia or relative hyperinsulinaemia (i.e. from recently administered insulin) may influence the [PG] response to exercise, however will also be influenced by exercise intensity. However, when subjects with type 1 DM are normoglycaemic prior to exercise, changes in [PG] during exercise are similar to non-diabetic subjects. In contrast, during recovery from intense exercise, the absence of the physiological rise in insulin (in response to exercise-induced

hyperglycaemia) in subjects with type 1 DM results in sustained hyperglycaemia of a clinically significant degree.

The marked rise in the glucose R_a during intense submaximal to maximal exercise is primarily attributable to the considerable rises in [Adr] and [NAdr], which markedly exceed their respective glucoregulatory threshold concentrations and are highly correlated with glucose R_a. An increase in the IRG/IRI ratio during exercise may play a small role in facilitating the increase in R_a, however the 'feed-forward' regulation by catecholamines is dominant. The increase in the glucose R_a exceeds that of R_d during exercise and early recovery and explains the rise in [PG]. In later recovery, R_d exceeds R_a and [PG] is progressively returned towards resting values. The fall in R_a in early recovery is thought to be mostly due to the falling catecholamine concentrations. Physiological hyperinsulinaemia is required to facilitate R_d and MCR in recovery, and due to the absence of such in subjects with type 1 DM, results in lower metabolic clearance of glucose and sustained hyperglycaemia in recovery, even with basal insulin infusion.

Few cross-sectional or longitudinal high-intensity training studies have examined the glucoregulatory response to maximal exercise, with no studies reporting IRG and IRI as well as catecholamine concentrations. Two studies have examined maximal exercise after sprint training, however, hormonal changes were not reported in one and the relation of such to glucoregulation in the other remains unclear. The results from these two studies may indicate that glucoregulation is enhanced when performing at the same absolute work rate after training. No sprint training studies have examined subjects with type 1 DM, thus the glucoregulatory response during either exhausting or matched-work exercise after training is unknown.

Section D – Ion Regulation

2.10 Potassium and sodium and fatigue during maximal exercise

2.10.1 Potassium, sodium and the muscle membrane

Potassium (K^+) loss from the exercising muscle may impair the excitability of the membrane and thereby contractility, and is considered a major factor in the development of muscle fatigue during intense exercise or electrical stimulation of muscle in humans (Bigland-Ritchie *et al.*, 1979; Jones, 1981; Hermansen *et al.*, 1984; Sjøgaard, 1986; Sjøgaard, 1991; Lindinger *et al.*, 1995; McKenna *et al.*, 1997a; McKenna *et al.*, 1997b; Juel *et al.*, 2000b). Fatigue has been defined as the "failure to maintain the required or expected power output" (Edwards, 1983). There is a close relationship between excitability and contractility with the reduction in the latter being an important factor in fatigue (Overgaard *et al.*, 1999). The transverse tubules (t-tubules), with their narrow lumen, and to a lesser extent the muscle sarcolemma, have been implicated as sites involved in the fatigue associated with high intensity exercise (Jones, 1981; Sjøgaard, 1990; Sjøgaard, 1991; Westerblad *et al.*, 1991).

The membrane potential (E_m) is largely determined by the distribution of K^+ across the membrane. Although the concentration gradients for K^+ and Na^+ between the inside and outside of the muscle cell are relatively similar (albeit opposite), the skeletal muscle membrane is ~100 times more permeable to K^+ than to Na^+ (Hodgkin & Horowicz, 1959a; Jones, 1987), and the Na^+,K^+ pump is electrogenic (Clausen *et al.*, 1998). These relationships are quantified in the modification (Hodgkin & Katz, 1949; Hodgkin & Horowicz, 1959a) of the constant field equation (Goldman, 1943), now termed the Goldman-Hodgkin-Katz (GHK) equation:

$$E_m = \frac{RT}{F} \ln \left\{ \frac{r\alpha^{o}_{K^+}[K^+]_o + \beta\alpha^{o}_{Na^+}[Na^+]_o + \gamma\alpha^{i}_{Cl^-}[Cl^-]_i}{r\alpha^{i}_{K^+}[K^+]_i + \beta\alpha^{i}_{Na^+}[Na^+]_i + \gamma\alpha^{o}_{Cl^-}[Cl^-]_o} \right\}$$

where E_m is the membrane potential, R is the universal gas constant, T is absolute temperature, F is Faraday's constant, r is the stoichiometric ratio of the Na^+,K^+ pump, the αs are the respective activity coefficients of the ions, sub- and superscripted o is

extracellular, β and γ are the permeabilities of the resting membrane to Na^+ and Cl^-, respectively, expressed relative to that of K^+, and sub- and superscripted i is intracellular [from (Cairns *et al.*, 1997)]. The calculated resting membrane potential is approximately -90 mV in humans (Sjøgaard *et al.*, 1985; Sjøgaard, 1991; Lindinger *et al.*, 1995).

Electrical stimulation of muscle resulted in a movement of Na^+ and K^+ down their concentration gradients, i.e. a net entry of Na^+ and loss of K^+ (Fenn & Cobb, 1936; Hodgkin & Horowicz, 1959b). In single frog fibres maintained at 21°C, one electrical impulse caused a net entry of 15.6 pmol·cm^{-2} of Na^+ and a net exit of 9.6 pmol·cm^{-2} of K^+, which resulted in an increase in $[Na^+]_i$ of 0.0077 mmol·kg H_2O^{-1}, and a decrease in $[K^+]_i$ of 0.0047 mmol·kg H_2O^{-1} (Hodgkin & Horowicz, 1959b). However, the authors caution that these values were determined on different fibres and using different methods. A magnified effect has been estimated to occur with each action potential in frog t-tubules with an increase in $[K^+]$ of 0.28 mmol·l^{-1} and a decrease in $[Na^+]$ of 0.5 mmol·l^{-1} (Adrian & Peachy, 1973). In humans, K^+ loss from the quadriceps during high-intensity dynamic knee extension or high-intensity bicycling was estimated to amount to ~1.7-1.8 μmol·kg^{-1} per action potential (Sjøgaard, 1990; Hallén, 1996).

2.10.2 K^+ and the effect of maximal exercise

The loss of K^+ during skeletal muscle stimulation or intense exercise occurs through various K^+ channels, as recently reviewed (Sjøgaard, 1991; McKenna, 1992; Pallotta & Wagoner, 1992; Fitts, 1994). It is thought that the net loss of K^+ exceeds that attributable solely to egress via the delayed rectifier channel (involved in repolarization after the action potential) (Sjøgaard, 1990). Potassium conductance was increased in metabolically exhausted frog skeletal muscle, suggested to be consequent to elevated intracellular calcium concentration (rather than to a disruption of t-tubular membranes which remained electrically connected to the sarcolemma) (Fink & Lüttgau, 1976). Increased K^+ conductance is thought to contribute to the net loss of intracellular K^+ during intense exhausting exercise in humans, and to be both a causal factor in muscular fatigue and to act as a safety mechanism to protect the muscle (Sjøgaard, 1990; Sjøgaard, 1991). It has been suggested that inactivation of

the t-tubule, primarily via high interstitial [K^+] may be an energy-saving mechanism that prevents muscle rigor due to cytosolic ATP depletion (Sjøgaard, 1996).

Exhausting one-legged dynamic knee extension, performed at 100% $\dot{V}O_{2peak}$ (after 10-20 min submaximal exercise) increased total muscle water by 21%, reduced $[K^+]_i$ from 165 to 129 mmol·l^{-1}, increased $[Na^+]_i$ from 10 to 24 mmol·l^{-1}, increased femoral venous plasma [K^+] from 4.5 to 6.4 mmol·l^{-1}, and was calculated to reduce the E_m from –89 to –75 mV (Sjøgaard *et al.*, 1985). Maximal cycle exercise of 30 s duration was calculated to have similarly depolarized the membrane potential by 17 mV based on resting values of 4.5 and 146 mmol·l^{-1}, and exhaustion values of 7 and 117 mmol·l^{-1} for extracellular and intracellular [K^+], respectively (Lindinger *et al.*, 1995). However, in each study (Sjøgaard *et al.*, 1985; Lindinger *et al.*, 1995) the E_m was likely depolarised more than the calculated values, since one-legged exhausting exercise resulted in a mean interstitial [K^+] of ~9 mmol·l^{-1} (Juel *et al.*, 2000b) and 30 s maximal cycling exercise increased femoral venous [K^+] to 8.2 mmol·l^{-1} (from 4.4 mmol·l^{-1}) (McKenna *et al.*, 1997a), with interstitial values likely to be markedly higher. Increased interstitial [K^+] has been calculated to contribute 70% to the depolarization of the membrane, whilst changes in $[K^+]_i$ account for the remaining 30% (Lindinger *et al.*, 1995). More direct measurement in rat soleus muscle demonstrated that tetanic force was little affected (11% reduction) when extracellular [K^+] was between 8-9 mmol·l^{-1}, however when [K^+] was increased to 12.5 mmol·l^{-1}, force was reduced 87% and an ~17 mV depolarization of the membrane potential was evident (Cairns *et al.*, 1995).

Maximal firing frequency during sustained contractions is ~30Hz (Bigland-Ritchie & Woods, 1984; Bigland-Ritchie *et al.*, 1986), and if applied to high-intensity cycling at a pedal frequency of 1 Hz, results in an average of six action potentials reaching the muscle per second (Hallén, 1996). Force during tetanic pulse trains of 30 Hz declined by 96% when rat soleus was exposed to 12.5 mmol·l^{-1} K^+ (Clausen *et al.*, 1993), and the loss of force induced by electrical stimulation was correlated with the increase in intracellular [Na^+] (Nielsen & Clausen, 1996). The inhibitory effect of high extracellular [K^+] on muscle contractility, via depolarization and inactivation of the voltage-dependent sodium channels (Hodgkin & Horowicz, 1959a; Ruff *et al.*, 1988)

reduced the velocity of the compound action potential (Overgaard *et al.*, 1999). This effect is counteracted by the action of the Na^+,K^+-ATPase pump, which is itself stimulated by muscle excitation and increases the transmembrane gradient for Na^+ and returns K^+ intracellularly (Clausen *et al.*, 1993).

2.10.3 The importance of Na^+, and interactions between Na^+ and K^+

Muscle excitability and contractility also depend upon the maintenance of steep electrochemical gradients for Na^+ across the muscle membrane and the t-tubules. The effect of a high extracellular [K^+] is far more detrimental to force generation when combined with low extracellular [Na^+]. Reduction of the electrochemical gradient for Na^+ from 116 mV at an extracellular concentration of 147 mmol·l^{-1} to 81 mV at a concentration of 25 mmol·l^{-1}, or combined extracellular concentrations of 8 mmol·l^{-1} [K^+] and 71 mmol·l^{-1} [Na^+], resulted in an ~70% loss of tetanic force at 30 Hz (Overgaard *et al.*, 1997). Addition of catecholamines or insulin decreased intracellular Na^+, hyperpolarized the membrane and produced a marked force recovery (Overgaard *et al.*, 1997). It was concluded that when the membrane is depolarized by high extracellular [K^+] that maximal force cannot be attained unless the Na^+ gradient is intact (Overgaard *et al.*, 1997).

Muscle excitation results in increased intracellular [Na^+] which is a potent stimulus for increasing Na^+,K^+-ATPase activity, however there is also an excitation-induced Na^+-independent stimulus that augments pump activity (Nielsen & Clausen, 1997). The Na^+-independent activation may be consequent to increased pump affinity for intracellular Na^+ (Nielsen & Harrison, 1998), however, it is also conceivable that the recent observation of exercise-induced Na^+,K^+ pump translocation in humans (Juel *et al.*, 2000a) may be involved in the Na^+-independent excitation-induced upregulation of Na^+,K^+-ATPase activity. Increased activation of the Na^+,K^+ pump by the Na^+-independent stimulus may represent a feed-forward mechanism that acts to protect the Na^+ and K^+ gradients and hence the muscle membrane potential (Nielsen & Clausen, 1997). Indeed, the initial 30 s of electrical stimulation of rat muscle at 30Hz resulted in a 14% decrease in tetanic force, but an 8% increase in the M wave (representing muscle sarcolemmal excitability), i.e. a hyperpolarization of the sarcolemma; the latter attributed to increased electrogenic activity of the Na^+,K^+ pump (Overgaard *et*

al., 1999). It was suggested that metabolic factors, e.g. accumulation of inorganic phosphate, and/or reduced t-tubule excitability (which cannot be detected by M wave recordings) may account for the loss of force during the first 30 s (Overgaard *et al.*, 1999). Continued electrical stimulation (for another 90 s) resulted in parallel reductions in the M wave and the force, supporting the hypothesis that reduced K^+ and Na^+ gradients are major causal factors in fatigue during intense muscular activity (Overgaard *et al.*, 1999).

Another important relationship with regard to fatigue is the capacity for Na^+ influx via the Na^+ channels, relative to the capacity for Na^+,K^+-ATPase pumping, i.e. the leak-to-pump ratio for Na^+ (Harrison & Clausen, 1998). The onset of fatigue was more rapid when an upregulation of Na^+ channels (induced by triiodothyronine injections) preceded the upregulation of Na^+,K^+-ATPase concentration in rat soleus muscle: supporting the significance of the transmembrane gradient for Na^+ in muscle fatigue (Harrison & Clausen, 1998).

2.10.4 Effects of hormones and muscle activation in K^+-paralyzed muscle

In K^+-paralyzed muscle, adrenaline, insulin and CGRP each effected a partial restoration of tetanic force via Na^+,K^+-ATPase pump stimulation (Andersen & Clausen, 1993; Cairns *et al.*, 1995). Muscle excitation *per se* also effected a marked recovery of force in K^+-inhibited fibres (Nielsen *et al.*, 1998). The recovery of force was not altered by pre-treatment with propranolol, however, muscle force did not recover during excitation if the content of CGRP in nerve endings was reduced by pre-treatment with capsaicin, leading the authors to suggest that the excitation-induced recovery of force was linked to activation of the Na^+,K^+-ATPase via the CGRP receptors (Nielsen *et al.*, 1998).

2.10.5 Effects of experimental diabetes

Intracellular Na^+ was elevated ~30% in rat soleus muscle within 2 days of fasting or STZ-induced diabetes (both of which result in hypoinsulinaemia), whilst plasma [Na^+] and [K^+] were unchanged (Moore *et al.*, 1983). A significant negative correlation was found between plasma insulin concentration and intracellular Na^+ (Moore *et al.*, 1983). It was suggested that a change in membrane transport had occurred and was

either attributable to reduced Na^+,K^+-ATPase pump activity or an increased Na^+ permeability (Moore *et al.*, 1983). Twice daily treatment with insulin for 7 days did not fully reduce intracellular Na^+ to control levels, perhaps secondary to downregulation of the number of Na^+,K^+-ATPase pumps (Moore *et al.*, 1983). The resting membrane potential was significantly depolarised in the EDL (Grossie, 1982), but not soleus muscle (Paulus & Grossie, 1983) of severely alloxan-diabetic rats after 2 days of insulin withdrawal. The authors postulated that this may have been due to transverse tubule damage induced by plasma hypertonicity, leading to increased intracellular chloride (Cl^-) and subsequent membrane depolarisation; or to elevated Cl^- short-circuiting the Na,K pump (Grossie, 1982). Additionally, a reduced amplitude and prolonged duration of induced action potentials that was associated with weakness in EDL muscle, unsteady gait and poverty of movement was evident after 1-2 days of insulin withdrawal from alloxan-diabetic rats; all effects were reversed by insulin treatment (Grossie, 1982).

The affect of altered insulin sensitivity on the membrane potential is unknown. However, insulin hyperpolarized the muscle membrane (half-maximal effect within 5 mins) in normotensive rats, however had no affect upon spontaneously hypertensive rats (Pickar *et al.*, 1994), demonstrating resistance to the action of insulin.

2.10.6 Effects of human type 1 DM

In subjects with type 1 DM vastus lateralis K^+ concentration at rest was significantly lower (6%) than in healthy controls, however plasma [K^+] did not differ (Sjögren *et al.*, 1986), and intra- and extracellular [Na^+] was not reported. Similarly, even after 6 weeks of insulin treatment in patients with newly diagnosed type 1 diabetes, whole body K^+ was reduced 5% compared to the norm (Walsh *et al.*, 1974). A reduced $[K^+]_i$ would effect a degree of depolarization of the muscle membrane.

Insulin has long been known to have a de-fatiguing effect in insulin-deficient patients with type 1 DM: an early anecdotal report described a patient, who prior to commencing insulin treatment was unable to ascend stairs without his legs aching with exhaustion, however, when tested after 3 years on insulin was able to exercise strenuously, cycling and rowing for 2.5 hours before fatigue (Lawrence, 1926).

Whilst insulin has a multiplicity of physiological effects it may be speculated that a small depolarization of the muscle membrane potential may contribute to fatigue during intense exercise of brief duration in subjects with type 1 diabetes who exercise when insulin deficient; however this has not been investigated.

2.11 Potassium regulation at rest

2.11.1 Plasma K^+, glucose and basal insulin in non-diabetics

The major sites of K^+ clearance which contribute to plasma [K^+] regulation include skeletal muscle, the liver, and excretion via the kidney (Knochel, 1977). Acute K^+ loads are cleared via extrarenal mechanisms, whilst renal K^+ excretion contributes to longer term regulation (Bia & DeFronzo, 1981). In non-diabetics, insulin is secreted in response to increments induced at rest in plasma K^+ that exceed 1-1.5 mM (Bia & DeFronzo, 1981). Insulin has long been known to acutely reduce plasma [K^+] (Briggs *et al.*, 1923-24; Harrop & Benedict, 1924) via an increase in muscle (Zierler, 1960; Andres *et al.*, 1962; Zierler & Rabinowitz, 1964) and hepatic K^+ uptake (Fenn, 1939; DeFronzo *et al.*, 1980). Conversely, acute reduction of basal insulin secretion, induced at rest by somatostatin administration in non-diabetic subjects, resulted in progressive hyperkalaemia (DeFronzo *et al.*, 1978). Basal insulin levels are thus intimately related to the maintenance of K^+ homeostasis at rest, being required for the prevention of resting hyperkalaemia (DeFronzo *et al.*, 1978).

In non-diabetic subjects at rest, mean plasma [K^+] has been reported to vary between 3.68- 5 $mmol \cdot l^{-1}$, with very similar concentrations in arterial and venous blood; hence the arteriovenous difference for plasma [K^+] is essentially zero (Juel *et al.*, 1990; Medbø & Sejersted, 1990; Bangsbo *et al.*, 1992a; Hallén *et al.*, 1994; Lindinger *et al.*, 1994; Vøllestad *et al.*, 1994; Gullestad *et al.*, 1995; McKenna *et al.*, 1997a; Wasserman *et al.*, 1997; Juel *et al.*, 1999; Verburg *et al.*, 1999). Recent work using microdialysis in vastus lateralis (Juel *et al.*, 2000b) or gastrocnemius muscle (Green, S. *et al.*, 1999) has demonstrated that interstitial [K^+] similarly varies between 3.90-4.53 $mmol \cdot l^{-1}$.

Over fifty years ago Seldin & Tarail (1949), albeit using few subjects (non-diabetics) and slightly different experimental protocols in each subject, demonstrated that

injections of either glucose or mannitol administered at a relatively fast rate, resulted in water movement from the intra- to the extracellular space, which reduced serum [Na^+]. Concomitantly, with the glucose injection only, serum [K^+] fell due to a movement of K^+ from the extracellular space into cells; an effect that the authors commented was similar to the action of insulin (Seldin & Tarail, 1949). In contrast, when glucose was administered comparatively slowly, little change occurred in fluid distribution; an observation attributed to an increment in cellular glucose utilisation that was closely matched to the administration rate (Seldin & Tarail, 1949). Later studies confirmed the hypokalaemic (Viberti, 1978; Nicolis *et al.*, 1981) and hyponatraemic effect of a glucose load in non-diabetic subjects (Moreno *et al.*, 1969).

2.11.2 Relationship between K^+, glucose and insulin in type 1 diabetes mellitus

An early study (Farber *et al.*, 1951) reported no difference in resting plasma [K^+] in subjects with type 1 diabetes mellitus (DM) from whom insulin had been withheld for 24 hours, compared to non-diabetics. However, a later study of subjects with type 1 DM, whose usual morning insulin dose had been withheld, demonstrated higher baseline plasma [K^+], blood glucose and plasma osmolality compared to non-diabetics (Viberti, 1978). Similarly, a larger study (McNair *et al.*, 1982) which evaluated 193 outpatients with type 1 DM (who had fasted overnight and delayed their morning insulin dose) and compared them with 371 non-fasting controls, found slightly higher serum [K^+] and decreased serum [Na^+] in the outpatients, which was attributed to relative insulin deficiency. When the outpatients were stratified according to blood glucose level (BGL), serum [K^+] and [Na^+] did not differ from controls in those outpatients with BGL between 2.5 - 10.5 $mmol \cdot l^{-1}$, whereas outpatients with BGL in the range of 11.0 - 29.5 $mmol \cdot l^{-1}$, had significantly higher and lower values for [K^+] and [Na^+], respectively (McNair *et al.*, 1982). Serum [K^+] was found to increase curvilinearly ($r = 0.37$) and serum [Na^+] to decrease linearly ($r = -0.61$) with increasing BGL (McNair *et al.*, 1982).

In contrast to the hypokalaemic effect of glucose in non-diabetic subjects, in those with type 1 DM, administration of an oral or intravenous glucose load induced hyperkalaemia that developed progressively over a period of 60-90 min (Viberti,

1978; Nicolis *et al.*, 1981). In patients with type 1 DM who had fasted overnight and withheld their morning insulin dose, ingestion of a glucose load was associated with a 0.2 $mmol \cdot l^{-1}$ rise in venous plasma [K^+] and a 1.3 $mmol \cdot l^{-1}$ fall in plasma [Na^+] for each 5.6 $mmol \cdot l^{-1}$ rise in glucose (Shalwitz *et al.*, 1991). The mechanism of hyperglycaemia-induced hyperkalaemia in subjects with type 1 DM has been suggested to be due to an effect of hyperosmolality, rather than to hyperglycaemia *per se* (Viberti, 1978). This notion was supported by significant rises in serum [K^+] (0.5–0.7 $mmol \cdot l^{-1}$) and plasma osmolality following administration of hypertonic saline or mannitol at rest in normally hydrated non-diabetic subjects (Moreno *et al.*, 1969; Bratusch-Marrain & DeFronzo, 1983). Several mechanisms have been postulated to explain this effect. An early view was that it may be secondary to a dilutional acidosis (caused by dilution of extracellular bicarbonate when P_{CO_2} was held constant) which may be buffered by intracellular exchange for K^+ (Winters *et al.*, 1964); or to a concomitant movement of water and K^+ to the extracellular space (Moreno *et al.*, 1969). Hyperosmolality has also been demonstrated to reduce insulin-mediated glucose metabolism, i.e. to acutely induce peripheral insulin resistance (Bratusch-Marrain & DeFronzo, 1983). However, when both glucose and insulin were infused in subjects with type 1 DM, arterial and venous plasma [K^+] fell (Farber *et al.*, 1951); mimicking the non-diabetic response. Thus, hyperglycaemia may contribute to an increased plasma osmolality, and subsequently to increased plasma [K^+] when portal and peripheral insulin levels are insufficient.

Severely insulin-depleted subjects who develop ketoacidosis require K^+ replacement for intracellular K^+ depletion. Whole-body K^+ was reduced in newly diagnosed, untreated subjects with type 1 DM, but was progressively, though not fully restored (95 % of the expected norm, based on body size) after 1 and 6 weeks of insulin treatment (Walsh *et al.*, 1974). Lean body mass increased with insulin treatment, and probably explained much of the initial K^+ depletion (Walsh *et al.*, 1974). A recent study (Goodship *et al.*, 1990) which examined patients with type 1 DM (in varying degrees of glycaemic control, with mean duration of diabetes 9 years), and compared them with age- and sex-matched non-diabetics, found neither evidence of depleted total-body K^+ stores, nor of an association between K^+ stores and glycaemic control. However, the study did not report whether or not subjects had fasted or administered insulin prior to evaluation. A cross-sectional study which included 25 subjects with

mean duration of type 1 DM of 19 years, who had fasted overnight (and had presumably delayed the morning insulin dose), reported significantly lower (6 %) vastus lateralis muscle [K^+] at rest compared to healthy non-diabetics, but no difference in plasma or erythrocyte [K^+], and no significant relationship between muscle or blood K^+ and HbA_{1c} (Sjögren *et al.*, 1986). The clinical significance of this finding remains to be tested.

2.11.3 Interaction of K^+, glucose and other hormones

Infusion of potassium chloride (KCl) into dogs increased secretion of glucagon and insulin after 10–20 min (Santeusanio *et al.*, 1973). It was suggested that the kalaeogenic increase in glucagon was required to antagonise the effects of the concomitant rise in insulin, which would otherwise induce hypoglycaemia (Santeusanio *et al.*, 1973). In alloxan-diabetic dogs, tolerance to KCl infusion was reduced and exaggerated rises in glucagon occurred; both were corrected by insulin infusion (Santeusanio *et al.*, 1973). In patients with type 1 DM, plasma glucagon began to rise 120 min after withdrawal of a basal insulin infusion and peaked at ~70% above basal values after 5 hours; whereas plasma [K^+] started to rise immediately after insulin withdrawal, peaked 0.73 $mmol \cdot l^{-1}$ above basal levels after 210 min, then slowly fell thereafter (Cagliero *et al.*, 1983). Plasma glucose concentration rose dramatically (by 12 $mmol \cdot l^{-1}$) with a similar time-course to the rise in plasma [K^+]. In a separate experiment, inhibition of glucagon secretion by somatostatin attenuated the rise in plasma [K^+] and prevented the rise in plasma glucose concentration (Cagliero *et al.*, 1983). The authors concluded that glucagon was likely to be mainly responsible for the hyperkalaemia, with a contribution from insulinopaenia (Cagliero *et al.*, 1983). However, given the similar time-courses of the rises in glucose and [K^+], and the dissimilar time-course of glucagon changes, it is more likely that the hyperkalaemia was induced by hyperglycaemia, which was secondary to insulinopaenia. Interestingly, an earlier study by the same group (Massara *et al.*, 1980) in non-diabetic subjects, demonstrated a greater rise in plasma [K^+] with somatostatin plus glucagon versus somatostatin plus glucose, with similar suppression of insulin and rise in glucose (however, glucagon concentrations were not reported); perhaps suggesting a role for glucagon in potassium regulation in man, and also, when interpreted in light of the later study (Cagliero *et al.*, 1983), suggesting altered

sensitivity to, or effectiveness of glucagon in subjects with type 1 DM (see section 2.7.1).

Intravenous adrenaline has long been known to exert a biphasic effect on arterial serum [K^+], whereby an initial rise was followed by a reduction (D'Silva, 1934). The initial increase in [K^+] has been attributed to α-adrenergic stimulation of the liver (Todd & Vick, 1971), inducing a net release of K^+ in cats (D'Silva, 1936; Craig & Honig, 1963) and dogs (Vick *et al.*, 1972). The hypokalaemic effect of adrenaline is due to β-adrenergic stimulation (Todd & Vick, 1971; Rosa *et al.*, 1980), specific to β_2-receptor action (Brown *et al.*, 1983) on the Na^+,K^+ pump (Clausen & Flatman, 1980). The latter enhances cellular K^+ uptake in cats (Craig & Honig, 1963), dogs (Vick *et al.*, 1972), rats (Clausen & Flatman, 1977), and man (Rosa *et al.*, 1980; Williams *et al.*, 1985). The effects of the catecholamines on the Na^+,K^+ pump are reviewed in detail below.

Although studies conducted at rest may not be indicative or predictive of responses during exercise [especially with regard to K^+ metabolism, since muscular activity is a very potent activator of the Na^+,K^+-ATPase pump (Everts & Clausen, 1994; Nielsen & Clausen, 1997)], both the pre-exercise status, and the period of recovery from exercise bear relevant comparison.

2.12 K^+ and maximal exercise

Electrical activity results in a gain of Na^+ and a loss of K^+ from muscle (Fenn & Cobb, 1936; Hodgkin & Horowicz, 1959b). The loss of muscle K^+ is proportional to the magnitude of the muscle contraction (Fenn, 1938), and thus the rate of muscle K^+ efflux increases step-wise with increasing exercise intensity (Hallén *et al.*, 1994; Vøllestad *et al.*, 1994). Femoral venous plasma [K^+] increases abruptly at the onset of exercise after a 5-10 s delay (Hallén *et al.*, 1994; Vøllestad *et al.*, 1994; Gullestad *et al.*, 1995; Wasserman *et al.*, 1997), which probably represents the time for blood to flow from the capillaries to the sampling site (Hallén *et al.*, 1994), including a few seconds or more for diffusion of K^+ through the interstitium (Hodgkin & Horowicz, 1959b). Shortly thereafter arterial [K^+] increases, and continues to rise throughout the duration of maximal exercise (Vøllestad *et al.*, 1994; Hallén, 1996).

2.13 Plasma [K^+]

2.13.1 [K^+] depends on sampling site

Femoral venous [K^+] consistently exceeded antecubital venous concentrations at similar exercise intensities, and arterial concentrations were somewhat higher than arterialized venous [K^+] (Table 2.10). Both active (Juel *et al.*, 1999) and inactive muscles (Kowalchuk *et al.*, 1988a) contribute to K^+ uptake during and after intense exercise. During intense leg exercise, antecubital venous [K^+] is therefore expected to be lower than femoral venous [K^+] (providing that the arms are relatively inactive) as a consequence of forearm K^+ uptake (Kowalchuk *et al.*, 1988a; Kowalchuk *et al.*, 1988b), whilst K^+ extraction by inactive muscles is minimized if superficial venous blood is well-arterialized (McKenna *et al.*, 1993).

Change in femoral venous [K^+] was thought to only slightly underestimate both the concentration and the rate of change of [K^+] in the interstitium, with a calculated 70-90% equilibration between interstitium and capillary after a sudden step in concentration (Hallén & Sejersted, 1993). However, recent work using microdialysis has suggested that venous [K^+] may markedly underestimate interstitial concentration (Juel *et al.*, 2000b). Mild to exhausting one-legged dynamic exercise resulted in a progressive increase in the calculated interstitial [K^+] to a mean value of ~9 mmol·l^{-1}, and although large variation was found for individual probes, some individual values approximated 14 mmol·l^{-1} (Juel *et al.*, 2000b). Previous femoral venous [K^+] measurements had yielded values between 6.2-6.8 mmol·l^{-1} for exhausting one-legged kicking, whereas exhausting two-legged cycling at 110% $\dot{V}O_{2max}$ or maximal 30-s cycle bouts increased femoral venous [K^+] to 8.0-8.2 mmol·l^{-1} (Table 2.10). Based upon the microdialysis-derived calculations, it is likely that interstitial [K^+] was considerably higher.

Table 2.10: *Plasma [K^+] at rest, and the peak during intense exercise*

Study	Exercise			Arterial [K^+] ($mmol \cdot l^{-1}$)		Venous [K^+] ($mmol \cdot l^{-1}$)		
	NExh, Exh	Mode	Duration, intensity	Rest	Ex peak	Site	Rest	Ex peak
(Vøllestad *et al.*, 1994)	NExh	cycling	10 min; 85% $\dot{V}O_{2max}$	~4.4	6.0	fv	4.4	6.4
(Wasserman *et al.*, 1997)	NExh	cycling	6 min; 85% $\dot{V}O_{2max}$	~4.4	~6.8	fv	~4.2	~6.9
(Wilkerson *et al.*, 1982)	NExh	treadmill run	20 min; 90% $\dot{V}O_{2max}$			acv	4.5	6.0
(Gullestad *et al.*, 1995)	NExh	knee ext	8 min; 100% max	4.10	5.07	fv	4.04	5.24
(Paterson *et al.*, 1990)	Exh	cycling	incremental	~3.9	~7			
(Laso *et al.*, 1991)	Exh	cycling	incremental			acv	3.8	4.74
(McCoy & Hargreaves, 1992)	Exh	cycling	incremental			artv	4.0	~5.7
(Hallén *et al.*, 1994)	Exh	cycling	incremental	4.24	6.44	fv	4.25	6.77
(McLoughlin *et al.*, 1994)	Exh	cycling	incremental			artv	~4.4	~5.4
(Sjøgaard *et al.*, 1985)	Exh	one-leg kick.	6.5 min	~4.9	~5.5	fv	~4.5	~6.3
(Bangsbo *et al.*, 1992a)	Exh	one-leg kick.	3.7 min	4.27	5.62	fv	4.17	6.15
(Juel *et al.*, 1990)	Exh	one-leg kick.	3.2 min	4.2	5.8	fv	4.2	6.8
(Vøllestad *et al.*, 1994)	Exh	cycling	3.8 min; 110% $\dot{V}O_{2max}$	~4.5	8.0	fv	~4.5	8.2
(Boulay *et al.*, 1995)	Exh	cycling	90 s; maximal			acv	~4.3	~5.2
	Exh	cycling	30 s; maximal			acv	~4.3	~4.8
	Exh	cycling	10 s; maximal			acv	~4.3	~5.2
(Kowalchuk *et al.*, 1988b)	Exh	cycling	30 s; maximal	5	7	fv	5	8
(Lindinger *et al.*, 1992)	Exh	cycling	30 s†; maximal	4.66	6.52	fv	4.77	6.14
(McKenna *et al.*, 1993)	Exh	cycling	30 s†; maximal			artv	4.71	6.5
(McKenna *et al.*, 1997a)	Exh	cycling	30 s; maximal	4.4	7.0	fv	4.4	8.2
(Hargreaves *et al.*, 1998)	Exh	cycling	30 s†; maximal			acv	4.3	6.2

NExh, non-exhausting exercise; Exh, exhausting exercise; Ex peak, peak value measured during or immediately after exercise; ~, value estimated from figure; fv, femoral venous; acv, antecubital venous; knee ext, knee extension; artv, arterialized venous; one-leg kick., one-legged kicking; †, data reported for the first of four 30-s exercise bouts.

2.13.2 The arteriovenous difference for K^+

Femoral venous [K^+] rose throughout the first 90 and 150 s of non-exhausting constant load exercise at 85% $\dot{V}O_{2peak}$ (Vøllestad *et al.*, 1994; Wasserman *et al.*, 1997) and 100% maximum power (Gullestad *et al.*, 1995) (Table 2.10), then was unchanged (Gullestad *et al.*, 1995; Wasserman *et al.*, 1997), or reduced (Vøllestad *et al.*, 1994) for the remainder of the exercise bout. Excepting one study (Lindinger *et al.*, 1992), femoral venous [K^+] consistently exceeded arterial concentration during intense exercise (Table 2.10), resulting in a negative arteriovenous difference for K^+ ([K^+]$_{a\text{-}fv}$). This difference had returned to zero by the third to sixth minute of exercise at 85% $\dot{V}O_{2peak}$ (Vøllestad *et al.*, 1994; Wasserman *et al.*, 1997), however a small [K^+]$_{a\text{-}fv}$ remained after 6 min of exercise at 100% maximum power output (Gullestad *et al.*, 1995). Similarly, a negative [K^+]$_{a\text{-}fv}$ persisted throughout the duration of one-legged kicking to exhaustion (Sjøgaard *et al.*, 1985; Juel *et al.*, 1990; Bangsbo *et al.*, 1992a), and two-legged cycling to exhaustion at 110% $\dot{V}O_{2max}$ produced a negative [K^+]$_{a\text{-}fv}$ that peaked after 20-40 s, then was gradually reduced for the remainder of exercise (Vøllestad *et al.*, 1994). The widening of the [K^+]$_{a\text{-}fv}$, which indicates K^+ release, is blunted by cellular re-uptake of K^+. Correction of the [K^+]$_{a\text{-}fv}$ to account for the marked decline in plasma volume with intense exercise diminished the difference, with only a very small net K^+ release evident in the final seconds of a maximal 30-s cycle sprint (McKenna *et al.*, 1997a), a consideration that has mechanistic significance, as recently reviewed (McKenna, 1999).

2.13.3 K^+ efflux, uptake and plasma [K^+]

The femoral venous [K^+], and the rates of K^+ efflux and uptake are linearly related to power output during bicycling, however the relationship between arterial [K^+] and power is curvilinear (Vøllestad *et al.*, 1994). This observation was explained (Vøllestad *et al.*, 1994) by an intensity-dependent decline in distribution volume for K^+, that was attributed to increasing sympathetic outflow, which would reduce flow to inactive tissues at high intensities (Rowell, 1993). Another possibility is that the increasing recruitment of type II muscle fibres at high exercise intensities increases K^+ efflux.

Conversely, when electrical activity ceases at the end of exercise, K^+ efflux ceases and redistribution and re-uptake rapidly restore intracellular [K^+], with the consequence that plasma [K^+] declines rapidly to below resting levels with a half-time of 25 – 31 s (Medbø & Sejersted, 1990; Vøllestad *et al.*, 1994; Wasserman *et al.*, 1997). The rate of decline in plasma [K^+] after exhausting uphill running is proportional to the deviation in peak concentration relative to the nadir observed post-exercise, and hence the half-time is constant regardless of exercise intensity (Medbø & Sejersted, 1990).

Renal K^+ excretion has a half-life of 4 – 6 hours (Bia & DeFronzo, 1981), so is too slow to be a contributor to K^+ regulation during and immediately following brief, intense exercise. Therefore the major sites of K^+ clearance during and following acute hyperkalaemia, such as that evoked by intense exercise, are extrarenal (Knochel, 1977). The mechanism by which K^+ is returned intracellularly, and hence removed from plasma, is the membrane-bound sodium-potassium-adenosinetriphosphatase (Na^+,K^+-ATPase) pump (see below).

2.13.4 Effect of exercise training

An absolute reduction in exercise-induced hyperkalaemia was demonstrated when performing the same submaximal workload after endurance training (Green *et al.*, 1993), and after general physical conditioning when exercising at a similar heart rate (Kjeldsen *et al.*, 1990).

2.13.5 Effect of sprint training

Given the marked increment in plasma [K^+] with intense exercise, it may be speculated that repeated elevations of plasma [K^+], such as are brought about by sprint training, may induce an adaptation. Indeed, K^+ regulation during and after four maximal 30-s cycle bouts was enhanced following 7 weeks of sprint training (McKenna *et al.*, 1993; McKenna *et al.*, 1997a). Whilst there was no difference in either the arterialized venous [K^+], or in the absolute rise of plasma [K^+] with each exhaustive bout (in which more work was done after training), a significant reduction was evident in both the rise in plasma [K^+] (corrected for fluid shifts) ($\Delta[K^+]$), and the $\Delta[K^+]$ relative to the work performed during bouts (McKenna *et al.*, 1993). In a

single 30-s cycle sprint to exhaustion, the absolute rise in each of arterial and femoral venous plasma [K^+] was unchanged, even though greater work was performed after sprint training; however, the $\Delta[K^+]\cdot work^{-1}$ ratio was not reduced (McKenna *et al.*, 1997a). Perhaps contributing to this finding, was an increased net K^+ uptake in contracting muscle during the final seconds of the maximal 30-s bout after training (McKenna *et al.*, 1997a). In addition, arterial and femoral venous plasma [K^+] were lower at rest, and at each measurement in exercise and recovery after sprint training (McKenna *et al.*, 1997a). These studies demonstrated for the first time improved K^+ regulation after sprint training, however the anticipated training effect of reduced exercise-induced hyperkalaemia was not evident (McKenna *et al.*, 1993; McKenna *et al.*, 1997a). When examined relative to work output, the rise in plasma [K^+] was lower after training when repeated exercise bouts were performed (McKenna *et al.*, 1993). Considered together, these results suggest that a reduction in hyperkalaemia may be evident if the work performed during sprinting was the same pre- and post-training; however, to date no sprint training study has examined this possibility.

In sprint- and endurance-trained subjects there was no reported difference in femoral venous and arterial plasma [K^+], which increased 2-fold to 8.34 and 8.17 $mmol\cdot l^{-1}$, respectively, after 60 s treadmill running to exhaustion (Medbø & Sejersted, 1990). Sixty seconds of intense, but non-exhausting treadmill running increased arterial plasma [K^+] to ~6.8 and ~7.0 $mmol\cdot l^{-1}$, in sprint- and endurance-trained subjects, respectively, again with no apparent difference between subjects despite the sprinters completing 14% more work (Hermansen *et al.*, 1984). Similarly, peak femoral venous plasma [K^+] did not differ significantly between sprint- and endurance-trained groups (8.58 and 8.08 $mmol\cdot l^{-1}$, respectively), despite the sprinters exercising at a higher relative intensity (172% vs 141%) and completing 12% more work during an ~60-s exhausting run (Medbø & Sejersted, 1994). These findings implied that K^+ regulation during intense exercise may have been enhanced to a greater extent in the sprint-trained subjects. Further, based upon a 14% faster half-time for decline to a lower post-exercise nadir in femoral venous plasma [K^+] after exercise in sprinters, it was suggested that K^+ regulation differed between sprint- and endurance-trained subjects (Medbø & Sejersted, 1994). However, 6 days of endurance training also improved K^+ regulation during exercise of moderate to high intensity (Green *et al.*,

1993). Further complicating interpretation, in the Hermansen *et al.* (1984) study, sprinters achieved greater work in the first two of five sprints (the second and subsequent bouts were all conducted to exhaustion and varied between 35-60 s duration), however the endurance-trained runners ran 8-42% further in each of the final 3 bouts, with no difference reported in arterial plasma [K^+] in any bout. Therefore, in the absence of an untrained control group, the extent of adaptation in the sprint-trained subjects in each study (Hermansen *et al.*, 1984; Medbø & Sejersted, 1990; Medbø & Sejersted, 1994) is difficult to evaluate since K^+ regulation was almost certainly improved in the endurance-trained runners.

The suggested mechanisms by which K^+ regulation may be improved during and after intense exercise following sprint training include increased Na^+,K^+-ATPase concentration, content and activation in active and inactive muscle (McKenna *et al.*, 1997a) (see below); changes in muscle blood flow and capillarization; and reduced K^+ release via attenuated K^+ channel opening (McKenna, 1995). The effects of training upon muscle K^+ channels remain unknown.

2.13.6 K^+ regulation and type 1 diabetes mellitus

Surprisingly, no studies to date have reported upon plasma [K^+] responses during and after intense exercise (or indeed any other form of exercise) in type 1 diabetes mellitus. Such study is of considerable interest, and perhaps of clinical significance, given the role of insulin in K^+ regulation at rest and the absence of the usual post-exercise increment in insulin concentration in response to an exercise-induced increase in glucose concentration in subjects with type 1 diabetes. Exercise in these subjects may be undertaken with markedly lower, similar or higher insulin concentrations (according to the timing of the antecedent dose) than in non-diabetics, which may influence K^+ regulation; however has never been investigated.

No studies have examined K^+ regulation following training in subjects with type 1 diabetes mellitus. Similarly, it is unknown whether insulin sensitivity is enhanced after sprint training: a factor that may conceivably contribute to improved K^+ regulation during early recovery in non-diabetic subjects, and to improved K^+ regulation in subjects with type 1 diabetes.

2.14 Intracellular K^+ regulation

2.14.1 Na^+-K^+-Adenosinetriphosphatase (ATPase) pump

The Na^+,K^+-ATPase pump, first identified by Skou (Skou, 1957), who was later awarded the Nobel prize in Chemistry for his contribution, transports K^+ into the cell and extrudes Na^+ from the cell, thus regulating and defending the ionic gradients for K^+ and Na^+ across the cell membrane. This vital function preserves the excitability of the sarcolemma (see section 2.11.1).

Quantification by ouabain binding in frog muscle demonstrated that the Na^+,K^+-ATPase pump is primarily localized to the sarcolemma, with a minor fraction (~20%) present in the t-tubules (Venosa & Horowicz, 1981). The functional Na^+,K^+-ATPase pump, the subunit isoforms of which have recently been characterised in human skeletal muscle (Hundal *et al.*, 1994; Juel *et al.*, 2000a), is comprised of a catalytic α subunit (α_1, α_2, α_3) and a glycosylated β_1 subunit (Hundal *et al.*, 1994; Ewart & Klip, 1995). The human α_1 (and α_3) subunit is largely localized within the plasma membrane, whereas the α_2 subunit is located both within the plasma membrane and in an intracellular pool (Hundal *et al.*, 1994). The β_1 subunit is abundant in the plasma membrane and also present in an internal membrane (Hundal *et al.*, 1994). The α_1 subunit is probably involved in basal metabolism, whereas the major catalytic isoform, α_2, and the β_1 subunit are regulated by various stimuli (Hundal *et al.*, 1992b).

The pump is subject to both acute and chronic regulation by a variety of stimuli, including hormones (e.g. insulin, catecholamines), contractile activity, exercise training, and electrolyte and nutritional status (Clausen, 1986; Ewart & Klip, 1995; Clausen, 1996a; Clausen, 1998). In terms of K^+ regulation, pump function is determined both by the density of pumps at the sarcolemma and by the activity of each pump (see below). It has recently been demonstrated that [^{3}H]ouabain binding site content and Na^+,K^+-ATPase activity, may be independently modified in the presence of disease (Druml *et al.*, 1988; Pickar *et al.*, 1994).

2.14.2 [^{3}H]ouabain binding site content

Since the α subunit of each Na^+,K^+-ATPase molecule binds only one molecule of digitalis glycoside, quantification of Na^+,K^+-ATPase pump number may be assessed

by use of ^{3}H-labelled glycosides such as ouabain or digoxin (Clausen, 1998). The α_1, α_2, and α_3 isoforms of human skeletal muscle have a high affinity for ouabain allowing detection by the [^{3}H]ouabain binding site content assay (Clausen, 1998), which quantifies all functional Na^+,K^+ pumps (Clausen *et al.*, 1987).

The range of values for vastus lateralis [^{3}H]ouabain binding sites obtained in healthy, untrained human subjects was 223 - 360 pmol·g^{-1} w.w (see Table 6.19). Adult age does not affect [^{3}H]ouabain binding site content (Nørgaard *et al.*, 1984), although a tendency for lower values with advancing age has been noted (Nørgaard *et al.*, 1984; Klitgaard & Clausen, 1989). Similarly, [^{3}H]ouabain binding site content was reported to be unrelated to gender (Nørgaard *et al.*, 1984), however a study of elite, junior skiers reported 18% lower content in women than men (Evertsen *et al.*, 1997). Interestingly, erythrocyte [^{3}H]ouabain binding site concentration was 26% lower in female than male subjects, with a significant negative correlation found between age and number of binding sites in men, but not women (Garner, 1996).

2.14.3 Effect of type 1 diabetes mellitus on [^{3}H]ouabain binding site content

Only one previous study (Schmidt et al., 1994) has examined skeletal muscle [^{3}H]ouabain binding site content in human diabetes. In 7 subjects with type 1 DM (2 – 10 years' duration) in moderate control (HbA_{1c} 10.5%), the mean vastus lateralis [^{3}H]ouabain binding site content was 223 ± 13 pmol·g^{-1} w.w, which was 22% higher than in non-diabetic controls (Schmidt et al., 1994). However, in erythrocytes, a log-normal rather than normal distribution for number of ouabain binding sites was found in 20 postprandial subjects with longer-term (18 yr) type 1 DM in excellent control (HbA_{1c}, 6.1%), with a significant 9% lower mean log (of ouabain binding sites per cell) than non-diabetic subjects (Garner, 1996). In further contrast to both skeletal muscle and erythrocytes, the number of binding sites in placental tissue did not differ between women with well-controlled (HbA_{1c} 7.6%) type 1 DM and non-diabetic women (Zolese et al., 1997), and incubation of purified Na^+,K^+-ATPase from placentas of non-diabetic women with plasma from subjects with type 1 DM had no

affect on the ouabain-binding capacity or the affinity for ouabain (Rabini *et al.*, 1998), although Na^+,K^+-ATPase activity was reduced in each study (see section).

Two studies in rodents have demonstrated reductions in skeletal muscle [^{3}H]ouabain binding site content of between 17 – 48% in untreated streptozotocin (STZ)-induced diabetes of 4 – 8 weeks' duration (Kjeldsen *et al.*, 1987; Schmidt *et al.*, 1994). Treatment of diabetic rats with insulin either increased [^{3}H]ouabain binding site content by 23% compared to controls (Schmidt *et al.*, 1994), or prevented the diabetes-induced decline (Kjeldsen *et al.*, 1987).

2.14.4 Acute effect of insulin on Na^+,K^+-ATPase subunit - translocation

Whilst it is not possible to determine whether the results obtained in these studies (Kjeldsen *et al.*, 1987; Schmidt *et al.*, 1994) reflect an acute, or a chronic effect of insulin (or lack of insulin), it is likely that a combined effect is evident. Insulin is an acute stimulator of skeletal muscle Na^+-K^+-ATPase activity (Gavryck *et al.*, 1975; Clausen & Kohn, 1977). One proposed mechanism by which insulin may increase pump activity is to promote the unmasking of preformed latent pumps or the translocation of pumps (or subunits) from an intracellular pool to the plasma membrane in amphibian (Grinstein & Erlij, 1974; Erlij & Grinstein, 1976; Erlij & Schoen, 1981; Omatsu-Kanbe & Kitasato, 1990) and mammalian muscle (Hundal *et al.*, 1992b; Marette *et al.*, 1993; Lavoie *et al.*, 1996). The α_2 and β_1 subunits of the Na^+,K^+-ATPase do not appear to be co-localized in the intracellular pool in rat muscle (Hundal *et al.*, 1992b), although may be co-localized in human muscle (Hundal *et al.*, 1994), and translocation of each of the subunits in response to insulin may occur independently (Hundal *et al.*, 1992b).

Given the above, the [^{3}H]ouabain binding site assay, performed on small pieces of muscle, should detect an increase in content of functional Na^+,K^+ pumps following insulin stimulation. Indeed, insulin acutely increases [^{3}H]ouabain binding site content in intact frog muscle (Grinstein & Erlij, 1974; Erlij & Grinstein, 1976). However, due to the more volatile nature of ouabain binding in rodent muscle (Tobin *et al.*, 1972), this effect is more difficult to demonstrate in rodents, and debate exists as to whether insulin acutely increases the number of [^{3}H]ouabain binding sites (Erlij & Schoen,

1981), or rather, primarily increases the rate of ouabain binding (Clausen & Hansen, 1977). However, it does seem that insulin-induced translocation occurs given that two different protocols which used immunoelectron microscopy to determine the cellular location of the gold-labeled α_2 subunit, yielded similar results, i.e. an acute increase in plasma membrane Na^+-K^+-ATPase following insulin stimulation (Marette *et al.*, 1993). Presuming that subunit translocation does occur, and the [^{3}H]ouabain binding site content assay is able to detect an increase in pump number following insulin administration in human muscle, then the results of Schmidt *et al.* (1994) may be due to an acute effect of the most recent insulin dose, since the type 1 DM subjects had three-fold higher plasma insulin concentrations (although these may have represented total, rather than free insulin levels) than non-diabetic controls.

With regard to translocation, and the quantification of such in studies which used a membrane fractionation procedure, interpretation and extrapolation to whole muscle must be made bearing in mind that in skeletal muscle the procedure recovers only a small fraction of the total amount of plasma membrane and therefore discards much (90-99%) of the enzyme activity (Hansen & Clausen, 1988; Clausen, 1996b). Comparisons derived following fractionation procedures are therefore qualitative rather than quantitative. However, at least one study (Hundal *et al.*, 1992a) has used both fractionation procedures and immunocytochemistry, with similar results, thus strengthening the conclusion that translocation does indeed occur.

2.14.5 Chronic effect of insulin and insulin deficiency on Na^+,K^+-ATPase

It has also been suggested that insulin concentration may be a chronic regulator of [^{3}H]ouabain binding site content (Ewart & Klip, 1995; Clausen, 1998). No change was demonstrated in α_2 or β Na^+,K^+-ATPase subunit mRNA levels after 2 days, but a dramatic increase in the level of the α_2 subunit mRNA, and a decrease in β mRNA level after 14 days of untreated STZ-induced diabetes in rats (Nishida *et al.*, 1992) . In partial agreement, 6 weeks of STZ-induced diabetes resulted in a marked increase in the α_2 mRNA subunit in hindlimb skeletal muscle, however also resulted in increases in both the α_1 and the β_1 mRNA levels (Ng *et al.*, 1993). It thus appears that insulin deficiency has a chronic effect on Na^+,K^+-ATPase gene expression with increased α_2 mRNA that is mediated within 2 – 14 days, and extends for at least 6

weeks. The duration of STZ diabetes may explain the difference in findings between the two studies (Ng *et al.*, 1993). Treatment of diabetic rats with insulin for 14 days partially reversed elevated α_1 and α_2 mRNA subunit levels, but did not restore β mRNA levels (Nishida *et al.*, 1992; Ng *et al.*, 1993).

2.14.6 Effect of exercise on Na^+,K^+-ATPase

Vastus lateralis muscle digoxin binding was increased compared to rest after 60 min exercise at a 'high' submaximal work rate in healthy subjects who had undergone pre-treatment with digoxin for 2 weeks (Joreteg & Jogestrand, 1983). This finding may support an increased presence of Na^+-K^+-ATPase pumps at the sarcolemma (Tsakiridis *et al.*, 1996), but has also been interpreted to indicate a higher rate of binding in existing pumps (Everts & Clausen, 1994). The former is likely since a recent report demonstrated increased appearance of pump subunits in sarcolemmal membrane of vastus lateralis after brief, exhausting exercise in humans (Juel *et al.*, 2000a). One-legged exercise to exhaustion (<5 min) resulted in a 70% and 26% increase in the presence of α_2 and β_1 subunits, respectively, in the membrane of giant sarcolemmal vesicles (representing sarcolemma produced from vastus lateralis biopsies), but no change in α_1 subunits, and no change in isoform content of homogenized muscle (Juel *et al.*, 2000a). Whilst the results are not quantitative, the authors concluded that the α_2 and β_1 subunit translocation to the sarcolemma was an important mechanism in the acute up-regulation of Na^+-K^+ pumps in human muscle in response to a brief, exhausting exercise bout (Juel *et al.*, 2000a).

Following 60 min of fast running on an incline, an increased plasma membrane content of Na^+-K^+-ATPase α_1 and α_2 isoforms, and increased α_1 mRNA was demonstrated in rat skeletal muscle (Tsakiridis *et al.*, 1996). Whilst no concomitant reduction in intracellular subunit content was demonstrated, the authors did not exclude the possibility of translocation of pump subunits from an intracellular compartment that had not been identified in the fractionation procedure. The authors (Tsakiridis *et al.*, 1996) suggest that pump subunits may initially be regulated post-transcriptionally, and then later in exercise (or perhaps in recovery) increased protein expression via increased mRNA levels may contribute to increased subunit protein.

2.14.7 Effect of exercise training on Na^+,K^+-ATPase

Sprint training, comprised of 7 weeks of thrice-weekly, repeated bouts of 30-s sprint cycling exercise, increased vastus lateralis Na^+-K^+-ATPase content by 16% in men (McKenna *et al.*, 1993) (Table 2.11). Intensification of an existing exercise programme with high-intensity intermittent or continuous training, also resulted in a 13 – 16% increase in Na^+,K^+ pump content (Madsen *et al.*, 1994; Evertsen *et al.*, 1997; Pilegaard *et al.*, 1999). Additionally, subjects who underwent 6 days (Green *et al.*, 1993) to 8 weeks (Green, H. *et al.*, 1999) of endurance exercise training evidenced a 14% increase in Na^+-K^+-ATPase content; and those who had undertaken swimming, running, or strength training for some years displayed 30 – 40% greater Na^+-K^+-ATPase content than age-matched, sedentary controls (Klitgaard & Clausen, 1989). Thus, a considerable range of exercise frequencies, modes, and training durations elicit increases in Na^+-K^+-ATPase concentration (Table 2.11). In contrast, 10 weeks of 'moderate physical training' in male military conscripts, resulted in no change in [^{3}H]ouabain binding site content; however, the difference between this study and others is difficult to assess since the training regimen was not specified. However, it has been concluded that relatively intense exercise training is required to induce up-regulation of Na^+-K^+-ATPase content (Green *et al.*, 1993; McKenna *et al.*, 1993; Clausen, 1996a).

Only one study (Evertsen *et al.*, 1997) which examined elite, junior skiers, included female subjects, and reported 18% lower Na^+-K^+-ATPase content compared to males. This difference was maintained after 5 months of intensified endurance training. Although the men had higher $\dot{V}O_{2peak}$ than the women, prior to intensification of the training programme the absolute [^{3}H]ouabain binding site content for each group fell well within the range reported for untrained subjects. The authors suggested that hormonal differences between men and women may have contributed to their finding (Evertsen *et al.*, 1997); however no studies have addressed this issue.

2.14.8 [^{3}H]ouabain binding site content, performance, and plasma [K^+]

Despite increased [^{3}H]ouabain binding site content and improved performance following training (or intensified training), and in trained subjects, the two parameters

Table 2.11: *The effect of exercise training upon vastus lateralis [^{3}H]ouabain binding site content in human skeletal muscle*

Study	Subjects			Training period	Training			[^{3}H]ouabain binding site content *
	n	Tr. Stat.	Gdr		*f*	mode	Intensity/duration	
(Kjeldsen *et al.*, 1990)	15	milit. c.	M	10 wk	Moderate physical training			No change
(Green *et al.*, 1993)	9	UT	M	6 d	7/wk	cycle	65% $\dot{V}O_{2max}$, 2h	↑ 14%
(McKenna *et al.*, 1993)	6	UT	M	7 wk	3/wk	cycle	4-10, 30-s max bouts	↑ 16%
(Green, H. *et al.*, 1999)	9	UT	M	8 wk	3/wk	1 leg cycle	75% $\dot{V}O_{2max}$, 30 min (6wk) max. 3 min bouts (2wk)	↑ 14%
(Pilegaard *et al.*, 1999)	7	A	M	8 wk	3-5/wk	1 leg kick	max. 30-60 s bouts	↑ 13%
(Madsen *et al.*, 1994)	39	ET	M	6 wk	3/wk	run	93%HRmax, 25 min	↑ 15%
(Evertsen *et al.*, 1997)	11	T	M	5 mo	7/wk	run/ski	65 or 85% $\dot{V}O_{2max}$ 12-16 h/wk	↑ 16%
	9	T	F					
(Klitgaard & Clausen, 1989)	5-6 each	T	M	12–17 yr	3/wk	Swim or run or strength training		30 – 40% higher than age-matched sed.

Tr. Stat., training status; Gdr, gender; * all changes $P<0.05$; milit. c., military conscripts; M, male; wk, week/s; h, hours; UT, untrained; A, active; ET, endurance trained; T, trained; F, female; mo, months; yr, years; sed., sedentary controls.

are not consistently correlated. Nor is there significant correlation between [^{3}H]ouabain binding site content and improved plasma [K^+] regulation (Kjeldsen *et al.*, 1990; McKenna *et al.*, 1993). In a cross-sectional study (Haller *et al.*, 1998) patients with McArdle's disease were compared with matched controls, and despite lower [^{3}H]ouabain binding site content and higher exercise-induced peak plasma [K^+] in the patients, no relationship was found between the two variables. In contrast to these findings, two other studies found significant positive correlations between [^{3}H]ouabain binding site content and maximal isometric strength (Klitgaard & Clausen, 1989) and treadmill performance (Evertsen *et al.*, 1997); however plasma [K^+] regulation was not examined.

2.14.9 Exercise training and [^{3}H]ouabain binding site content in type 1 DM

No studies have examined the effects of any form of intense exercise training on [^{3}H]ouabain binding site content in subjects with type 1 DM. However, rats rendered diabetic via a partial pancreatectomy and not treated with insulin maintained [^{3}H]ouabain binding site content at non-diabetic control levels by endurance training 5 days per week, whereas their inactive diabetic counterparts displayed a 19% reduction after 10 weeks (Schmidt *et al.*, 1994). Thus, in rodents, acute exercise or a chronic effect of repeated exercise bouts during training can prevent the diabetes-induced decline in [^{3}H]ouabain binding site content.

2.14.10 Na^+-K^+-ATPase activity

Alterations in the activity of the Na^+,K^+-ATPase enzyme may be brought about by an increased number of sarcolemmal pumps, an increased activity of each existing pump, or a combination of both. As recently reviewed (Clausen & Nielsen, 1994; Ewart & Klip, 1995; Clausen, 1996a; Clausen, 1998), the major acute physiological stimuli for the Na^+,K^+-ATPase pump include muscle contraction, insulin and catecholamines.

In human muscle it is very difficult to detect and measure maximal Na^+,K^+-ATPase activity due to formation of vesicles during homogenization, the presence of other active ATPases (especially Mg^{2+}-ATPase), and the inhomogeneity of suspensions (Benders *et al.*, 1992). Instead, the K^+-dependent 3-*O*-methylfluorescein phosphatase (MFPase) activity, which performs part of the Na^+-K^+-ATPase cycle (Clausen, 1998),

has been determined *in vitro* in human muscle samples (Benders *et al.*, 1992; Benders *et al.*, 1993; Fraser & McKenna, 1998). Recent modification to the K^+-dependent 3-*O*-MFPase activity assay, that optimized enzymatic conditions, yielded a mean value of 292 ± 10 $nmol \cdot min^{-1} \cdot g^{-1}$ in vastus lateralis biopsies from young, untrained men (Fraser & McKenna, 1998).

2.14.11 Effect of insulin on Na^+-K^+-ATPase activity

Insulin acutely stimulates skeletal muscle Na^+-K^+-ATPase activity (Gavryck *et al.*, 1975; Clausen & Kohn, 1977), having an effect that is evident within 5 (Clausen & Kohn, 1977) to 30 min (Marette *et al.*, 1993; Lavoie *et al.*, 1996). In rat skeletal muscle, insulin acts both to increase Na^+ efflux and K^+ influx, thus reducing the intracellular Na^+ pool and increasing the K^+ content (Clausen & Kohn, 1977). The muscle membrane is hyperpolarised consequent to the effect of insulin on ionic gradients (Zierler, 1959; Flatman & Clausen, 1979; Clausen & Flatman, 1987; Overgaard *et al.*, 1997). In addition to acute regulation of Na^+-K^+-ATPase activity, insulin is also an extensively documented regulator of muscle glucose uptake, and although the two processes are regulated independently (Andres *et al.*, 1962; Zierler & Rabinowitz, 1964; Clausen & Flatman, 1987; Ferrannini *et al.*, 1988), recent work in transgenic mice suggests that there may be common translocating organelles for GLUT4 and Na^+-K^+-ATPase subunits (Ramlal *et al.*, 1996).

Insulin-like growth factor I (IGF-I) which has a number of actions in common with insulin, increased intracellular K^+ content and reduced Na^+ content within 10 min in rat soleus and extensor digitorum longus (EDL) muscle, and these can be attributed to Na^+-K^+-ATPase since the effects were blocked by ouabain (Dørup & Clausen, 1995). Thus, IGF-I also stimulates the muscle Na^+,K^+-ATPase pump, although probably via its own receptor rather than through the insulin receptor, and may play a functional role in ionic regulation (Dørup & Clausen, 1995).

2.14.12 Effect of type 1 DM on Na^+-K^+-ATPase activity

It is thought that Na^+,K^+-ATPase dysfunction could play a major role in many diabetic complications (Greene *et al.*, 1987; Garner, 1996). Postabsorptive subjects with type 1 DM who had delayed their morning insulin dose had 22-30% lower Na^+-K^+-ATPase

activity in erythrocyte membranes than fasted non-diabetics (Rahmani-Jourdheuil *et al.*, 1987; Garner, 1996). In addition, Na^+,K^+-ATPase activity was log-normally, rather than normally distributed in the subjects with diabetes (Garner, 1996). It was suggested that post-translational modification was the primary cause of the inhibition of Na^+,K^+-ATPase activity in erythrocytes (Garner, 1996). No correlation was found between the log of pump activity and either HbA_{1c} or blood glucose, indicating that changes were probably not directly due to nonenzymatic glycation (Garner, 1996); however, experimental glycation *in vitro* did reduce Na^+,K^+-ATPase activity in one study (Santini *et al.*, 1996). It was recently demonstrated that both insulin and C-peptide (which is co-secreted with insulin) directly increased Na^+-K^+-ATPase activity *in vitro* in erythrocyte membranes from subjects with type 1 DM (HbA_{1c}, 8.9%) who had fasted overnight and delayed their morning insulin dose (BGL, 11.2 $mmol \cdot l^{-1}$) (Djemli-Shipkolye *et al.*, 2000); unfortunately no non-diabetic subjects were included for comparison. In support, a 24 hr period of insulin treatment (via an artificial pancreas) increased pump activity to non-diabetic values in postabsorptive subjects with type 1 DM (Rahmani-Jourdheuil *et al.*, 1987).

Na^+-K^+-ATPase activity was reduced ~20% in both the crude membrane fraction and per mg of protein in enzyme purified from placentas of women with well-controlled type 1 DM compared to non-diabetic women (Zolese *et al.*, 1997); and plasma from subjects with type 1 DM inhibited the activity of purified placental Na^+-K^+-ATPase from non-diabetic women, but did not alter binding site number or affinity for ouabain (Rabini *et al.*, 1998). Na^+-K^+-ATPase activation energy was increased ~25% in the women with diabetes; a modification suggested to indicate subconformational changes of the protein, possibly linked to a higher fluidity of the lipid membrane (Zolese *et al.*, 1997).

Na^+,K^+-ATPase activity is reduced in type 1 diabetes mellitus in a variety of other tissues in animals, including peripheral nerves, renal glomeruli, and the retina, as discussed in Greene *et al.* (1987). A mechanism proposed to explain the reduction involves an alteration in cellular metabolism, induced by hyperglycaemia, in which abnormalities in the phosphoinositide cascade result in less protein kinase C activity, which modulates Na^+,K^+-ATPase activity (Greene *et al.*, 1987).

To date no studies have examined skeletal muscle Na^+,K^+-ATPase activity in human diabetes. In rat abdominal muscle, 2 days after induction of diabetes by STZ, Na^+,K^+-ATPase activity was reduced 50% compared to control rats, and remained 31% lower after a further 12 days of diabetes (Nishida *et al.*, 1992). Seven days treatment with insulin partially restored Na^+,K^+-ATPase activity to 76% of control values (Nishida *et al.*, 1992). In contrast, in rodent hamstring muscles which are predominantly composed of fast-twitch fibres, Na^+,K^+-ATPase activity was not altered after 7, 14, 28, or 56 days of STZ-induced diabetes, nor after 2 weeks of insulin treatment (Taira *et al.*, 1991). Similarly, in rodent mixed hindlimb muscle, 42 days of STZ-induced diabetes had no affect upon K^+-dependent *p*-nitrophenylphosphatase (K^+-pNPPase) activity (which reflects Na^+,K^+-ATPase activity), but did alter subunit isoform expression (Ng *et al.*, 1993).

The affect of altered insulin sensitivity on the activity of the Na^+,K^+ pump in humans is not known. Insulin resistance occurs in subjects with type 1 DM (see section 2.2.1). Hypertensive rats had increased Na^+-K^+-ATPase pump number in skeletal muscle, but reduced activity; showing that pump number and activity may be uncoupled (Pickar *et al.*, 1994). Subjects with type 1 DM had a greater number of ouabain binding sites than non-diabetics (Schmidt *et al.*, 1994): it is possible that the pump number may have been upregulated as a compensation for reduced Na^+-K^+-ATPase activity, however this remains untested. An impaired effect of insulin to stimulate whole body K^+ uptake has been demonstrated in obesity in humans (DeFronzo, 1988), and it has been speculated that the Na^+-K^+-ATPase pump may not be normally responsive to insulin (DeFronzo & Ferrannini, 1991). It is possible that insulin resistance of the pump may contribute to each of these abnormalities (Sweeney & Klip, 1998).

2.14.13 Effect of exercise on Na^+,K^+-ATPase pump activity

Muscle contraction *per se* elicits a marked increase in Na^+,K^+-ATPase pump activity, not solely attributable to the rise in intracellular Na^+ content (Everts & Clausen, 1994; Nielsen & Clausen, 1997). However, a net loss of K^+ still occurs during exercise, and it has been shown that this is due initially to a lag in activation (Medbø & Sejersted,

1990; Hallén *et al.*, 1994; Gullestad *et al.*, 1995), and later to insufficient continuing Na^+,K^+-ATPase pump activation (Verburg *et al.*, 1999).

One preliminary report examined the effect of exhaustive knee extensor exercise on vastus lateralis muscle Na^+,K^+-ATPase activity in men, and showed a 17 % reduction in maximal K^+-dependent 3-*O*-MFPase activity at exhaustion (Fraser & McKenna, 1996). The authors proposed that this may have been as a consequence of exercise-induced structural damage to the Na^+,K^+-ATPase.

2.14.14 Effect of exercise on Na^+,K^+-ATPase activity in type 1 DM

In human subjects with type 1 DM, the prevailing insulin concentration prior to commencing exercise may be the same, higher, or lower than in non-diabetic subjects, depending on the timing of the antecedent dose. Neither the effect of differing insulin concentration nor of exercise on Na^+-K^+-ATPase activity has been investigated in type 1 DM in humans.

2.14.15 Effects of hormones on Na^+,K^+-ATPase activity

2.14.15.1 Catecholamines

In addition to muscle contraction, catecholamines stimulate pump activation (Clausen & Flatman, 1977; Rogus *et al.*, 1977; Pfliegler *et al.*, 1983). There are both α- and β-adrenergic modulated components to the Na^+,K^+-ATPase pump (Akaike, 1981; Clausen, 1986; Lindinger & Sjøgaard, 1991). Alpha-adrenergic stimulation induces a slow net release of K^+, which may serve as a protective buffer to maintain K^+ homeostasis (Akaike, 1981), whereas β-adrenergic stimulation results in increased uptake of K^+ and extrusion of Na^+ and muscle membrane hyperpolarisation (Clausen & Flatman, 1977; Clausen & Flatman, 1987; Ballanyi & Grafe, 1988; Overgaard *et al.*, 1997). The latter effect is consistent with an earlier description of adrenaline as having a 'defatiguing effect' on skeletal muscle (Bowman & Nott, 1969). Both adrenaline and noradrenaline concentrations are markedly elevated during intense exercise (see Table 2.4).

Studies examining K^+ balance during exercise with and without β-adrenoceptor blockade have shown that catecholamines are important in accelerating the activation of the Na^+,K^+-ATPase pump at the start of exercise, and maintaining activation at the

cessation of exercise when muscle contraction is absent (Hallén *et al.*, 1994; Gullestad *et al.*, 1995). The net effect is to reduce the pump lag at the onset of exercise, and cause a rapid undershoot after exercise (Hallén *et al.*, 1994; Gullestad *et al.*, 1995). However, the post-exercise elevation of noradrenaline, although transient, may also exert a protective α-adrenergic influence on the pump, preventing dangerous hypokalaemia (Williams *et al.*, 1985; Laso *et al.*, 1991). Hallén *et al.* (1994) estimated that the time constant for pump activation during bicycling was probably less than 90 s, however, more recent work (Harrison *et al.*, 1997) in rodent muscle using the Na^+,K^+-ATPase pump blocker ouabain, demonstrated that pump activation occurs within a few seconds of commencing electrical stimulation at 90 Hz. The extent to which catecholamines provide an ongoing stimulus to pump activation during steady state exercise is uncertain, with several studies failing to show significant effects of either β-adrenoceptor blockade (Hallén *et al.*, 1994; Gullestad *et al.*, 1995) or stimulation (Rolett *et al.*, 1990) on K^+ loss. In addition, studies in rat muscle showed no further effect on $^{86}Rb^+$ uptake (reflecting K^+ uptake) of combining electrical stimulation with adrenaline, than when either was applied alone (Everts *et al.*, 1988). However, exercise to exhaustion (~45–70 min) during non-selective β-blockade in humans resulted in higher serum [K^+] than without blockade, and a significant reduction in muscle K^+ (Cleroux *et al.*, 1989). Further, based on a switch from net loss to net uptake of K^+ in the quadriceps during knee extension, following the addition of intense arm cranking (a consequence of which was an increased catecholamine concentration), it was suggested that catecholamines (in addition to high arterial [K^+]) may be an important stimulus for K^+ re-uptake by the exercising muscle (Juel *et al.*, 1999). In contrast, in inactive sites, K^+ redistribution has been suggested to be impaired during intense exercise due to markedly elevated catecholamine concentrations (Hallén *et al.*, 1994; Vøllestad *et al.*, 1994).

2.14.15.2 Insulin

Although insulin concentration usually declines or is unchanged during intense exercise, insulin delivery may be anticipated to be markedly increased consequent to increased blood flow to exercising muscle. Additionally, insulin is markedly elevated during recovery from intense exercise in response to increased plasma glucose concentration. The effects of insulin and β-adrenergic agonists on Na^+,K^+ pump

activity are additive since the hormones act via different intracellular pathways (Flatman & Clausen, 1979).

2.14.15.3 Calcitonin gene-related peptide

Calcitonin gene-related peptide (CGRP), appears to be released from the high-threshold muscle sensory nerve fibres (Aδ or C) in response to nerve stimulation (Sakaguchi *et al.*, 1991), and appears to stimulate the Na^+,K^+ pump in a similar manner to the catecholamines (Andersen & Clausen, 1993).

2.14.16 Effect of exercise training on Na^+-K^+-ATPase activity

A cross-sectional comparison of endurance-trained, strength-trained and untrained subjects demonstrated no differences in Na^+-K^+-ATPase activity (as assessed by K^+-stimulated 3-*O*-MFP activity) between the groups at rest or after fatiguing maximal knee extensions ; however, in all groups Na^+-K^+-ATPase activity was significantly reduced at fatigue (Fraser *et al.*, 1997). The effects of sprint training remain unknown.

In summary, the distribution of K^+ and Na^+ across the muscle membrane determines the membrane potential, and hence defence of the gradients is crucial in maintaining excitability. In maximal exercise loss of muscle K^+ and accumulation of K^+ in the t-tubular areas has been implicated in fatigue. The Na^+,K^+-ATPase pump acts to restore the electrochemical gradients. Little is known about the effect of type 1 DM on fatigue, however, lower intracellular K^+ was reported in one study.

Basal insulin levels are required for the maintenance of K^+ homeostasis at rest, acting to prevent resting hyperkalaemia. In subjects with type 1 DM, hyperkalaemia and hyponatraemia can be induced by hyperglycaemia. In a large study, patients with blood glucose levels >11 mM, had significantly higher and lower values for [K^+] and [Na^+], respectively, compared with non-diabetics.

K^+ efflux increases abruptly at the onset of maximal exercise. Interstitial K^+ after exhausting exercise may reach 9 mM. Sprint training has been reported to enhance K^+ regulation during maximal exercise, however the expected reduction in hyperkalaemia has not been demonstrated, possibly due to greater work being

performed after training. No studies have examined the effect of maximal exercise or exercise training on K^+ regulation in subjects with type 1 DM.

Both exercise and insulin induce a translocation of Na^+,K^+-ATPase pump subunits from distinct intracellular pools to the muscle membrane. Na^+,K^+-ATPase content is reduced in insulin-deficient diabetes in animals. However, only one study has examined Na^+,K^+-ATPase content in human type 1 DM, and reported higher levels than non-diabetics. Sprint training has been demonstrated to increase Na^+,K^+-ATPase content in non-diabetics.

2.15 H^+ regulation

2.15.1 $[H^+]$ is a dependent variable

The $[H^+]$ (and $[HCO_3^-]$) in plasma and in the resting and exercising muscle cell is dependent upon three independent variables, according to the concepts revitalised by Stewart (Stewart, 1981), as recently reviewed (Stewart, 1983; Jones, 1987; Jones & Heigenhauser, 1992; Jennings, 1994; Heigenhauser, 1995; Kowalchuk & Scheuermann, 1995; Lindinger, 1995). The net charge difference between strong (largely dissociated in solution) cations and anions - the strong ion difference (SID), is an independent variable, thus capable of effecting change in dependent variables such as $[H^+]$. A reduction in the SID increases $[H^+]$; conversely, an increased SID effects an increase in $[HCO_3^-]$. Intracellular SID (~130 mM) is ~3-4-fold higher than the SID of plasma (40-48 mM) (Jones, 1987; Kowalchuk *et al.*, 1988b). A second independent variable is the total concentration of CO_2 (effectively represented by PCO_2) which is regulated by alveolar ventilation, and acts to increase $[H^+]$; an effect potentiated by a reduction in SID. In terms of $[H^+]$ regulation, the effect of an increased PCO_2 is of considerably more import in plasma than in skeletal muscle (Kowalchuk *et al.*, 1988b). The third is the total weak anion concentration ($[A_{tot}]$), which acts to increase $[H^+]$, and in plasma is constituted by the albumin component of protein. Plasma $[A_{tot}]$ tends not to change during acid-base disturbances, e.g. hypercapnia (Jennings, 1994), although may be increased during intense exercise consequent to a reduction in plasma volume (Lindinger *et al.*, 1992; McKenna *et al.*, 1997a). Intracellular phosphates and proteins constitute an $[A_{tot}]$ (~200 mM) that is

~10-fold higher than in plasma (~20 mM) (Jones, 1987; Kowalchuk *et al.*, 1988b). The interaction of the three independent variables, acting within an aqueous solution and obeying the constraints of electrical neutrality and conservation of mass, determines the $[H^+]$ and $[HCO_3^-]$ of body fluids (Jones, 1987).

2.15.2 Plasma $[H^+]$ regulation during maximal exercise

2.15.2.1 A single exercise bout

In plasma, the major strong ions are Na^+, K^+, and Cl^-, with $[Lac^-]$ being a major additional factor during intense exercise. At rest the SID in arterial plasma (SID_a) was 37-45 $mmol \cdot l^{-1}$ and $[H^+]$ 36-39 $nmol \cdot l^{-1}$, and immediately following a maximal 30-s exercise bout SID_a was reduced to 34-43 $mmol \cdot l^{-1}$ whilst arterial $[H^+]$ ($[H^+]_a$) was increased to 40-49 $mmol \cdot l^{-1}$ (Kowalchuk *et al.*, 1988b; Lindinger *et al.*, 1992; McKenna *et al.*, 1997a). Several minutes after exercise, when peak arterial $[Lac^-]$ ($[Lac^-]_a$) was evident, the SID_a had further reduced to 29-30 $mmol \cdot l^{-1}$ and $[H^+]_a$ increased to 54-66 $nmol \cdot l^{-1}$ (Kowalchuk *et al.*, 1988b; McKenna *et al.*, 1997a). In femoral venous plasma, SID (SID_{fv}) at rest was 42-47 $mmol \cdot l^{-1}$ and $[H^+]$ ($[H^+]_{fv}$) was 41 $nmol \cdot l^{-1}$ (Kowalchuk *et al.*, 1988b; McKenna *et al.*, 1997a). Immediately after 30 s maximal exercise SID_{fv} was either transiently increased (McKenna *et al.*, 1997a) or unchanged (Kowalchuk *et al.*, 1988b), and then proceeded to fall sharply, with the nadir occurring between 3.5 to 10 min of recovery. In contrast, peak $[H^+]_{fv}$ (95-107 $nmol \cdot l^{-1}$) occurred 30 to 60 s after exercise, after which $[H^+]_{fv}$ fell slowly, remaining 1.5- to 2-fold above resting values after 10 min recovery (Kowalchuk *et al.*, 1988b; McKenna *et al.*, 1997a). Arterial and venous plasma $[A_{tot}]$ ($[A_{tot}]_a$, $[A_{tot}]_{fv}$) increased with exercise, peaked 15-19% above resting values (~17 $mmol \cdot l^{-1}$) in the first minutes of recovery, and remained 8% higher than rest after 10 min recovery (McKenna *et al.*, 1997a). Arterial PCO_2 ($PaCO_2$) fell with exercise and reached a nadir of 30-32 mmHg after 3.5 to 5 min recovery; in contrast femoral venous PCO_2 ($PvCO_2$) rose sharply with exercise, peaked at 103-106 mmHg 30 to 60 s post-exercise, and fell for the remainder of recovery (Kowalchuk *et al.*, 1988b; McKenna *et al.*, 1997a). In arterial plasma, the sharp post-exercise rise in $[H^+]_a$ was attributable to the decline in SID_a, primarily consequent to the rise in $[Lac^-]_a$ (with increased $[K^+]$ and $[Na^+]$ attenuating the effect) (McKenna *et al.*, 1997a), with the concurrent small fall in $PaCO_2$ and rise in $[A_{tot}]_a$ playing minor roles in respectively reducing and increasing $[H^+]_a$. In

contrast, in femoral venous plasma, the sharp rise in $[H^+]_{fv}$ was mostly due to the marked rise in $PvCO_2$, with the reduced SID_{fv} playing a greater role in later recovery (Kowalchuk *et al.*, 1988b; McKenna *et al.*, 1997a). As discussed by Jones (Jones, 1995) and demonstrated by Kowalchuk *et al.* (1988b) and McKenna *et al.* (1997a), the fall in $[HCO_3^-]_a$ approximately mirrors the rise in $[Lac^-]_a$ since the latter is the major contributor to the SID_a change in intense exercise in arterial plasma, however, highlighting the value of the Stewart approach, in femoral venous blood, $[HCO_3^-]_{fv}$ transiently increases with exercise concurrent with the increase in $[Lac^-]_{fv}$; in opposition to the concept (Wasserman, 1984) of bicarbonate buffering of lactic acid.

A recent paper which used the base deficit (negative of the base excess) to quantify changes in acid-base status in arterial and venous blood with intense, exhausting exercise of 2.1 min duration, concluded that H^+ left skeletal muscle at a faster rate than Lac^-, and that intracellular H^+ was exchanged one-to-one with plasma Na^+ (corrected for fluid shifts) (Medbø *et al.*, 2000). The authors suggested that muscle lactic acidosis activated a Na^+/H^+ membrane transporter which extruded H^+ independently of Lac^- release (Medbø *et al.*, 2000). However, as acknowledged by the authors, their explanation may be incomplete since increased cellular Na^+ may be anticipated to increase the activity of the Na^+-K^+-ATPase pump, however, no net muscle K^+ accumulation was evident from arteriovenous data (Medbø *et al.*, 2000). Whilst these authors (Medbø *et al.*, 2000) criticised the approach of Stewart (1981) in quantifying acid-base status, it is interesting to consider that in Medbø *et al.* (2000) the arterial $[Lac^-]$ and base deficit were relatively similar in magnitude, however the v-a difference was approximately twice as great for the latter. Again, supporting the discussion of Jones (1995) regarding differences between causative factors in arterial and femoral venous H^+ generation, the excess H^+ in the femoral venous blood would be expected (Kowalchuk *et al.*, 1988b; McKenna *et al.*, 1997a) to be due in great part to the marked increase in $PvCO_2$, rather than increased $[Lac^-]$. Hence, it may be misleading to compare the v-a differences for base deficit and Lac^-. However, the role of Na^+/H^+ exchange requires further investigation in human muscle, and as has been previously suggested (Kowalchuk *et al.*, 1988b), change in $[H^+]$ consequent to translocation of strong ions (e.g. Na^+), would effectively 'translocate' H^+.

2.15.2.2 Repeated bouts of maximal exercise

$[H^+]_a$ peaked at 65 nmol·l^{-1} immediately after the final bout of four, 30-s bouts (4 min recovery between each bout) (Lindinger *et al.*, 1992). The major effector of increased $[H^+]_a$ was the reduction in SID_a, with increased $[A_{tot}]_a$ contributing (14%) to the rise, and the reduced $PaCO_2$ effectively acting to reduce $[H^+]_a$ by 37% (Lindinger *et al.*, 1992). In femoral venous plasma, the peak $[H^+]_{fv}$ after bout 4 was similar to that after the preceding three bouts, however in contrast to the 75% contribution to $[H^+]_{fv}$ genesis from $PvCO_2$ after bout 1, the rise in $PvCO_2$ in bout 4 accounted for only 20% of the increase in $[H^+]_{fv}$ (Lindinger *et al.*, 1992). The balance of the increase in $[H^+]_{fv}$ in bout 1 was effected equally by increased $[A_{tot}]_{fv}$ and reduced SID_{fv}, however after bout 4, the reduction in SID_{fv} accounted for 67% of the rise in $[H^+]_{fv}$, then remained the main determinant of $[H^+]_{fv}$ during recovery (with $PvCO_2$ acting to alkalinize the plasma) (Lindinger *et al.*, 1992).

$[HCO_3^-]_a$ was progressively reduced during the four bouts, with reduced SID_a responsible for 78%, and increased $[A_{tot}]_a$ for 19% of the reduction after bout 4 (Lindinger *et al.*, 1992). The small increase in $[HCO_3^-]_{fv}$ after bouts 1 and 4 was accounted for by the marked increase in $PvCO_2$ (Lindinger *et al.*, 1992).

2.15.3 $[H^+]$ regulation in skeletal muscle during maximal exercise

In skeletal muscle at rest, SID was 154 mmol·l^{-1}, PCO_2 46 mmHg (assumed to be equal to $PvCO_2$), and $[A_{tot}]$ was assumed to be 180 mmol·l^{-1}; with $[H^+]$ being 132 nmol·l^{-1} and $[HCO_3^-]$ 9 mmol·l^{-1} as a consequence (Kowalchuk *et al.*, 1988b). After 30 s of maximal exercise $[H^+]$ rose to 328 nmol·l^{-1}, with a further increase to 417 nmol·l^{-1} after 3.5 min recovery (Kowalchuk *et al.*, 1988b). Total tissue water was unchanged immediately after exercise, but was ~7% greater than at rest after 3.5 to 9.5 min recovery (Kowalchuk *et al.*, 1988b); a factor that would ameliorate increases in $[H^+]$ (McKenna, 1992). $[HCO_3^-]$ was little changed immediately after exercise (at which time Lac^- had increased nearly 8-fold), then fell significantly after 3.5 min recovery, and remained at 3 mM for the duration of recovery (10 min) (Kowalchuk *et al.*, 1988b). The SID fell by ~30% immediately after exercise, of which 87% of the decrease was attributable to the rise in Lac^-, and remained at a similar value

throughout recovery, with the influence of Lac^- waning and a greater effect of lowered K^+ on SID evident in later recovery (Kowalchuk *et al.*, 1988b). Although $PvCO_2$ rose to at least 106 mmHg with exercise, the reduction in SID accounted for 93% of the rise in $[H^+]$ immediately after exercise; whilst the high $PvCO_2$ (and the reduction in the dissociation constant for weak acids) acted to maintain $[HCO_3^-]$ in spite of the reduction in SID (Kowalchuk *et al.*, 1988b). The return of $PvCO_2$ towards resting values, whilst the SID remained low, accounted for the delayed reduction in $[HCO_3^-]$ (Kowalchuk *et al.*, 1988b).

2.15.4 Buffering capacity (β)– *in vitro, in vivo*

The buffering capacity of human skeletal muscle has been assessed *in vitro* ($\beta_{in\ vitro}$) by examining changes in muscle homogenate pH with acid titration. The buffers comprise muscle phosphates and proteins, i.e. intracellular $[A_{tot}]$. $\beta_{in\ vitro}$ has been reported to vary from ~91 (Parkhouse *et al.*, 1985), through 156 to ~215 (Bell & Wenger, 1988; Mannion *et al.*, 1993; Mannion *et al.*, 1994) to ~283 mmol $H^+ \cdot kg^{-1}dw \cdot pH^{-1}$ (Nevill *et al.*, 1989) in muscle from untrained or active subjects.

The *in vivo* buffering capacity ($\beta_{in\ vivo}$), i.e. the ratio of the change in muscle Lac^- to the change in muscle pH from rest to exercise, varied between 164 to ~192 mmol $H^+ \cdot kg^{-1}dw \cdot pH^{-1}$ in vastus lateralis muscle from untrained or active subjects (Sahlin & Henriksson, 1984; Sharp *et al.*, 1986; Mannion *et al.*, 1993).

Both methods of estimating buffering capacity have their limitations. As briefly discussed in McKenna *et al.* (1996), $\beta_{in\ vitro}$ does not account for dynamic fluxes in intracellular concentrations of e.g PCr, Lac^- or K^+, whilst $\beta_{in\ vivo}$ infers that Lac^- is the sole contributor to muscle $[H^+]$ changes; and neither accounts for changes in work-rate or duration with perturbations, e.g. exercise training. Consequently a more physiological measure may be the ratio of the change in $[H^+]$ ($\Delta[H^+]$) to the work performed during exercise, which encompasses all processes affecting $[H^+]$ (McKenna *et al.*, 1996).

2.15.4.1 Effects of sprint training on muscle in vitro and in vivo buffering capacities

Seven weeks of one-legged sprint cycling training (Bell & Wenger, 1988) increased $\beta_{in\ vitro}$ in the trained leg by 16%. Trained sprinters (Parkhouse *et al.*, 1985) had 41% higher $\beta_{in\ vitro}$ than sedentary controls. In contrast, two other studies of sprint training revealed no change in $\beta_{in\ vitro}$ (Nevill *et al.*, 1989; Mannion *et al.*, 1994).

Effects of sprint training on $\beta_{in\ vivo}$ are also equivocal. $\beta_{in\ vivo}$ was increased in one study (Sharp *et al.*, 1986), but not another (Nevill *et al.*, 1989).

2.15.5 Sprint training and H^+ regulation

Muscle pH, H^+ release, and arterial and venous pH, did not differ significantly between trained and untrained legs during one-legged exercise to exhaustion at a set power output following 8 weeks of high intensity one-legged training (Pilegaard *et al.*, 1999), however, since more work was performed by the trained leg, H^+ regulation as assessed by the $\Delta[H^+]\cdot work^{-1}$ ratio was improved. Calculation (McKenna *et al.*, 1996) of $\Delta[H^+]\cdot work^{-1}$ from data in Bell & Wenger (1988) also demonstrated marked reductions in the ratio for both the trained and untrained legs after a 60-s one-legged maximal cycling test, following a 7-week one-legged sprint training programme. Similarly, calculations using the data of Nevill *et al.* (1989) demonstrated a 17% reduction in $\Delta[H^+]\cdot work^{-1}$ during a 30-s maximal sprint after 8 weeks of sprint training. A cross-sectional study (Sahlin & Henriksson, 1984) found similar isometric work performed by highly trained anaerobic athletes and sedentary men, but a higher muscle pH at fatigue in the trained subjects: calculations from their data revealed a 53% lower $\Delta[H^+]\cdot work^{-1}$ ratio in the trained subjects. Thus, in those who have undergone sprint training and in the sprint-trained, muscle H^+ regulation during intense exercise is improved when the work performed during exercise is considered. However, in the only study to match the work performed during intense exercise (110% pre-training $\dot{V}O_{2max}$) before and after training, muscle pH did not differ with training (Nevill *et al.*, 1989); although the metabolic perturbation was not extensive and may have been insufficient to allow discrimination of an effect. The effect of sprint training on muscle H^+ accumulation during intense matched work exercise

therefore remains to be investigated, however, based upon findings when exercising to exhaustion, it would be anticipated that a marked improvement would be evident.

With regard to the mechanism of enhanced $[H^+]$ regulation during exhausting exercise following sprint training, one study (McKenna *et al.*, 1997a) applied the physicochemical approach of Stewart (1981). Although muscle measurements were not reported, arterial and femoral venous blood was sampled and arteriovenous differences examined. Maximal exercise performance was improved after training, whilst the arteriovenous difference for $[H^+]$ ($[H^+]_{a-v}$) was unchanged (McKenna *et al.*, 1997a), suggesting improved muscle $[H^+]$ regulation. Interestingly, improved performance occurred at the expense of greater $[H^+]_a$; accounted for by a lower SID_a, produced by higher $[Lac^-]_a$ and lower arterial plasma $[K^+]$ and $[Na^+]$ (McKenna *et al.*, 1997a). Each of $PaCO_2$ and $PvCO_2$ was lower at rest, during exercise, and in recovery after sprint training, as was $[A_{tot}]_{fv}$, however $[A_{tot}]_a$ was not affected by sprint training (McKenna *et al.*, 1997a). $[H^+]_{fv}$ was not affected, although the SID_{fv} was lower after sprint training (McKenna *et al.*, 1997a), implying that the reduction in $[A_{tot}]_{fv}$ and $PvCO_2$ with training was sufficient to offset the greater reduction in SID_{fv} after training. Both $[HCO_3^-]_a$ and $[HCO_3^-]_{fv}$ were lower after training. Thus whilst sprint training enhanced muscle $[H^+]$ regulation and improved performance during maximal exercise, the improvements in regulation of the plasma strong ions K^+ and Na^+ (combined with higher $[Lac^-]_a$) resulted in greater systemic acidosis (McKenna *et al.*, 1997a). Examination of muscle $[H^+]$ before and after sprint training would provide a useful extension of these data.

Following 8 weeks of one-legged high intensity interval training in habitually active men, the rate of Lac^-/H^+ transport in sarcolemmal giant vesicles was 12% higher in the trained leg than in the untrained leg (Pilegaard *et al.*, 1999), consistent with a 33-70% greater content of monocarboxylate transporters in the trained leg (Pilegaard *et al.*, 1999). Additionally, after high-intensity training in rats (Juel, 1998), the Na^+/H^+ exchange capacity was increased and may contribute to enhanced muscle $[H^+]$ regulation in human muscle after sprint training, although this has not been investigated.

In summary, $[H^+]$ is a dependent variable. Peak $[H^+]$ occurs several minutes after maximal exercise. The sharp post-exercise rise in muscle and in arterial $[H^+]$ was primarily attributable to the decline in SID, whereas in femoral venous plasma, the sharp rise in $[H^+]_{fv}$ was mostly due to the marked rise in $PvCO_2$, with the reduced SID_{fv} playing a greater role in later recovery. Muscle $[H^+]$ regulation, as assessed by the $\Delta[H^+]\cdot work^{-1}$ ratio, is improved during maximal exhausting exercise following sprint training, and it may be anticipated that an even more marked improvement would be evident during matched-work exercise. The primary mechanism for such does not appear to be related to changes in muscle buffering capacity, but rather a complex interplay between training-induced changes in independent variables, and perhaps also in membrane transport proteins.

Section E – Muscle metabolism

2.16 Muscle glucose utilization

2.16.1 Muscle glucose transporters, insulin, and glucose uptake

Glucose is hydrophilic and cannot freely permeate the muscle membrane, but instead enters by facilitated diffusion, effected via specific carrier proteins, as recently reviewed (Klip & Pâquet, 1990; Birnbaum, 1992; Kahn, 1992; Rea & James, 1997; Goodyear & Kahn, 1998). Of the family of facilitative glucose transporters, by far the most abundant in human skeletal muscle is GLUT4 which is primarily sequestered within an intracellular compartment, but is also present in the triads (terminal cisternae and tranverse tubules – *t*-tubules) (Friedman *et al.*, 1991; Gumà *et al.*, 1995). Much less abundant, GLUT1 mRNA was originally identified in muscle from two patients with type 2 DM (Flier *et al.*, 1987), and GLUT1 protein is evident in muscle membranes of healthy men and women (Kahn *et al.*, 1992), and in the plasma membrane of healthy men (Gumà *et al.*, 1995). Although as much as 60% of the GLUT1 found in plasma membrane fractions in rats may arise from perineural sheaths (Handberg *et al.*, 1992), GLUT1 is thought to be involved in basal glucose transport (Rodnick *et al.*, 1992) and in human myocytes is responsive to changes in glucose availability (Baqué *et al.*, 1998). GLUT5 has also been identified in the plasma membrane of human muscle (Hundal *et al.*, 1992a; Gumà *et al.*, 1995).

Insulin has long been known to promote the uptake and storage of glucose in skeletal muscle (Best *et al.*, 1926). Skeletal muscle is the major site for insulin-mediated glucose utilization *in vivo* (DeFronzo *et al.*, 1981a; DeFronzo *et al.*, 1981b), and glucose transport is rate-limiting for glucose utilization under most conditions, as recently reviewed (Rodnick *et al.*, 1992; Goodyear & Kahn, 1998). GLUT4 is the primary insulin-sensitive glucose transporter in skeletal muscle (James *et al.*, 1988). Insulin was first demonstrated to redistribute glucose transporters from an intracellular pool (low-density microsomes) to the plasma membranes of rat adipocytes (Cushman & Wardzala, 1980; Suzuki & Kono, 1980), and was later shown to have a similar effect in rat muscle (Klip *et al.*, 1987; Douen *et al.*, 1990). Recently, in human muscle, insulin was also demonstrated to elicit translocation of GLUT4 from an intracellular membrane pool to the surface membrane (Gumà *et al.*, 1995). Ingestion of a physiological dose of glucose also effected a translocation of GLUT4 in

human muscle (Goodyear *et al.*, 1996). GLUT1 is constitutively targeted to the plasma membrane (Rea & James, 1997) and does not appear to undergo translocation in human skeletal muscle (Goodyear *et al.*, 1996). Similarly, GLUT5 does not appear to be affected by insulin in human muscle (Gumà *et al.*, 1995).

2.16.2 GLUT4 and the effect of exercise

Exercise also stimulates GLUT4 translocation from an intracellular pool to the muscle membrane (Douen *et al.*, 1990), with enhanced glucose uptake evident in the absence of insulin (in severely STZ-diabetic rats), albeit to a diminished degree (Wallberg-Henriksson & Holloszy, 1985). It is thought that the intracellular pools of GLUT4 that respond to exercise or insulin stimulation are distinct (Douen *et al.*, 1990), and are activated or mobilized as a consequence of different molecular mechanisms or 'switches', as recently reviewed (Goodyear & Kahn, 1998). Indeed the former was recently confirmed, with identification and characterisation of separate intracellular insulin- and exercise-sensitive GLUT4 pools in rat hind limb muscle, which were selectively depleted according to the stimulus (insulin injection or swimming), or simultaneously depleted with combined stimuli (Coderre *et al.*, 1995). The effects of high-frequency electrical stimulation plus high-dose insulin are additive in rats (Gao *et al.*, 1994).

In humans, hyperinsulinaemia and low intensity exercise acted synergistically on glucose uptake (DeFronzo *et al.*, 1981a; Wasserman *et al.*, 1991). Submaximal exercise for 10 min followed immediately by exercise to exhaustion at 100% $\dot{V}O_{2peak}$ was recently demonstrated to increase glucose transport and GLUT4 protein content in sarcolemmal giant vesicles produced from muscle biopsy samples; the latter providing evidence that GLUT4 translocation also occurs in human muscle in response to exercise (Kristiansen *et al.*, 1996). In addition to translocation, the intrinsic activity of GLUT4 was increased by exercise (Kristiansen *et al.*, 1996). However, an inverse correlation was found between total crude membrane GLUT4 and glucose uptake, supporting an earlier study that used exercise at 72% $\dot{V}O_{2peak}$ (McConell *et al.*, 1994), and hence it was suggested that the exercise-induced activation of GLUT4 is reduced when total muscle GLUT4 protein content is high (Kristiansen *et al.*, 1996).

2.16.3 Muscle glucose phosphorylation – effects of insulin and moderate exercise

Whilst membrane transport of glucose is rate-limiting under most conditions of glycaemia and insulinaemia (see above), at high glucose flux rates glucose phosphorylation may become limiting (Kruszynska *et al.*, 1998). Regulated co-ordinately with GLUT4 is the non-equilibrium enzyme hexokinase (HK) (Phillips *et al.*, 1996), which phosphorylates muscle glucose to form glucose 6-phosphate (G 6-P). Human skeletal muscle expresses two isoforms of HK, of which HKII comprises about 47% of total HK at rest (Kruszynska *et al.*, 1998). HK activity is inhibited allosterically by accumulation of G 6-P (Newsholme & Leech, 1983).

HKII mRNA was doubled (Vogt *et al.*, 2000), and HKII activity was increased ~1.4- to 1.8-fold by 2-6 h of euglycaemic hyperinsulinaemia in humans (Mandarino *et al.*, 1995; Kruszynska *et al.*, 1998; Vogt *et al.*, 2000). Total HK activity also increased after 3 h of hyperinsulinaemia, solely attributable to the increase in HKII activity (Kruszynska *et al.*, 1998). Of interest, in type 2 DM in which marked insulin resistance is a characteristic clinical feature, HKII was not increased by sustained hyperinsulinaemia (Kruszynska *et al.*, 1998). Sixty min of exercise at 60% $\dot{V}O_{2peak}$ increased HKII activity by ~1.8-fold and increased HKII mRNA by ~2.1-fold in sedentary men and women (Koval *et al.*, 1998). The effect of maximal exercise on HKII has not been determined.

2.16.4 Glucose uptake and phosphorylation, and intense exercise

Leg glucose uptake (LGU) increased continuously with maximal exercise (97% $\dot{V}O_{2max}$) and at fatigue was 48-fold higher than at rest (Katz *et al.*, 1986). Concomitantly, leg blood flow during maximal exercise was increased ~10-fold over resting values (Katz *et al.*, 1986). The rate of intracellular glucose accumulation was similar to the rate of LGU, with blood glucose only contributing ~1% of carbohydrate utilized during maximal exercise (Katz *et al.*, 1986). Muscle glucose increased by 13-fold, and G 6-P by 7-fold at fatigue, suggesting that rather than membrane transport being rate-limiting for glucose utilization during maximal exercise, that HK activity was inhibited by G 6-P, and hence limited glucose phosphorylation (Katz *et al.*, 1986). The glycogenolytic rate is reduced when intense exercise is repeatedly

performed, and if the rate of G 6-P accumulation is reduced and HKII activity is relatively less inhibited, then glucose may contribute more to carbohydrate-derived ATP generation in later bouts. To the author's knowledge, no human studies have reported upon the effect of intense, exhausting exercise on HK activity. However, 0.5 to 1 hr after swimming to exhaustion (30-40 min) a 24% reduction in maximal *in vitro* HK activity was found in rats (Boström *et al.*, 1974). HK activity had returned to be similar to resting values by 8 hr post-exercise (Boström *et al.*, 1974). In contrast, HK was increased 48 hr after a bout of exhausting exercise in untrained guinea pigs, whilst HK activity was reduced 48 hr after 15-day endurance-trained guinea pigs ran to exhaustion (~75 min) (Barnard & Peter, 1969). The reason for the difference was not clear, but was not related to a change in free versus bound enzyme (Barnard & Peter, 1969).

2.16.5 Glucose transport and phosphorylation in type 1 diabetes mellitus

2.16.5.1 Effect of insulin and insulin resistance

Basal leg glucose uptake (LGU) at rest did not differ between non-diabetics and patients with type 1 DM (who had insulin withdrawn for 24 h), and averaged 0.16 and 0.20 $mmol \cdot min^{-1}$, respectively (Wahren *et al.*, 1975). During euglycaemic hyperinsulinaemia, men and women with moderately controlled type 1 DM (HbA_{1c} 8.6%) of long-standing (17 yr) had similar glucose disposal rates to non-diabetic subjects (Cohen *et al.*, 1996). However, in subjects with poorly controlled type 1 DM (HbA_{1c} 13.7%) of long-standing (17 yr), who had fasted overnight, glucose utilization during each of euglycaemia and hyperglycaemia (with physiological increments in free insulin levels) was substantially lower than that of matched non-diabetic subjects; a defect that was not overcome by acute high-dose hyperinsulinaemia (Proietto *et al.*, 1983). Similar results were found even when the subjects with type 1 DM commenced the clamp with normoglycaemia, achieved by overnight i.v. insulin (Cline *et al.*, 1997). Such a defect may be attributable to reduced uptake and/or reduced intracellular glucose metabolism (Proietto *et al.*, 1983), and probably reflects the characteristic insulin resistance (Yki-Järvinen & Koivisto, 1986) that develops in subjects with type 1 DM. Using NMR spectroscopy, it was demonstrated that the defect lay either in glucose transport or phosphorylation rather than in glycogen synthesis (Cline *et al.*, 1997). The defect in glucose utilization in type 1 DM was

further localized to glucose transport (Yki-Järvinen *et al.*, 1990). However, vastus lateralis GLUT1 and GLUT4 mRNA, and GLUT4 protein did not differ between subjects with type 1 DM (11.4 yr duration; mean HbA_{1c} 8.6%) who had fasted overnight and delayed the morning insulin dose, and non-diabetics (Kahn *et al.*, 1992). Although GLUT1 mRNA was correlated with HbA_{1c} in the subjects with DM, there was no association between transporter expression and fasting insulin level or daily insulin dose; however, GLUT4 protein expression was negatively correlated with body mass index (Kahn *et al.*, 1992). In view of the results, the authors suggested that impaired translocation of glucose transporters, reduced activity, or altered affinity for glucose may explain the insulin resistance of type 1 DM (Kahn *et al.*, 1992). This suggestion has recently been affirmed in rats with STZ-induced diabetes. Both basal *t*-tubule GLUT4 content, and insulin-stimulated GLUT4 translocation to *t*-tubules (which represent 70-90% of the cell surface area), were impaired in rats with STZ-induced diabetes; whereas basal plasma membrane GLUT4 content and translocation to the plasma membrane was unimpaired (Dombrowski *et al.*, 1998). Further, insulin-mediated glucose disposal was correlated with GLUT4 content in the *t*-tubular, but not plasma membrane (Dombrowski *et al.*, 1998).

2.16.5.2 Effect of exercise

Despite insulin withdrawal for the preceding 24 hours, LGU in subjects with type 1 DM during 40 min of cycling at 55-60% $\dot{V}O_{2peak}$ did not differ from that of non-diabetics of similar fitness (Wahren *et al.*, 1975). In contrast, when using the stable isotope technique (compared with arteriovenous differences), the rate of disappearance (R_d) of glucose was ~25% lower in subjects with type 1 DM (with a basal insulin infusion) than in non-diabetics during 30 min exercise at 45% $\dot{V}O_{2peak}$, even though plasma glucose was a little higher in the former group (Raguso *et al.*, 1995). However, no difference was evident between groups at 75% $\dot{V}O_{2peak}$ (Raguso *et al.*, 1995). It was suggested that at moderate intensity exercise the recruitment of GLUT4 from the insulin-sensitive pool may be reduced and contribute to the lower R_d, whereas at higher intensities the muscle contraction-sensitive pool may become predominant, and GLUT4 translocation from such may not be impaired in subjects with type 1 DM (Raguso *et al.*, 1995). Alternatively, it was suggested that glucose phosphorylation may be rate-limiting at higher intensity rather than glucose uptake

(Raguso *et al.*, 1995). The R_d during exercise for 60 min at 35% $\dot{V}O_{2peak}$ did not differ when the same subjects with type 1 DM were either insulinized prior to exercise or had delayed their morning dose (and hence were hyperglycaemic), however, the metabolic clearance rate (MCR) was markedly lower in the latter condition (Benn *et al.*, 1992). In agreement, R_d during exhausting exercise at 89-100% $\dot{V}O_{2peak}$ did not differ between subjects with type 1 DM who were either eu- or hyperglycaemic, and non-diabetics, however MCR was lower in those with diabetes (Purdon *et al.*, 1993; Sigal *et al.*, 1994b). Glucose uptake is increased during hyperglycaemia via a mass action effect, whether in non-diabetic subjects or in insulin deprived subjects with type 1 DM (Sanders *et al.*, 1964). Thus, hyperglycaemia in intense exercise compensates for the reduced MCR (Purdon *et al.*, 1993; Sigal *et al.*, 1994b).

No studies have examined the translocation of GLUT4 during exercise in subjects with type 1 DM. Three hours of mild to moderate exercise had no affect upon muscle total GLUT4 protein content or GLUT4 mRNA in non-diabetics (Koivisto *et al.*, 1993). However, in subjects with type 1 DM in good metabolic control (HbA_{1c} 7.8%), whilst total GLUT4 protein was unchanged, GLUT4 mRNA was reduced following exercise (Koivisto *et al.*, 1993). It was concluded that GLUT4 mRNA synthesis and/or degradation differed from non-diabetics during exercise, however, the mechanism for such remains unclear (Koivisto *et al.*, 1993).

2.16.5.3 Hexokinase activity

No studies to date have reported HKII activity in subjects with type 1 DM, although STZ-induced diabetes reduced HKII expression in rats (Frank & Fromm, 1986). Total HK activity was very similar in men with type 1 DM (unspecified HbA_{1c}) and non-diabetic men (Costill *et al.*, 1979). However, total HK activity also tended ($P<0.1$) to be lower (20%) in men with type 1 DM of longer duration who were in moderate control (HbA_{1c} 9.8%) than in non-diabetic men (Wallberg-Henriksson *et al.*, 1984), or was lower in men with type 1 DM than non-diabetics (Saltin *et al.*, 1979). Two other studies (Wallberg-Henriksson *et al.*, 1982; Mandroukas *et al.*, 1986) which included only a group with type 1 DM (in moderate to poor control, HbA_{1c} 10.5%) reported mean total HK activities of 2.6 and 0.8 $\mu mol \cdot min^{-1} \cdot g\ w.w.^{-1}$, which although 3-fold different, were similar to the range of values reported in non-diabetics (Costill

et al., 1979; Saltin *et al.*, 1979), highlighting the importance of comparing type 1 DM and non-diabetic groups within the same study.

2.16.6 Effects of sprint training upon glucose transport and phosphorylation during exercise

No studies have examined the effect of sprint training on GLUT4 or muscle glucose uptake during exercise in humans. Total HK activity at rest was increased 56% after 7 weeks of sprint training (MacDougall *et al.*, 1998), however in two other studies HK was not affected by 7-15 weeks of sprint training (Simoneau *et al.*, 1986; Linossier *et al.*, 1993). Five-second sprints were employed in one study (Linossier *et al.*, 1993), which may provide insufficient glucose flux for an increase in maximal HK activity. The other study (Simoneau *et al.*, 1986) utilized a combination of continuous (30 min at 70% heart rate reserve) and interval (15-30 s and 60-90 s at 60-70% of the maximum work rate) training, and given the increase in HK activity observed after endurance training (Costill *et al.*, 1979; Phillips *et al.*, 1996) it is somewhat surprising that HK activity was not increased in the former study. Given that HK activity may be increased after sprint training, the lack of effect of 8 weeks of sprint training on the increase in muscle glucose content during maximal exercise (Nevill *et al.*, 1989) may suggest that glucose transport was concomitantly increased, the inhibition of HK during exercise was attenuated (although this is unlikely given that the calculated glycogenolytic rate was greater after training), or that 30 s of exercise is too brief to allow discrimination of an effect. The effect of sprint training on HK activity during intense exercise has not been examined.

Higher muscle GLUT4 content is evident in skeletal muscle of the endurance-trained (Houmard *et al.*, 1991; Dela *et al.*, 1993; Ebeling *et al.*, 1993), and following 5 days to 14 weeks of endurance training (Houmard *et al.*, 1993; Phillips *et al.*, 1996; Cox *et al.*, 1999; Kristiansen *et al.*, 2000). However, when glycogen replete, both glucose uptake and GLUT4 translocation to the sarcolemma are reduced when exercising at the same absolute workload after endurance training (Richter *et al.*, 1998). In contrast, when exercising to fatigue with low resting muscle glycogen content, glucose uptake was higher in the trained than in the untrained leg following 3 weeks of one-legged endurance training (Kristiansen *et al.*, 2000).

2.16.7 Effects of sprint training upon glucose transport and phosphorylation in type 1 DM

No studies have investigated the effect of sprint training in type 1 DM. However, in the only study to examine GLUT4 and glucose uptake in trained subjects with type 1 DM, athletes with type 1 DM who trained both aerobically and anaerobically had similar GLUT4 mRNA and protein levels and similar basal and insulin-stimulated glucose uptake rates to sedentary subjects with type 1 DM (Ebeling *et al.*, 1995). These findings contrast with studies comparing athletic and sedentary non-diabetics (see above).

Endurance training for 8-10 weeks increased muscle total HK activity by 23-28% in subjects with either unspecified (Costill *et al.*, 1979) or moderately controlled type 1 DM (HbA_{1c} 9.8%) (Wallberg-Henriksson *et al.*, 1984), and either effected a similar increase (Costill *et al.*, 1979) or no change in non-diabetics (Wallberg-Henriksson *et al.*, 1984). In subjects with moderately to poorly controlled type 1 DM (mean HbA_{1c} 10.5%), total HK activity was either increased 1.5-fold after 10 weeks of endurance training (Mandroukas *et al.*, 1986) or unchanged after 16 weeks of jogging, games and gymnastics (Wallberg-Henriksson *et al.*, 1982).

In summary, the uptake of blood glucose into skeletal muscle is effected by glucose transporters. GLUT4 is translocated to the plasma membrane from distinct intracellular pools by insulin or exercise stimuli, whereas GLUT1 and GLUT5 are thought to be constitutively located in the membrane. At rest, lower intensity exercise, and under most conditions of glycaemia and insulinaemia, glucose transport is rate-limiting for glucose metabolism. The mechanism of translocation of GLUT4 via insulin stimulation may be impaired in subjects with type 1 DM, whereas the translocation induced by exercise may be intact, however neither has been investigated. Glucose uptake is similar in nondiabetics and in subjects with type 1 DM during moderate to intense exercise, regardless of insulinaemia. Similarly to GLUT4, HK II activity and mRNA are increased by each of insulin and non-exhausting, moderate intensity exercise. It is apparent that different regulatory mechanisms operate during high intensity exercise as compared to rest (or altered

conditions of glycaemia and/or insulinaemia) or lower intensity exercise. The phosphorylation of glucose by HK is rate-limiting for glucose entry into glycolysis under conditions of maximal exercise, since the accumulation of G 6-P may allosterically inhibit the activity of HK. Although the contribution of glucose to carbohydrate utilization during maximal exercise has been estimated to constitute only ~1%, the rate of carbohydrate utilization is reduced in subsequent bouts of intense exercise, and if HK activity is less inhibited (lower rate of G 6-P accumulation) glucose may contribute relatively more to ATP generation in later bouts. Resting HK activity may be increased by sprint training, and may be similar or lower than nondiabetics in subjects with type 1 DM. No studies have examined the effect of intense exercise on HK activity, or upon the effect of sprint training on such in either non-diabetics or in those with type 1 DM.

2.17 Muscle high-energy phosphates

2.17.1 Muscle cell ATP provision

Adenosine 5'-triphosphate (ATP) is required as a substrate by specialized ATPases for three principal cellular processes, as recently reviewed (Green, 1995): the restoration/ maintenance of the membrane potential via activation of the sarcolemmal (and transverse-tubular) Na^+K^+-ATPase pump; activation of the myosin heavy chain by myosin ATPase in preparation for cross-bridge cycling; and for the re-sequestration of calcium (Ca^{2+}) from the cytoplasm to the sarcoplasmic reticulum (SR) by the SR Ca^{2+}-ATPase. The ATPases effect hydrolysis of ATP and in so doing release energy:

$$ATP^{4-} + H_2O \rightarrow ADP^{3-} + Pi^{2-} + H^+ + \text{work}$$

where ADP is adenosine 5'-diphosphate and Pi is inorganic phosphate. Muscle ATP concentration is determined by the relative rates of ATP synthesis and utilization, and is highly protected to prevent rigor. When the rates of synthesis and utilization are similar, e.g. rest or mild to moderate exercise, ATP concentration changes little and energy coupling is very tight (Hochachka & Matheson, 1992).

ATP requirements increase enormously during intense exercise, therefore rapid resynthesis is essential and is provided via:

i) the transfer of high-energy phosphate from phosphocreatine (PCr) to ADP in the near-equilibrium reaction catalysed by creatine kinase:

$$PCr^{2-} + ADP^{3-} + H^{+} \rightleftharpoons ATP^{4-} + \text{creatine}$$

ii) the transfer of high-energy phosphate from ADP to ATP in the near-equilibrium adenylate kinase reaction:

$$ADP^{3-} + ADP^{3-} \rightleftharpoons ATP^{4-} + AMP^{2-}$$

where AMP is adenosine 5'-monophosphate.

iii) substrate-level phosphorylation in glycolysis (from glucose) and glycogenolysis (from glycogen - glucosyl units). Figure 2.1 (modified from Newsholme & Leech, 1983) depicts the metabolic pathway of glycolysis/ glycogenolysis which occurs in the cytosol of the muscle cell, and also shows the initial step in the oxidation of pyruvate which occurs in the mitochondria. Bidirectional arrows indicate near-equilibrium reactions and unidirectional arrows indicate non-equilibrium reactions (enzyme names appear in smaller print adjacent to the arrows). The common entry point for glucose (derived from the liver and phosphorylated by hexokinase) and for glucosyl units (derived from muscle glycogen and phosphorylated by glycogen phosphorylase) is glucose 6-phosphate (G 6-P). Glycolysis from glucose is summarized as:

$$\text{glucose} + 2ADP^{3-} + 2Pi^{2-} \rightarrow 2\ \text{lactate}^{-} + 2H^{+} + 2ATP^{4-}$$

When glycogen is the substrate 3 ATP are generated per glucosyl unit (see Figure 2.1).

iv) and the coupling of electron transport with oxidative phosphorylation (Figures 2.2, 2.3). Acetyl CoA, formed from the oxidative decarboxylation of pyruvate (from glucose or glycogen), from the β–oxidation of fatty acids (from adipose tissue or intramuscular triglycerides), or from protein degradation, reacts with oxaloacetate in the matrix of the mitochondria, catalyzed by citrate synthase, and forms citrate which is then processed in the Krebs cycle (or tricarboxylic acid cycle) to yield the electron carriers reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$). The electron carriers react with ubiquinone (coenzyme Q) in the electron transfer chain in the inner mitochondrial membrane (Figure 2.3, modified from (Houston, 1995), and a series of oxidation-reduction reactions transfer electrons to oxygen and generate free energy. The latter is used to pump protons to the cytosolic side of the inner mitochondrial membrane, which generates an electrochemical gradient. The protons return down the gradient and release free energy that drives the phosphorylation of ADP to ATP. The oxidation of NADH results in 3 ATP being synthesised, assuming no uncoupling of the pump from ATP synthesis (whilst the oxidation of $FADH_2$ produces 2 ATP):

$$NADH + \tfrac{1}{2}O_2 + 3ADP^{3-} + 3Pi^{2-} + H^+ \rightarrow NAD^+ + 3ATP^{4-} + H_2O$$

The generation of ATP from the transfer of phosphate from PCr, the adenylate kinase reaction and glycolysis does not require oxygen and is termed 'anaerobic ATP production' (in which the contribution of the small ATP store is also included), and may be calculated (Spriet, 1992) as follows:

$$\text{Anaerobic ATP production} = \Delta PCr + 1.5(\Delta \text{lactate}) + 2(\Delta ATP - \Delta ADP)$$

The metabolism of pyruvate, fatty acids, and amino acids via the Krebs cycle, electron transport and oxidative phosphorylation pathways requires oxygen and therefore ATP is generated oxidatively or aerobically. Total ATP production comprises ATP derived from anaerobic and oxidative sources.

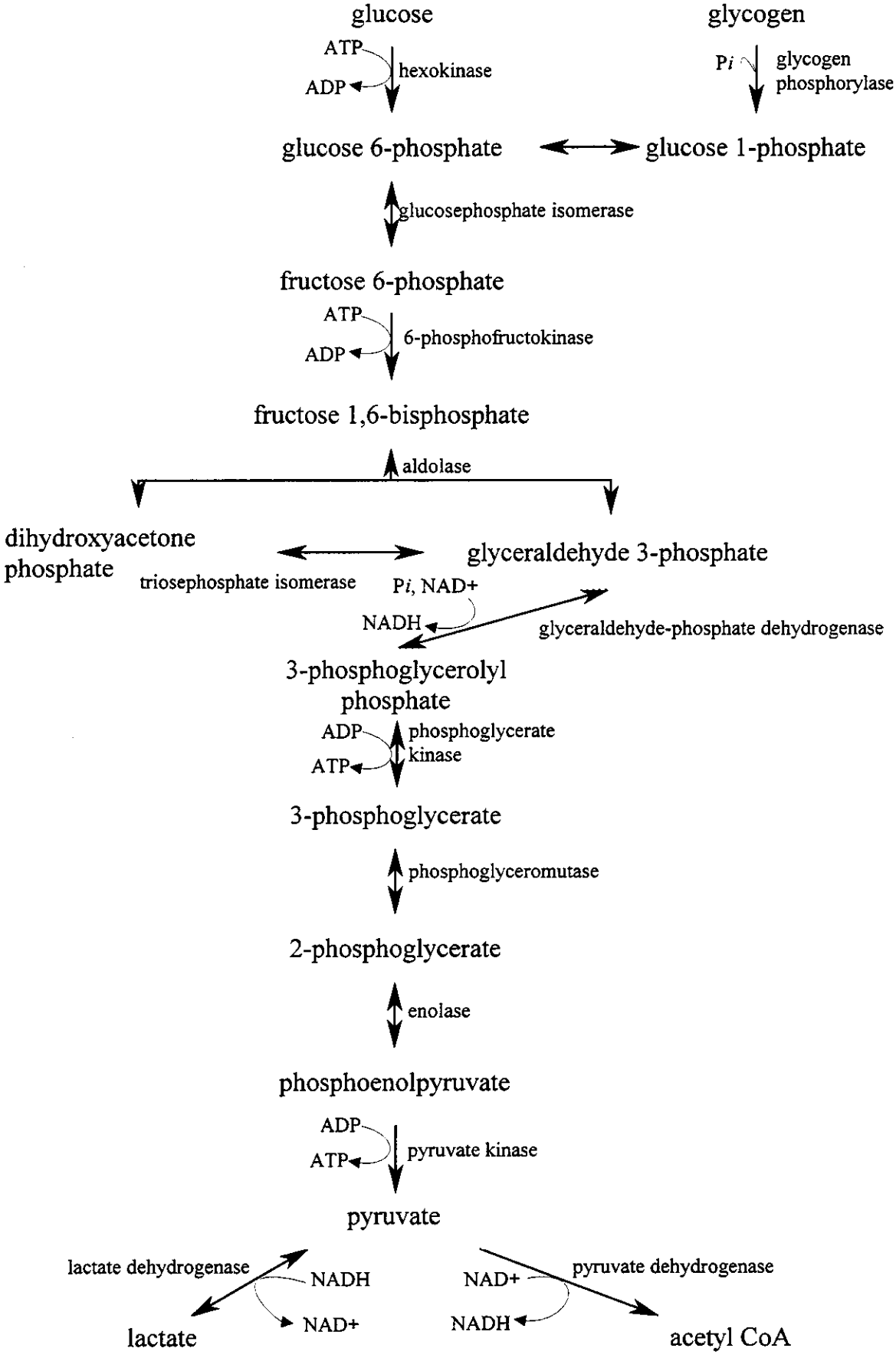


Figure 2.1 The metabolic pathway of glycolysis/ glycogenolysis (Modified from Newsholme & Leech, 1983)

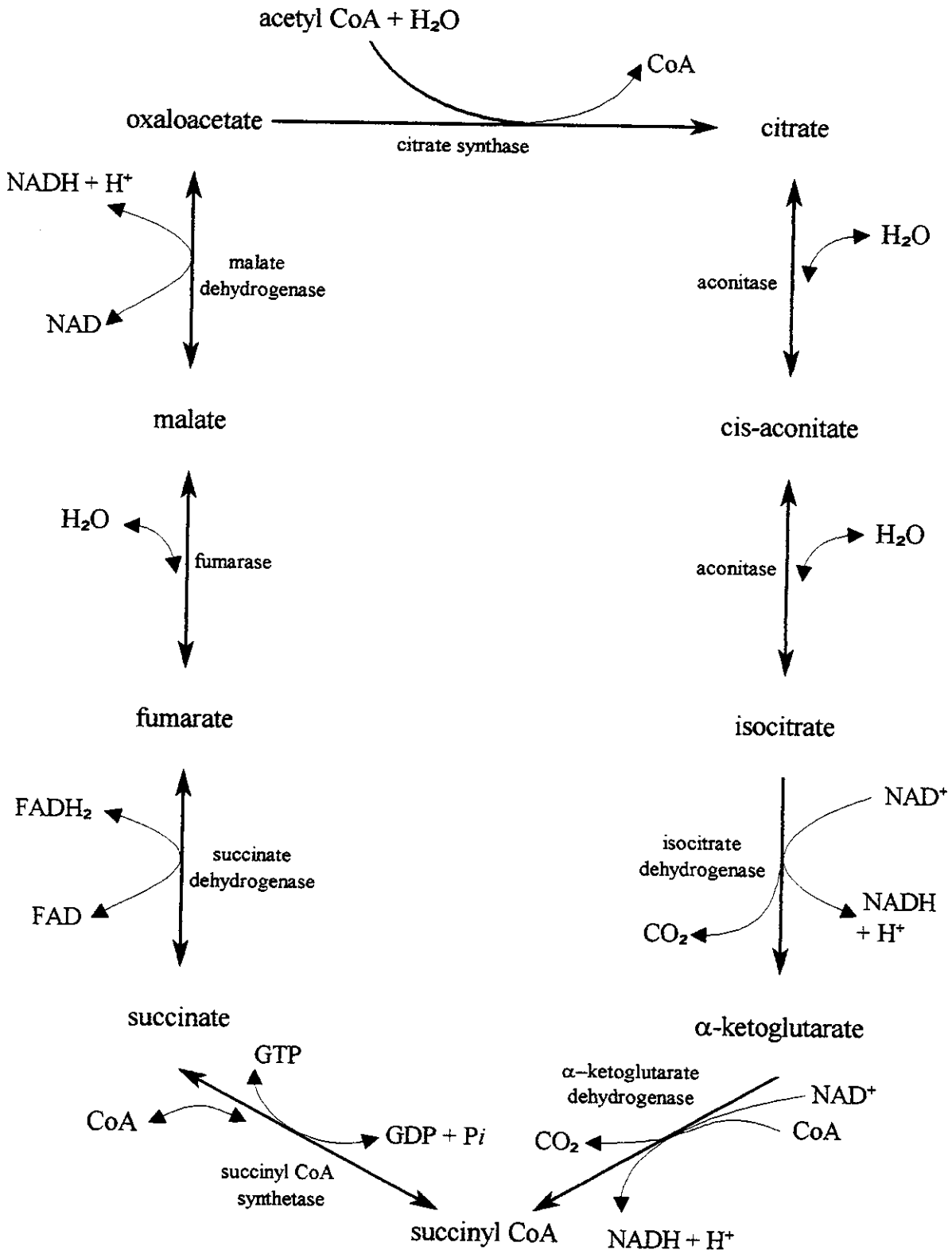


Figure 2.2 The tricarboxylic acid (or Krebs) cycle (modified from Houston, 1995)

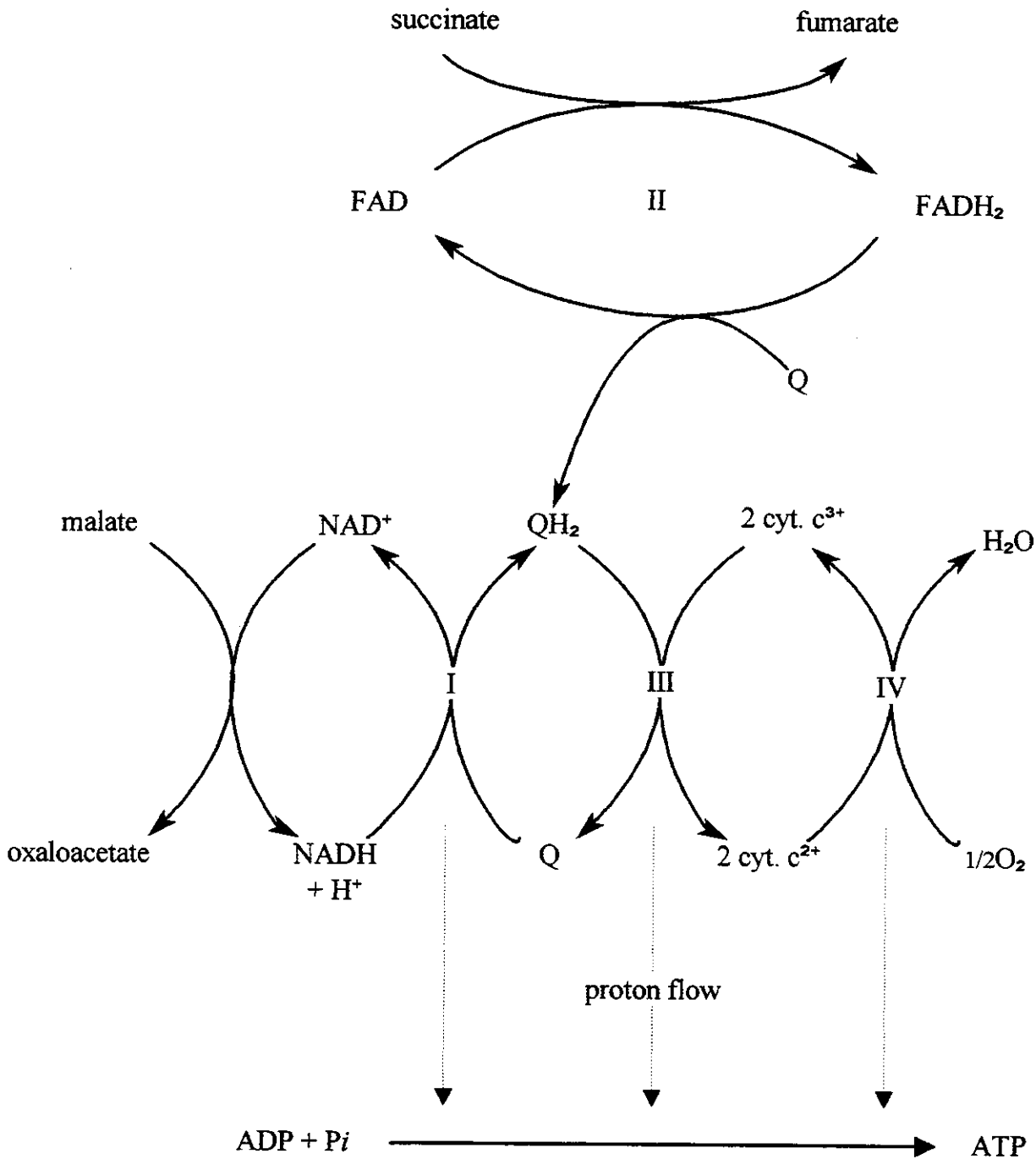


Figure 2.3 The electron transfer system (modified from Houston, 1995). The Roman numerals indicate electron transfer complexes, of which I, III and IV also generate proton flow, the free energy from which is subsequently used to phosphorylate ADP. Q is coenzyme Q (ubiquinone), QH_2 is reduced Q and cyt. c^{3+} and cyt. c^{2+} are reduced and oxidized cytochrome c, respectively.

2.17.2 High energy phosphate content at rest

In humans, ATP content at rest in mixed-fibre analysis of vastus lateralis varied between ~16.3 (Karlsson *et al.*, 1971) and 28.2 mmol·kg d.m.$^{-1}$ (Cheetham *et al.*, 1986), and averaged 22.6 mmol·kg d.m.$^{-1}$; whilst that of PCr ranged between 59.3 (Withers *et al.*, 1991) and 94.4 mmol·kg d.m.$^{-1}$ (Boobis *et al.*, 1983), and averaged 78 mmol·kg d.m.$^{-1}$ (Table 2.12). A large study (n = 81) similarly reported mean ATP and PCr contents of the vastus lateralis at rest to be 24.0 and 75.5 mmol·kg d.m.$^{-1}$, respectively (Harris *et al.*, 1974). ATP measured in vastus lateralis type I and type II fibres averaged 22.0-24.0 mmol·kg d.m.$^{-1}$ at rest, with no fibre type or gender differences evident, however PCr at rest was 5-11% higher in type II than type I fibres (Greenhaff *et al.*, 1994; Esbjörnsson-Liljedahl *et al.*, 1999) (Table 2.12).

2.17.3 High energy phosphate content at rest - effect of type 1 DM

Resting levels of skeletal muscle high-energy phosphates did not differ in subjects with type 1 diabetes mellitus (DM) on continuous subcutaneous insulin infusion (CSII) who had been either euglycaemic or hyperglycaemic for the preceding 24 hours (with same insulin dose, but either a saline or glucose infusion) (Vuorinen-Markkola *et al.*, 1992). The combined means (euglycaemia and hyperglycaemia) for resting ATP (19 mmol·kg d.m.$^{-1}$) and PCr (58 mmol·kg d.m.$^{-1}$) (Vuorinen-Markkola *et al.*, 1992) are at the lower end of the range evident in non-diabetic subjects (see Table 2.12). Similarly, resting ATP content did not differ between insulin-replete subjects with type 1 DM or nondiabetics (Yki-Järvinen *et al.*, 1990). Following an overnight fast, muscle PCr at rest did not differ between non-diabetics and subjects with moderately (HbA_{1c} 10.6%) (Yki-Järvinen *et al.*, 1990) or poorly controlled type 1 DM (HbA_{1c} 13.6%) (Cline *et al.*, 1997) who were rendered normoglycaemic prior to testing. A 3- to 5-hour hyperinsulinaemic clamp had no affect upon ATP or PCr contents in either those with or without type 1 DM (Yki-Järvinen *et al.*, 1990; Vuorinen-Markkola *et al.*, 1992). The effect of delaying the morning insulin dose on muscle ATP content in subjects with type 1 DM remains to be investigated in humans.

In rats, heart mitochondrial ATP concentration was reduced 28% after 40-48 hrs of alloxan-diabetes (Kerbey *et al.*, 1977). Hypoinsulinaemia, produced either by 7 days

Table 2.12 *High-energy phosphates in skeletal muscle at rest and during intense voluntary dynamic exercise; and the anaerobic ATP production rate.*

Study	Subjects	Exercise	ATP			PCr			An. ATP
			Rest	Ex.	Rate	Rest	Ex.	Rate	rate
(Howlett *et al.*, 1998)	4M,3F A	10min cycle 90% $\dot{V}O_{2peak}$:0-1 min	25.0	23.9	0.02	87.8	42.0	0.76	1.6†
		0-10 min		19.6	0.01		17.2	0.12	0.4†
(Odland *et al.*, 1998)	4M,4F A	10min cycle 90% $\dot{V}O_{2peak}$:0-1 min	25.2	24.1	0.02	88.7	42.7	0.77	1.6†
		0-10 min		19.8	0.01		18.2	0.12	0.4†
(Sutton *et al.*, 1981)	5M A	rest	20.6*			60.7			
		cycle 95% $\dot{V}O_{2peak}$, 4.6 min #	11.4	11.7	0	32.0	21.4	0.04	0.2†
(Sahlin *et al.*, 1989)	10M A	cycle 100% $\dot{V}O_{2peak}$, 4.8 min	26.1	20.2	0.02	83.1	17.3	0.23	0.8†
(Nevill *et al.*, 1989)	4M,4F A	2 min 110% $\dot{V}O_{2peak}$, non-exh	25.4	25.3	0.00	85.2	50.7	0.29	0.6†
(Karlsson *et al.*, 1971)	15M U	3-6 min max cycle	16.3*	11.2	0.02	72.7	13.3	0.22	0.6‡
	13F T	2-3 min max cycle	20.2*	12.0	0.05	62.4	12.5	0.33	1.4‡
(Bangsbo *et al.*, 1992a)	6M A	one-leg exh kicking, 3.7 min	26.4*	21.8	0.02	84.9	15.6	0.31	1.2†
(Graham *et al.*, 1990)	8M	one-leg exh kicking, 3.2 min	26.7*	17.6	0.04	96.0	34.4	0.32	-
(Bangsbo *et al.*, 1992b)	6M A	one-leg exh kicking, 2.8 min	20.4*	16.0	0.03	65.0	20.2	0.27	1.5
(Karlsson & Saltin, 1970)	3M T	exh cycle, 2.4 min	17.2*	12.0	0.04	67.2	10.3	0.40	1.1†
(Essén *et al.*, 1977)	5M T	3 min I/T max cycle	19.4*	~16	0.02	~77	~32	0.25	2.4†
(Medbø & Tabata, 1993)	16M A;9	exh cycle, 154s	18.5*	15.9	0.02	81.3	25.8	0.36	1.6
	8	exh cycle, 70s		15.5	0.04		32.7	0.69	3.4
	7	exh cycle, 34.4s		15.9	0.08		42.6	1.13	4.9
(Withers *et al.*, 1991)	6M T	90s max cycle	19.0*	12.2	0.08	59.3	15.2	0.49	2.3†
		60s max cycle	21.5*	12.1	0.16	66.7	14.5	0.87	3.7†
		30s max cycle	18.3*	11.7	0.22	58.1	18.9	1.31	5.4†

Table 2.12 (cont.): *High-energy phosphates in skeletal muscle at rest and during intense voluntary dynamic exercise; and the anaerobic ATP production rate*

Study	Subjects	Exercise	ATP			PCr			An. ATP
			Rest	Ex.	Rate	Rest	Ex.	Rate	rate
(Denis *et al.*, 1992)	8 ST	45s max cycle	23.0	14.1	0.20	88	35	1.18	5.6†
	8 MD		20.7	15.4	0.12	81	50	0.69	3.8†
(Boobis *et al.*, 1982)	4	30s max cycle	24.4	13.7	0.36	84.3	28.8	1.85	6.6†
(Jacobs *et al.*, 1982)	9F A	30s max cycle	20.9	13.8	0.24	62.7	25.1	1.25	4.3†
(Boobis *et al.*, 1983)	7M	30s max sprint	21.2	12.2	0.30	94.4	33.1	2.04	7.2†
(Jones *et al.*, 1985)	5M	30s max cycle – 60 rpm	21.1*	13.3	0.26	69.7	27.5	1.41	8.2†
		30s max cycle – 140 rpm	18.9*	18.9	0	64.1	42.6	0.72	6.5†
(Cheetham *et al.*, 1986)	8F T	30s max t/m sprint	28.2	17.9	0.34	87.7	31.2	1.88	6.1
(Boobis *et al.*, 1987)	4M,4F	30s max t/m sprint	26.5	19.2	0.24	85.1	28	1.90	6.6†
(Nevill *et al.*, 1989)	4M,4F A	30s max t/m sprint	26.7	19.2	0.25	84.0	28.0	1.87	6.7†
(Greenhaff *et al.*, 1994)	5M,1F A	30s max t/m sprint	M25.7	22.4	0.11	81.2	21.1	2.00	6.6†
			I 24.0	20.6	0.11	71.3	12.2	1.97	-
			II 24.0	19.0	0.17	79.3	5.0	2.48	-
(Stathis *et al.*, 1994)	6M,2F A	30s max cycle	22.8	13.6	0.31	85.4	27.6	1.93	6.8†
(Bogdanis *et al.*, 1995)	8M T	30s max cycle	25.6	18.1	0.25	77.1	15.1	2.07	8.5
(Esbjörnsson-Liljedahl *et*	20M,19F	30s max cycle	I 22.5	18.5	0.13	75	16	1.97	5.5†
al., 1999)	A		II 22.0	10.5	0.38	78.5	13	2.18	7.6†
(Bogdanis *et al.*, 1996)	8M A	1st bout of 2 x 30s max cycle:0-30s	27	19.6	0.25	75.2	12.6	2.09	7.8
		2nd bout of 2 x 30s max cycle:0-10s	22.2	19.7	0.25	58.5	15.3	4.32	9.8
		2nd bout: 0-30s		20.5	0.06		8.8	1.66	4.6
(Putman *et al.*, 1995a)	7M	1st bout of 3 x 30s max cycle	22.4	23.1	0	75.9	39.7	1.21	5.4†
		2nd bout	21.7	18.5	0.11	66.7	27.8	1.30	5.8†
		3rd bout	16.1	15.9	0.01	61.1	33.2	0.93	2.4†
(Hargreaves *et al.*, 1998)	6M A	1st bout of 3 x 30s max cycle: rest	25.6	-		90.6	-		-
		3rd bout: rest	14.0	-		55.6	-		-

Table 2.12 (cont.): *High-energy phosphates in skeletal muscle at rest and during intense voluntary dynamic exercise; and the anaerobic ATP production rate*

Study	Subjects	Exercise	ATP			PCr			An. ATP
			Rest	Ex.	Rate	Rest	Ex.	Rate	rate
(Parolin *et al.*, 1999)	6M	1st bout of 3 x 30s max cycle:0-6s	20.8	22.4	0	88.2	46.2	7.00	13.2
		0-15s		20.4	0.03		28.2	4.00	9.8†
		0-30s		16.2	0.15		7.6	2.69	6.5†
		3rd bout of 3 x 30s max cycle:0-6s	17.6	16.4	0.2	69.7	25.7	7.33	9.0†
		0-15s		18.1	0		14.9	3.65	3.8†
		0-30s		17.8	0		9.8	2.00	2.5†
(Trump *et al.*, 1996)	7M A	3 x 30s max cycle-3rd bout	20.7	19.8	0.03	63.0	15.5	1.58	2.7†
		occluded circ pre 3rd bout	18.9	19.4	0	20.7	17.6	0.10	0.7†
(McCartney *et al.*, 1986)	8M U	1st bout of 4 x 30s max cycle	22.4*	13.5	0.30	61.3	18.2	1.44	7.9†
		2nd bout		12.6			5.2		-
		3rd bout		12.8			7.3		-
		4th bout		13.9			2.6		-
(Hirvonen *et al.*, 1987)	5M ST	100m sprint (~11s)	22.4*	15.9	0.59	44.9§	15.1	2.71	7.5†
		80m sprint (~8.5s)	24.1*	17.8	0.74	48.6§	17.0	3.72	9.0†
		60m sprint (~6s)	22.4*	15.9	1.08	45.2§	20.2	4.17	9.7†
(Howlett *et al.*, 1999b)	7M,3F A	10s max cycle	22.4	17.0	0.54	93.7	40.2	5.35	13.6
(Boobis *et al.*, 1982)	4	6s max cycle	24.4	22.2	0.37	84.3	54.8	4.91	10.4†
(Gaitanos *et al.*, 1993)	8M A	1st bout of 10 x 6s max cycle	24.0	20.9	0.52	76.5	32.9	7.38	14.9
		10th bout	16.4	16.4	0.00	37.5	12.2	4.22	5.3

Studies arranged according to increasing exercise intensity; ATP, adenosine 5'-triphosphate; PCr, creatine phosphate; ATP and PCr contents expressed as mmol·kg d.m.$^{-1}$, and rates expressed as mmol·kg d.m.$^{-1}$·s^{-1}; Ex., exercise; An. ATP rate, anaerobic ATP production rate, expressed as mmol·kg d.m.$^{-1}$·s^{-1}; M, male subjects; F, female subjects; A, active; †, calculated from reported values of ATP, PCr, lactate (and pyruvate or adenosine 5'-diphosphate, ADP, if given), and exclusive of correction for lactate release; #, exercise at 95% preceded by 20 min exercise at each of 33 and 66% $\dot{V}O_{2peak}$;*, all metabolite values in paper converted from wet to dry weight by multiplication by 4.3; non-exh, non-exausting exercise; ‡, mean exercise time used (i.e. 2.5 and 4.5 min); U, untrained; T, trained; max, maximal; exh, exhausting exercise; I/T, intermittent; rpm, revolutions per minute; ST, sprinters; MD, middle distance runners; t/m, treadmill; M, mixed fibre analysis; I, type I fibres; II, type II fibres; circ, circulation; § PCr values measured after a 60-min warm up; actual resting PCr content was 93.3 mmol·kg d.m.$^{-1}$.

of STZ-induced diabetes or 48 hours of fasting, reduced soleus ATP content by ~20% and 8%, respectively, and increased intracellular Na^+ concentration by ~30% and 25%, respectively, in rats (Moore *et al.*, 1983). Reduction in ATP content only occurred after the elevation of intracellular Na^+ (Moore *et al.*, 1983). The mechanism of such a reduction has been thought to be due to reduced Na^+-H^+ exchange (and secondarily to reduced Na^+,K^+-ATPase), which may result in cellular acidosis and thence inhibition of ATP production via glycolysis (Moore *et al.*, 1983). Twice-daily insulin treatment gradually reversed the fall in muscle ATP, so that by the sixteenth day of treatment ATP content had returned to control levels (Moore *et al.*, 1983). Consistent with Moore *et al.* (1983), in the perfused hindquarter ATP and PCr at rest did not differ between non-diabetic rats (either fed or starved) and rats with 72-hour-untreated STZ-diabetes (Hagg *et al.*, 1976).

2.17.4 Energy coupling in intense exercise

During intense exercise ATP utilization exceeds synthesis, hence energy coupling is loose and cellular ATP concentration will fall (Hochachka & Matheson, 1992). The percent imbalance in energy coupling may be estimated by the following relation (Hochachka & Matheson, 1992):

$$\% \text{ imbalance} = \frac{\Delta[\text{ATP}] \times 100}{\text{total ATP turnover}}$$

where Δ[ATP] is the change in muscle ATP concentration. Alternatively, inosine 5'-monophosphate (IMP), which is formed by the deamination of AMP by AMP deaminase in the following reaction:

$$AMP^{2-} + H_2O + H^+ \rightarrow IMP^{2-} + NH_4^+$$

(in which NH_4^+ is ammonium), accumulates approximately stoichiometrically with the net reduction in total adenine nucleotides (TAN = ATP + ADP + AMP) (largely reflective of a reduction in ATP) during intense exercise (Sahlin *et al.*, 1989), and may also be used to assess the degree to which ATP degradation exceeds ATP resynthesis (Sahlin *et al.*, 1989; Hochachka & Matheson, 1992). IMP may be re-

aminated to AMP, however the activity of the two enzymes required to perform such is greatly exceeded by the activity of AMP deaminase; hence IMP accumulates (Lowenstein, 1990), although a small proportion may be further degraded to inosine, hypoxanthine, xanthine and urate during intense exercise (Sahlin & Broberg, 1990).

Muscle PCr content was curvilinearly and negatively correlated with muscle IMP content after exercise at various intensities up to maximum (Sahlin *et al.*, 1989), closely correlated with muscle lactate after contraction, and thought to reflect the equilibrium state of the creatine kinase reaction (Sahlin *et al.*, 1975). The apparent equilibrium constant of the creatine kinase reaction (K_{CK}) was considerably increased by intense exercise (isometric) to fatigue in humans, with the ratios of creatine to PCr and ATP to ADP markedly increased and slightly decreased, respectively (Sahlin *et al.*, 1975). The major determinant of the change in equilibrium was thought to be muscle pH, which was linearly related to the log of the apparent K_{CK} (Sahlin *et al.*, 1975).

2.17.5 Muscle ATP and PCr contents and hydrolysis rates during a single bout of intense exercise

A single bout of intense exercise, ranging in intensity from 90-~250% $\dot{V}O_{2peak}$, resulted in progressive reduction throughout exercise in both net ATP and PCr contents in the vastus lateralis (Howlett *et al.*, 1998; Odland *et al.*, 1998; Parolin *et al.*, 1999) (Table 2.12). Exhausting exercise of 90 s duration or less generally induced somewhat greater absolute reduction in net ATP content (~30%) than exercise of 2-10 min duration (~20%); however, the ~70% reduction in PCr with exhausting exercise was very similar between different intensities (Table 2.12). These data are consistent with protection of the small muscle ATP store, but effective depletion of PCr evident within ~10-30 s of intense exercise (Hirvonen *et al.*, 1987; Withers *et al.*, 1991; Bogdanis *et al.*, 1996; Parolin *et al.*, 1999). The rates of ATP and PCr hydrolysis increased with increasing exercise intensity, with a maximum calculated rate for ATP of 1.08 mmol·kg d.m.$^{-1}$·s^{-1} during 6 s of exhausting sprinting (Hirvonen *et al.*, 1987), and a maximum rate of 7.38 mmol·kg d.m.$^{-1}$·s^{-1} for PCr during 6 s of maximal cycling (Gaitanos *et al.*, 1993). A 30-s maximal treadmill run or cycle sprint resulted in

statistically similar (albeit 43% higher) (Greenhaff *et al.*, 1994) or ~3-fold greater (Esbjörnsson-Liljedahl *et al.*, 1999) net ATP degradation in type II (~52%) than type I muscle fibres; and a 1.1-1.3-fold greater net reduction in PCr content in type II (83-94%) than type I fibres (Table 2.12), with no difference between men and women.

2.17.6 Muscle ATP and PCr contents during intense intermittent exercise

When intense intermittent exercise (30 s exercise, 4 min recovery or 6 s exercise, 30 s recovery) was undertaken, muscle ATP (Gaitanos *et al.*, 1993; Putman *et al.*, 1995a; Bogdanis *et al.*, 1996; Hargreaves *et al.*, 1998) and PCr contents (Gaitanos *et al.*, 1993; Bogdanis *et al.*, 1996; Hargreaves *et al.*, 1998; Parolin *et al.*, 1999) were significantly reduced prior to commencing the second, third, or tenth exercise bout, due to incomplete resynthesis during recovery (Table 2.12). The mean half-time for resynthesis of PCr after a maximal 30-s cycle sprint was ~57 s, with 85% recovery evident after 6 min, and a predicted average time of 13.6 min (range 3.9-25 min) for resynthesis to 95% of the resting value (Bogdanis *et al.*, 1995). Circulatory occlusion applied immediately after the completion of two 30-s bouts of maximal cycling prevented the partial restoration of muscle PCr content during the 4 min recovery that preceded the third cycling bout (Trump *et al.*, 1996) (Table 2.12). This supported the earlier reports in which it was suggested that the early phase of PCr resynthesis was dependent upon an intact circulation (Sahlin *et al.*, 1975), which permitted ATP resynthesis via oxidative phosphorylation (Sahlin *et al.*, 1979).

2.17.7 ATP and PCr contribution to total anaerobic ATP production during intense exercise

During the first of ten 6-s maximal sprints, PCr degradation and muscle ATP stores provided ~50 and ~6%, respectively, of the total anaerobic ATP production, whereas in the final sprint, although absolute ATP production from anaerobic metabolism was ~66% lower, ~80% of the total was derived from PCr and 4% from ATP (Gaitanos *et al.*, 1993). Since power output only declined 27% by the tenth sprint, it was suggested that a significant shift to aerobic metabolism occurred during the later 6-s sprints (Gaitanos *et al.*, 1993). In the first 6 s of an exhausting 30-s cycle sprint 53% of the total anaerobic ATP production was provided by PCr breakdown (44% of total ATP production), whilst in the last 15 s of the bout PCr provided ~40% of anaerobic

ATP and ~20% of total ATP production (Parolin *et al.*, 1999). Averaged over the entire 30-s bout, ATP stores contributed ~6% (Bogdanis *et al.*, 1996) and PCr degradation accounted for 22-27% of anaerobic ATP production (Putman *et al.*, 1995a; Bogdanis *et al.*, 1996); and 16% of total ATP production was attributable to PCr (Putman *et al.*, 1995a). In a second 30-s sprint bout in which power output was 17-18% lower, anaerobic ATP production was not changed (Putman *et al.*, 1995a) or reduced 41% (Bogdanis *et al.*, 1996). The absolute contribution of PCr degradation to anaerobic ATP production in the second sprint was unchanged in one study (Putman *et al.*, 1995a), but reduced 23% in another (Bogdanis *et al.*, 1996); whilst the relative proportion of anaerobically-generated ATP attributable to PCr degradation was similar in the first and second bouts (Putman *et al.*, 1995a; Bogdanis *et al.*, 1996) and ATP stores contributed less than 1% (Bogdanis *et al.*, 1996).

2.17.8 Effect of intense exercise on ATP and PCr contents in type 1 DM

No studies have examined the effect of intense exhausting exercise on high-energy phosphates in skeletal muscle in subjects with type 1 diabetes mellitus. Neither resting content nor the reduction of muscle ATP and PCr induced by electrical stimulation of the perfused hindquarter differed between non-diabetic rats and rats with 72 hours of STZ-diabetes (Hagg *et al.*, 1976).

2.17.9 Effects of sprint training on muscle ATP and PCr contents at rest

ATP content in the vastus lateralis at rest was reduced 13-24% after 2 days to 7 weeks of sprint training (Green *et al.*, 1987b; Hellsten-Westing *et al.*, 1993; Stathis *et al.*, 1994); although other studies found no change after sprint training (Thorstensson *et al.*, 1975; Boobis *et al.*, 1983; Boobis *et al.*, 1987; Nevill *et al.*, 1989) (Table 2.13). One study reported a small reduction in resting PCr content after sprint training (Boobis *et al.*, 1983), however all other studies reported no change (Table 2.13). In a cross-sectional study, resting concentrations of ATP or PCr did not differ between well-trained sprinters and middle-distance runners (Denis *et al.*, 1992), but tended to be higher in sprinters than long-distance runners (Rehunen *et al.*, 1982). Lower resting ATP after sprint training was suggested to be due to depletion of the adenine nucleotide pool as a consequence of repeated severe metabolic stress (Green *et al.*, 1987b), supported by the finding of increased oxypurines and uric acid in the

Table 2.13 *Effects of sprint training upon muscle high-energy phosphate content at rest and during intense exercise*

Study	Training					Test	Effect of training			
							ATP		PCr	
	Dur.	*f*	no.	Int.	E:R		rest	Δex	rest	Δex
(Green *et al.*, 1987b)	2-3 d	1/d	12-24	120%	60s: 4min	training session	20%↓	54%↓	↔	↔
(Hellsten-Westing *et*	1 wk	2/d	15	max	10s: 50s	rest	24%↓	-	↔	-
al., 1993)	6 wk	3/wk	15	max	10s: 50s	rest	13%↓	-	↔	-
(Stathis *et al.*, 1994)	7 wk	3/wk	3-10	max	30s: 3-4min	30s max cycle	19%↓	52%↓	↔	↔
(Linossier *et al.*, 1993)	7 wk	4/wk	2(8-13)	max	2(5s: 55s) 15min b/w	2(8(5s:55s))	↔	↔	↔	↔
(Thorstensson *et al.*, 1975)	8 wk	3-4/wk	20-40	max	5s: 25-55s	rest	↔	-	↔	-
(Boobis *et al.*, 1983)	8 wk	5/wk	NR	NR	NR	30s max	↔	↔	8%↓	↔
(Boobis *et al.*, 1987)	8 wk	NR	NR	NR	NR	30s max run	↔	↔	↔	↔
(Sharp *et al.*, 1986)	8 wk	4/wk	8	max	30s: 4min	Incr. to exhn	-	-	↔	↔
(Nevill *et al.*, 1989)	8 wk	3-4/wk	2; 6-10; 2-5	max; max; 110%	30s: 10min; 6s:54s; 2min: 5min	30s max run	↔	↔	↔	↔
						2min 110% run	↔	↔	↔	↔

Dur., duration; *f*, frequency; no., number; Int., intensity; E:R, exercise to rest ratio; Δex, change in content with exercise; d, day; s, seconds; min, minutes; max, maximal; wk, week; 2(8-13), 2 sets of 8-13 repetitions; b/w, between (time between sets); NR, not reported; Incr., incremental; exhn, exhaustion.

circulation after strenuous exercise (Sutton *et al.*, 1980). Maximal or fatiguing submaximal exercise increased muscle ammonia (NH_3) and IMP, and increased plasma NH_3 (Broberg & Sahlin, 1989; Graham *et al.*, 1990; Bangsbo *et al.*, 1992a; Stathis *et al.*, 1994) and hypoxanthine (Stathis *et al.*, 1994), further supporting the suggestion of Green *et al.* (1987). Further, the slow resynthesis of ATP via *de novo* pathways (animal data) was suggested to account for the reduced ATP measured prior to commencing exercise on the third consecutive day of exhausting intermittent sprint training (Green *et al.*, 1987b). Reduced resting ATP concentration was also evident at 3, 24, and 48-72 hours after the cessation of 7 days of intense, twice-daily training and 6 (Hellsten-Westing *et al.*, 1993) to 7 weeks (Stathis *et al.*, 1994) of thrice-weekly sprint training; all of which resulted in no change in total creatine (TCr = PCr + creatine). However, since ATP had not been restored to pre-training resting levels by 48-72 hours, it was suggested (Stathis *et al.*, 1994) that either a downregulation had occurred as a consequence of training, or that *de novo* synthesis was a more protracted process in human muscle than that reported (Tullson *et al.*, 1988; Tullson & Terjung, 1991) in animal muscle. The lack of training effect on resting ATP content in other studies (Table 2.13) was thought to have been due to the use of less intensive sprint training regimens or shorter sprint duration combined with longer rest periods between efforts (Hellsten-Westing *et al.*, 1993; Stathis *et al.*, 1994); all of which would act to reduce the absolute loss of adenine nucleotides from the exercising muscle. In terms of cross-sectional comparisons, the similar resting ATP content in muscle of sprinters and middle-distance runners (Denis *et al.*, 1992) may reflect the incorporation of common training factors, or may suggest that a protracted period of sprint training is required to allow the muscle to more fully recover from, or limit, adenine nucleotide loss.

2.17.10 Effect of lowered resting ATP content on fibre type and cost of muscle contraction?

In rats, resting ATP content was reduced by 40% (and PCr by 87%) after 9 weeks of feeding with β–guanidinopropionate (β-GPA), a creatine analogue (Foley *et al.*, 1994).

Treatment with β-GPA also resulted in a change in myosin heavy chain distribution in the superficial gastrocnemius, with increased type IIA and reduced type IIB fibres

(Foley *et al.*, 1994). Interestingly, this shift towards more IIA and less IIB fibres also occurs with sprint training (Table 2.14).

Table 2.14 *Changes in fibre type with sprint training*

Study	Training duration	Fibre type change I	IIA	IIB
(Jansson *et al.*, 1990)	4-6 weeks	↓9%	↑6%	
(Allemeier *et al.*, 1994)	6 weeks	↓3% n.s.‡	↑9%‡	↓6%‡
(Allemeier *et al.*, 1994)	6 weeks	↔	↔	↔
(Jacobs *et al.*, 1987)	6 weeks	↓9%	↑7%	
(Esbjörnsson *et al.*, 1993)	7 weeks	↓7%	↑12%	↓6%
(Lynch *et al.*, 1994)	7 weeks	↓15%†		
(Linossier *et al.*, 1993)	7 weeks	↑8%	↓9%	
(Thorstensson *et al.*, 1975)	8 weeks	↔	↔	↔
(Andersen *et al.*, 1994)	3 months*	↓7%	↑16%	↓8%
(Andersen *et al.*, 1994)	3 months*	↓11%‡	↑18%‡	

Fibre typing based on myosin ATPase staining, except where otherwise specified; n.s. non-significant; ‡, myosin heavy chain analysis using SDS-PAGE; †, fibres typed according to force-pCa and force-pSr characteristics. *, Andersen *et al.* (1994) used a combined programme of intensified sprint and strength training in trained sprinters.

Gradual ATP depletion with a β-GPA diet had no affect upon peak isometric or twitch tension, or the ATP cost of muscle contraction (twitch or tetanus) as assessed by gated ^{31}P-nuclear magnetic resonance (Foley *et al.*, 1994). However, acute depletion of ATP by 46%, induced by tetanic stimulation and maintained for 75 min by administration of hadacidin (inhibits IMP reamination), was associated with a 39% reduction in ATP cost of contraction (twitch), with no change in peak twitch tension (Foley *et al.*, 1994). It was concluded that ATP depletion *per se* was not responsible for the enhanced economy of contraction (Foley *et al.*, 1994). In humans, acute reduction of resting ATP content (by 15%) by three prior 30-s, 'all out' exercise bouts (followed by a prolonged recovery period) did not alter peak power and total work in a fourth bout when compared with the first bout (Hargreaves *et al.*, 1998), further suggesting that reduced resting ATP content *per se* does not affect performance.

2.17.11 Effects of sprint training on ATP degradation during intense exercise

The net degradation of ATP during intense exhaustive exercise was markedly and similarly reduced after either 3 days or 7 weeks of sprint training (Green *et al.*, 1987b;

Stathis *et al.*, 1994) (Table 2.13), and occurred despite the production of more work in the maximal cycle test after training (Stathis *et al.*, 1994). In further support, a cross-sectional study found that 3 x 300m exhausting sprints significantly reduced muscle ATP concentration in long-distance runners, but not in sprinters (Rehunen *et al.*, 1982). Less percent imbalance (Hochachka & Matheson, 1992), demonstrated by reduced net ATP degradation and markedly attenuated IMP accumulation, confirmed that ATP resynthesis and hydrolysis were more closely matched during exhausting exercise after sprint training (Stathis *et al.*, 1994). Whilst the mechanisms resulting in tighter energy coupling remain unclear, it was suggested that training may have enhanced the glycolytic capacity (Stathis *et al.*, 1994). However, several studies (Table 2.13) in which the anaerobic ATP production rate was increased after sprint training (Boobis *et al.*, 1987; Nevill *et al.*, 1989; Linossier *et al.*, 1993) failed to find attenuated net degradation of ATP during intense exercise: however more work was performed during exercise after training and thus ATP degradation was reduced relative to work. Another possibility is that the shift towards a more oxidative fast-twitch fibre pool with sprint training (see Table 2.14) may allow tighter energy coupling and reduce IMP accumulation. The notion that sprint training may enhance muscle oxidative metabolism (see section 2.20) is consistent with tighter energy coupling after training, although this has been little investigated.

In the only study to include a test in which pre- and post-training work was matched (2-min treadmill run at 110% pre-training $\dot{V}O_{2max}$), ATP content did not change during exercise either before or after sprint training (Nevill *et al.*, 1989) (Table 2.13). The pre-training test was not conducted to exhaustion, evinced by only mild metabolic perturbation, and thus an effect of sprint training may have been difficult to detect. Based upon improved ATP regulation during exhausting exercise in other studies (Green *et al.*, 1987b; Stathis *et al.*, 1994), it is likely that improvement may be even more pronounced during matched-work exercise after sprint training; however this remains to be examined.

2.17.12 Effect of sprint training on PCr degradation during intense exercise

Whilst 5 days to 8 weeks of endurance training attenuated PCr degradation during submaximal exercise (Green *et al.*, 1992; Cadefau *et al.*, 1994; Green *et al.*, 1995), the degradation of PCr with intense exhausting exercise does not appear to be altered by 3 days to 8 weeks of sprint training (Table 2.13). Although trained sprinters had greater PCr degradation in type I fibres (but similar degradation in type II fibres) than long-distance runners during 300m sprints (Rehunen *et al.*, 1982), and 3 faster sprinters had lower mixed-fibre PCr concentration after maximal efforts over distances of 80 and 100 m than four slightly slower sprinters (Hirvonen *et al.*, 1987), neither study included untrained subjects, and thus interpretation of the effect of sprint training is difficult. It has been suggested that even in the absence of changes in PCr content and degradation during intense exercise after sprint training, that the rate of breakdown may be faster in the first few seconds of exercise (Nevill *et al.*, 1989), and that the capacity to do work was related to the ability to use the high-energy phosphate pool (Hirvonen *et al.*, 1987). Thermodynamics would dictate that the maximum rate of PCr breakdown would be reduced when PCr content is reduced and that PCr availability may therefore be a determinant of fatigue during intense exercise (Sahlin *et al.*, 1998). In support, a positive correlation was found between resting PCr and pedalling velocity: however only when resting PCr was relatively low (Linossier *et al.*, 1993). Consistent with this finding, elevated resting PCr, induced by creatine supplementation in a double-blind study, did not improve performance during repeated 10-s maximal sprint bouts and was not related to peak power or cumulative work during exercise (McKenna *et al.*, 1999); although the contribution of PCr to ATP provision during exercise was not measured.

2.17.13 Effects of sprint training on ATP and PCr contents at rest and during exercise in type 1 DM

No studies have investigated the effect of sprint training in subjects with type 1 DM.

In summary, in non-diabetic subjects, intense exercise induces a near complete depletion of muscle PCr content, and up to 40% reduction in net ATP content (up to 50% reduction in type II fibres). In maximal exercise of 6-30 s duration, PCr degradation contributes between ~25-50% of the total anaerobic ATP production.

The greatest rates of ATP and PCr degradation occur during the most intense exercise. ATP content in resting muscle was reduced after sprint training programmes which utilized repeated 10- to 60-s maximal exercise bouts, with exercise: recovery ratios of 1:4 to 1:8. These regimens also resulted in reduced net ATP degradation during exhausting exercise after training. Trained sprinters may also show enhanced ATP regulation during maximal sprinting. Sprint training programmes employing briefer sprints, fewer repetitions, and longer recovery intervals induced no significant change in resting ATP content and no attenuation of net ATP degradation during intense exercise. PCr degradation during intense exhausting exercise does not appear to be altered by sprint training of 3 days to 8 weeks' duration, although may be increased in trained sprinters. The effect of sprint training on ATP and PCr degradation when work is matched before and after training has been little investigated, however based upon the results obtained after exhausting exercise, a marked attenuation of the former may be anticipated. Only three studies have reported resting content of high-energy phosphates in subjects with type 1 DM. Values were at the lower end of the range for non-diabetic subjects in one study, but were similar to non-diabetic groups in the other two studies. The effect of intense exercise or exercise training on high-energy phosphate degradation in subjects with type 1 DM has not been reported.

2.18 Glycolysis and glycogenolysis

2.18.1 Rest

The metabolic pathway of glycolysis-from-glucose spans several tissues since the flux-generating step is either the breakdown of liver glycogen or the absorption of glucose from the intestine (Newsholme & Leech, 1983). In the basal state BGL declines by ~1% per hour, i.e. whole-body glucose uptake exceeds whole-body glucose production, with skeletal muscle contributing ~20% to uptake (Zierler, 1999). At rest the rate of glycolysis in human skeletal muscle is ~0.05 $\mu mol \cdot min^{-1} \cdot g$ w.w.$^{-1}$ (Newsholme & Leech, 1983).

The flux-generating and rate-limiting enzyme for glycogenolysis is glycogen phosphorylase (GP) which exists in two interconvertible forms: the more active GP*a*

and less active GP*b*, as recently reviewed (Connett & Sahlin, 1996). GP has a K_m of 1 mM and is thus saturated with its substrate (glycogen) at rest (Newsholme & Leech, 1983). The glycolytic or glycogenolytic pathway proceeds via various intermediates to form pyruvate, which is either reduced to lactate by the near equilibrium enzyme lactate dehydrogenase (LDH) or oxidized to acetyl CoA via the non-equilibrium pyruvate dehydrogenase (PDH) enzyme complex (see section 2.19).

2.18.2 Effect of type 1 DM on glycolytic/ glycogenolytic substrates, intermediates, and products at rest

2.18.2.1 Glucose

Muscle glucose concentration was similar to non-diabetic values (Harris *et al.*, 1974; Katz *et al.*, 1986) in subjects with type 1 DM on continuous subcutaneous insulin infusion (CSII) who underwent either 24 hours of euglycaemia (7 $mmol \cdot l^{-1}$) or hyperglycaemia (20 $mmol \cdot l^{-1}$) (Table 2.15), produced by the same insulin dosage combined with either a saline or glucose infusion (Vuorinen-Markkola *et al.*, 1992). In contrast, after 24 hours of insulin withdrawal, which resulted in a BGL of 18.3 $mmol \cdot l^{-1}$, muscle glucose was extremely high (Table 2.15), consistent with lower HK activity (Saltin *et al.*, 1979).

2.18.2.2 Glycogen

Insulin stimulates glycogen synthase (GS; or UDPG-glycogen transglucosylase) in skeletal muscle, by promoting the conversion of the inactive to the active form (Villar-Palasi & Larner, 1960; Villar-Palasi & Larner, 1968), and promotes the accumulation of glycogen when stores are low, however has little effect on the rate of synthesis when glycogen content is high (Newsholme & Leech, 1983). However, during a hyperinsulinaemic euglycaemic clamp, percent active GS and the increment in muscle glycogen was lower in subjects with type 1 DM (either on CSII or insulin injections) than in non-diabetics, despite overnight euglycaemia, and normal pre-clamp GS activity and glycogen content in the former; thus demonstrating an effect of insulin resistance on glycogen accumulation in type 1 DM (Kruszynska *et al.*, 1986).

Muscle glycogen content in the postabsorptive state did not differ between subjects with type 1 DM who were maintained at euglycaemia with an overnight insulin

Table 2.15 *Resting values for muscle glycolytic/ glycogenolytic substrates, intermediates, and products in subjects with type 1 diabetes mellitus (and, when included in the same study, non-diabetic subjects)*

Study	Subjects	Condition	Glucose	Glycogen	G 6-P	Pyruvate	Lactate
(Hildes *et al.*, 1949)	11M,4F,NP	NR		525†			
	4M,2F,D‡	I w/d ≥ 48h		379			
(Bergström *et al.*, 1963)	5M,1F,UtD	Pre-I		158†			
(Roch-Norlund *et al.*, 1970)	26,NP	O/n fast		334†			
	15M,9F,Ut D	O/n fast; pre-I		152*			
(Roch-Norlund, 1972)	21,N	O/n fast		310†			
	9,UtD	O/n fast; pre-I		169*			
(Roch-Norlund *et al.*, 1972)	6,N	O/n fast		320†			
	3,UtD	O/n fast; pre-I		200			
(Mæhlum *et al.*, 1977)	6M,N	O/n fast		329†	1.9		
	6M,D	O/n fast, I delayed		267*	1.7		
(Saltin *et al.*, 1979)	8M,D	I w/d 24h	17.2†	310	0.9		9.9
(Standl *et al.*, 1980)	10M,N	1h PP		373†			
	10M,D	I pre B/f, 1h PP		373			
	10M,D	I w/d 24h, 1h PP		235*			

Table 2.15 (cont.): *Resting values for muscle glycolytic/ glycogenolytic substrates, intermediates, and products in subjects with type 1 diabetes mellitus (and, when included in the same study, non-diabetic subjects)*

Study	Subjects	Condition	Glucose	Glycogen	G 6-P	Pyruvate	Lactate
(Wallberg-Henriksson *et al.*, 1982)	9M,D	O/n fast, I delayed		353†			
(Kruszynska *et al.*, 1986)	5M,1F,N	O/n fast		199†			
	4M,1F,D	O/n fast, O/n i.v. I		199			
(Mandroukas *et al.*, 1986)	6M,3F,D	B/f, I		383†			
(Yki-Järvinen *et al.*, 1990)	10M,N	O/n fast	0.2	321	1.9		8.7
	13M,D	O/n fast, i.v. I	0.6	323	4.3		11.9
(Vuorinen-Markkola *et al.*, 1992)	8M,D	24h eugly., CSII	1.2	238	1.7		
		24h hypergly., CSII	0.5	234	2.0		
(Ebeling *et al.*, 1993)	12,D	O/n fast, I delayed		343			
(Cline *et al.*, 1997)	2M,8F,N	O/n fast			0.56§		
	4M,2F,D	O/n fast, O/n i.v. I			0.47		

Studies are arranged chronologically in the Table. All muscle metabolites expressed as mmol·kg d.m.$^{-1}$. Muscle samples were obtained from vastus lateralis in all studies except Hildes *et al.* (1949) in which samples were obtained from pectoralis major. G 6-P, glucose 6-phosphate; M, males; F, females; NP, non-diabetic patients hospitalized for another cause; D‡, most subjects in this study probably had type 2 DM; NR, not reported; I, insulin dose; w/d, withdrawn; †, all values converted to dry weight from wet weight (and from g·kg^{-1} w.w. or mg·g protein^{-1}); UtD, untreated patients with newly diagnosed type 1 DM; pre-I, biopsy taken prior to insulin treatment commencing; o/n, overnight; *, significantly lower than other group/s in same study; N, non-diabetic; D, type 1 diabetes mellitus; PP, postprandial; B/f, breakfast; O/n i.v. I, overnight intravenous insulin infusion to maintain euglycaemia; eugly., euglycaemia; CSII, continuous subcutaneous insulin infusion; hypergly., hyperglycaemia; §, values determined by NMR spectroscopy.

infusion, and non-diabetics (Kruszynska *et al.*, 1986) (Table 2.15). Muscle glycogen content was also similar when the same subjects with type 1 DM on CSII were rendered either euglycaemic or hyperglycaemic for the preceding 24 hours (Vuorinen-Markkola *et al.*, 1992). Mean glycogen content in vastus lateralis from subjects with type 1 DM, obtained after an overnight fast with the morning insulin dose delayed (Wallberg-Henriksson *et al.*, 1982; Ebeling *et al.*, 1995), and after breakfast and insulin (Mandroukas *et al.*, 1986), was comparable to mean values reported (Hultman, 1967; Karlsson *et al.*, 1971; Harris *et al.*, 1974) for non-diabetic subjects. Further, and somewhat surprisingly, even after 24 hours of insulin deprivation (BGL 18.3 $mmol \cdot l^{-1}$), muscle glycogen stores at rest in men with type 1 DM remained within normal limits for non-diabetic subjects (Saltin *et al.*, 1979). An early study (Hildes *et al.*, 1949), in which the pectoralis major was biopsied in 6 patients with DM who had not had insulin for at least 2 days (mean BGL 14 $mmol \cdot l^{-1}$) and in 15 non-diabetic hospitalized patients, demonstrated that although glycogen content was lower in the former group, it was well within the normal range for non-diabetic subjects and did not differ significantly (Table 2.15). However, although not specified, based on the clinical data most patients probably had type 2 DM.

However, in contrast, subjects with poorly controlled type 1 DM, studied after 24 hr insulin withdrawal (BGL 19 $mmol \cdot l^{-1}$), had ~40% lower muscle glycogen content than both non-diabetics and subjects with type 1 DM who were well controlled and had administered 80-90% of the morning insulin dose 75 min prior to biopsy (Standl *et al.*, 1980). Similarly, in another study in which subjects with type 1 DM were compared with non-diabetic subjects within the same study, muscle glycogen content was 19% lower after an overnight fast (BGL ~14 $mmol \cdot l^{-1}$) than in the non-diabetics (Mæhlum *et al.*, 1977). Patients with untreated type 1 DM (recently diagnosed, prior to commencing insulin treatment) had lower muscle glycogen content than non-diabetic subjects (Bergström *et al.*, 1963; Roch-Norlund *et al.*, 1970; Roch-Norlund, 1972; Roch-Norlund *et al.*, 1972) (Table 2.15). In patients with newly diagnosed type 1 DM who were re-biopsied after 1-16 days of insulin treatment, muscle glycogen had increased to be similar to values in non-diabetic subjects (Bergström *et al.*, 1963; Roch-Norlund *et al.*, 1970; Roch-Norlund, 1972).

As illustrated by the above, with regard to muscle glycogen content at rest, the state of metabolic control (i.e. presence or absence of adequate insulin) in subjects with type 1 DM is a prime determinant. However, since only one study (Ebeling *et al.*, 1995) specified both the exercise and dietary conditions for the 24 hours preceding testing it is also possible that differences in resting glycogen content between studies may be explained by different diets and levels of physical activity prior to testing. In addition, due to the wide range of normal values for muscle glycogen content (Hultman, 1967), the presence or absence of a non-diabetic group within the same study will also have bearing upon the interpretation of the results.

2.18.2.3 Glycolytic intermediates and products

As illustrated by Table 2.15, very few studies have examined glycolytic intermediates or products in subjects with type 1 DM. Glucose 1-phosphate (G 1-P) and fructose 6-phosphate (F 6-P) contents at rest in subjects with type 1 DM did not differ after 24 hours of either euglycaemia or hyperglycaemia (Vuorinen-Markkola *et al.*, 1992), and were similar to values reported for non-diabetic subjects (Harris *et al.*, 1974). Similarly, G 6-P content was unaffected by euglycaemia, hyperglycaemia, delaying the morning insulin dose, or 24 hours of insulin withdrawal (Table 2.15). In contrast, muscle lactate was ~2-fold higher than non-diabetic values after 24 hours insulin withdrawal (Saltin *et al.*, 1979), however did not differ from non-diabetic values when insulin replete (Yki-Järvinen *et al.*, 1990). Muscle lactate dehydrogenase (LDH) activity at rest was 40-50% higher in subjects with type 1 DM (24 hr insulin withdrawal or condition prior to biopsy not reported) than in non-diabetic subjects included in the same study (Saltin *et al.*, 1979; Wallberg-Henriksson *et al.*, 1984), and 0.5- to 2-fold higher (Wallberg-Henriksson *et al.*, 1982; Mandroukas *et al.*, 1986) than values in non-diabetics (Newsholme & Leech, 1983). It was suggested that higher LDH activity may have been a compensation for lower PDH activity (Saltin *et al.*, 1979; Wallberg-Henriksson *et al.*, 1984), although the latter was not measured. Each of the above studies (Table 2.15) was conducted at rest.

2.18.3 Effect of type 1 DM on glycolytic/ glycogenolytic rates at rest

Basal glycolytic rates (from plasma glucose), which averaged ~0.01 $\mu mol \cdot min^{-1} \cdot g$ body $weight^{-1}$, did not differ between subjects with type 1 DM (rendered euglycaemic

prior to testing by an insulin infusion) and non-diabetic subjects (Cohen *et al.*, 1996). Although the effect of insulin withdrawal has not been reported in humans, in rats the resting glycolytic rate was unaffected by 72 hours of STZ-induced diabetes or 48 hours starvation (Berger *et al.*, 1976).

2.18.4 Effects of intense exercise on the rates of glycolysis and glycogenolysis in non-diabetics

Flux through the glycolytic pathway can be accelerated at least 1000-fold above resting values to ~60 $\mu mol \cdot min^{-1} \cdot g$ w.w.$^{-1}$ (4.3 $mmol \cdot kg$ d.m.$^{-1} \cdot s^{-1}$) during intense exercise (e.g. sprinting) (Newsholme & Leech, 1983). The *in vivo* glycolytic rate during exercise may be calculated from the accumulation of muscle metabolites:

$$\text{Glycolytic rate} = 0.5(\Delta([Lac^-] + [\text{Glyc 3-P}]) \text{ (Spriet } et\ al.\text{, 1987)}$$

where Δ is the difference between concentration in exercise and at rest and glyc 3-P is glyceraldehyde 3-phosphate. Pyruvate or a proportion of lactate (estimating pyruvate) may be substituted for Glyc 3-P in the same equation, e.g.:

$$0.5(\Delta[Lac^-] + 0.1(\Delta[Lac^-])) \text{ (Hultman \& Sjöholm, 1983)}$$

The calculation of the anaerobic glycolytic rate from lactate accumulation assumes that blood glucose uptake, muscle glycolytic intermediate accumulation, muscle lactate oxidation, and lactate efflux from the exercising muscle is minimal (Spriet, 1995); conditions which are met to varying degrees depending upon the exercise conditions (e.g. occluded versus intact circulation; exercise duration and intensity).

The *in vivo* glycogenolytic rate may also be estimated from the accumulation of muscle metabolites:

$$\Delta([\text{G 1-P}] + [\text{G 6-P}] + [\text{F 6-P}]) + 0.5(\Delta([Lac^-] + [\text{Glyc 3-P}])) \text{ (Spriet } et\ al.\text{, 1987)}$$

where G 1-P is glucose 1-phosphate, G 6-P is glucose 6-phosphate, and F 6-P is fructose 6-phosphate. Similarly, the change in pyruvate concentration may be substituted for Δ[Glyc 3-P], or a proportion of the change in G 6-P and lactate, e.g.

$\Delta[G\ 6\text{-}P] + (0.33(\Delta[G\ 6\text{-}P])) + 0.5(\Delta[Lac^-] + 0.1(\Delta[Lac^-]))$ (Hultman & Sjöholm, 1983)

The glycolytic/ glycogenolytic rate during intense exercise (100% $\dot{V}O_{2peak}$) has also been determined by the use of stable isotopic tracers for glucose, pyruvate and lactate (Williams *et al.*, 1995), however for maximal fatiguing exercise of short duration (seconds to several minutes) in which fluxes change markedly and rapidly, muscle biopsies may provide superior data.

The *in vivo* glycolytic and glycogenolytic rates in human skeletal muscle were progressively reduced during the course of a dynamic maximal 30-s sprint (Boobis *et al.*, 1982; Jacobs *et al.*, 1983; Parolin *et al.*, 1999) (Table 2.16). Peak glycolytic and glycogenolytic rates of 2.9 and 5.3 mmol·kg d.m.$^{-1}$·s^{-1}, respectively, were evident following a 10-s cycle sprint (Table 2.16). Intense exercise of several minutes' duration was associated with a marked reduction in both glycolytic and glycogenolytic rates in comparison to briefer exercise (Karlsson & Saltin, 1970; Sutton *et al.*, 1981; Howlett *et al.*, 1998).

Resting glycogen content was higher in type II than in type I human muscle fibres, and whilst the reduction in content with a maximal 30-s sprint was similar between fibre types in men, it was ~2-fold greater in type II than type I fibres in women (Esbjörnsson-Liljedahl *et al.*, 1999) (Table 2.16). It was suggested that slightly lower resting glycogen content, lower activity of glycolytic/glycogenolytic enzymes, and/or lower catecholamine stimulation may have accounted for the finding (Esbjörnsson-Liljedahl *et al.*, 1999). The accumulation of lactate during a 30-s sprint was ~1.4-fold greater in type II than type I fibres, with no gender difference (Esbjörnsson-Liljedahl *et al.*, 1999) (Table 2.16), however accumulation was lower in type I fibres in women. In mixed fibre analysis, lactate accumulation during a 30-s sprint was lower in women

than men, however once normalized for power output, no difference was apparent (Jacobs *et al.*, 1983).

2.18.5 Accumulation of hexose monophosphates and PFK activity

G 1-P, G 6-P, and F 6-P constitute the hexose monophosphates (HMPs). HMPs occur in the metabolic pathway between the enzymes GP (or HK) and 6-phosphofructokinase (PFK). As illustrated by Table 2.16, maximal exercise results in an accumulation of HMPs; whilst the glycolytic intermediates occurring after PFK accumulate to a lesser extent. Hence, PFK is considered to be the rate-limiting enzyme in glycolysis, evidently either operating at maximum catalytic activity or having activity inhibited in maximum exercise (Jones & Heigenhauser, 1992). As previously reviewed, accumulation of G 6-P allosterically inhibits HK activity; G 6-P also serves to inhibit GP activity (Newsholme & Leech, 1983), thus facilitating (amongst other regulators) co-ordinated regulation of glucose entry or glycogen breakdown, and flux through PFK.

PFK is inhibited by increased $[H^+]$, ATP, PCr, citrate and K^+, whereas inhibition is relieved by AMP, P_i, F 6-P, F 1,6-bisphosphate, and ammonia (NH_4^+) (Newsholme & Leech, 1983; Jones & Heigenhauser, 1992). The presence of positive modulators, singly or in combination, extends the pH range through which PFK can continue to operate, and stabilizes it against citrate inhibition (Dobson *et al.*, 1986). Muscle citrate has been demonstrated to have little effect under *in vitro* conditions that replicated concentrations found during maximal exercise (Bosca *et al.*, 1985). It has been suggested that during intense muscle contraction NH_4^+ accumulation helps to counteract the inhibitory effect of increasing $[H^+]$ on PFK activity, although may be overridden at fatigue when acidosis is particularly high (Spriet *et al.*, 1987).

2.18.6 Regulation of glycogenolysis in skeletal muscle during intense exercise

Marked Lac^- accumulation was evident after only 10 s of a 30-s sprint bout, leading the authors to conclude that glycolysis was activated very rapidly in maximal exercise (Jacobs *et al.*, 1983) (Table 2.16). This finding was subsequently confirmed using

Table 2.16 *Muscle glycolytic intermediate and product accumulation during intense exercise in non-diabetic subjects*

Study	*n*, gdr	Exercise*		Δ in muscle metabolite content (mmol·kg d.m.$^{-1}$)								Glyc rate	Ggn rate
		mode	*t* (s)	Gluc	Gly	G1-P	G6-P	F6-P	G3-P	Pyr	Lac		
(Howlett *et al.*, 1998)	4M,3F,A	90% $\dot{V}O_{2peak}$,	60	0.7			4.0				30.7	0.3†	0.4†
		NE	600	10.8			7.3				102	0.1†	0.1†
(Åstrand *et al.*, 1986)	5M,2F,A	Arm/leg cycle	318		206		17.3#				89.5	0.2†	0.2†
(Sutton *et al.*, 1981)	5M	95% $\dot{V}O_{2peak}$	274		231§‡	0.0	0.3	0.1		0.3	54.7	0.1†	0.9†
(Karlsson & Saltin, 1970)	3M,A	Cycle sprint	143		108‡		10.0				63.8	0.2†	0.3†
(Nevill *et al.*, 1989)	7,A	110% $\dot{V}O_{2peak}$, NE	120	1.0	34.7	0.9	8.1	2.0		0.4	24.5	0.1†	0.2†
(Jacobs *et al.*, 1982)	9F,A	Cycle sprint	30		82						51.5	0.9†	
(Boobis *et al.*, 1983)	7M	Dynamic ex.	30		55.5		18.1	3.6		2.6	88.7	1.5†	2.4†
(Jones *et al.*, 1985)	5M	Isokin. cycle	30				4.7	1.3	1.3		27	0.5†	0.7†
(McCartney *et al.*, 1986)	8M	Cycle sprint	30		78‡		27.3	4.8			118	2.2†	3.4†
(Boobis *et al.*, 1987)	4M,4F	Tm sprint	30		94.8					2.9	82.1	1.4†	
(Nevill *et al.*, 1989)	6,A	Tm sprint	30	2.1	103	2.3	19.5	5.3		2.8	81.9	1.4†	2.3†
(Greenhaff *et al.*, 1994)	5M,1F,A	Tm sprint	30	3.4	82	1.4	24.0	6.2	6.2		86.6	1.6†	2.6†

Table 2.16 (cont.): *Muscle glycolytic intermediate and product accumulation during intense exercise in non-diabetic subjects*

Study	*n*, gdr	Exercise*		Δ in muscle metabolite content (mmol·kg d.m.$^{-1}$)								Glyc	Ggn
		mode	*t* (s)	Gluc	Gly	G1-P	G6-P	F6-P	G3-P	Pyr	Lac	rate	rate
(Bogdanis *et al.*, 1995)	8M,A	Cycle sprint	30	3.6	110	2.0	21.6	6.3		3.7	115	2.0	3.0
(Bogdanis *et al.*, 1996)	8M,A	Cycle sprint	30	5.3	99.2	2.9	26.0	9.3		3.6	102	1.8†	3.0†
(Esbjörnsson-Liljedahl *et*	20M,A	Cycle sprint	30		I 126						72	1.3†	
al., 1999)					II 131						96	1.8†	
	19F,A				I 73						59	1.1†	
					II 149						89	1.6†	
(Spriet *et al.*, 1987)	7M,A	ES occ. circ.	25.6	2.7		0.5	8.6	1.8	3.9	2.1	60.5	1.3	1.7
(Jacobs *et al.*, 1983)	15M,A	Cycle sprint	10								19.2	1.1†	
			30								41.4	0.8†	
	7F,A		10								40.1	2.2†	
			30								67.9	1.2†	
(Parolin *et al.*, 1999)	6M	Isokin. cycle	6	2.7			8.1	1.3	3.5	~1.2	~22	2.1†	4.0
			15	0.7			17.5	3.2	6.9	~1.9	~60	2.2†	3.8†
			30	3.0	89.2		15.9	3.7	6.0	~1.5	73.4	1.3†	2.1†
(Boobis *et al.*, 1982)	4	Cycle sprint	6		37.9		9.7				19.1	1.8†	3.9†
			30		65.4		18.3				80.0	1.5†	2.3†

Table 2.16 (cont.): *Muscle glycolytic intermediate and product accumulation during intense exercise in non-diabetic subjects*

Study	*n*, gdr	Exercise* mode		*t* (s)	Δ in muscle metabolite content (mmol·kg d.m.$^{-1}$)								Glyc rate	Ggn rate
					Gluc	Gly	G1-P	G6-P	F6-P	G3-P	Pyr	Lac		
(Howlett *et al.*, 1999b)	7M,3F,A	Isokin. cycle		10	1.8			17.6		4.1	1.9	53.3	2.9†	5.3†
(Gaitanos *et al.*, 1993)	8M,A	Cycle sprint		6	1.1	43.5	0.3	9.0	2.2		1.4	24.8	2.3	4.4
(Spriet *et al.*, 1989)	7M,1F,A	Isokin. cycle	B2	30	1.5	47.2	0.2	3.5	0.6	2.4	1.8	33.3	0.6†	0.7†
			B3	30	1.4	15.1	0.02	2.7	0.3	0.9	0.5	22.9	0.4†	0.5†
(Parolin *et al.*, 1999)	6M	Isokin. cycle	B3	6	0.1			-1.5	0.2	-0.3	~-0.2	~2	~0.2	~0.4
				15	-3.1			3.3	0.8	0.6	~-0.2	~0.0	~0	~0.3
				30	-2.7	~0		2.4	0.4	0.5	~-0.4	~3	~0.1	~0.2

Table arranged in order of increasing exercise intensity, and when intensity similar, arranged chronologically, except for the last two studies which present data on subsequent exercise bouts. *n*, number of subjects; gdr, gender; *, all exercise bouts maximal unless indicated otherwise; *t* (s), time of exercise in seconds; Δ, difference in exercise and rest content expressed to one decimal place: all values represent net accumulation, except for glycogen which is net degradation; Gluc, total glucose; Gly, glycogen; G 1-P, glucose 1-phosphate; G 6-P, glucose 6-phosphate; F 6-P, fructose 6-phosphate; G 3-P, glyceraldehyde 3-phosphate; Pyr, pyruvate; Lac, lactate; Glyc rate, glycolytic rate, mmol·kg d.m.$^{-1}$·s^{-1}; Ggn rate, glycogenolytic rate, mmol·kg d.m.$^{-1}$·s^{-1}; M, male; F, female; A, active subjects; NE, exercise not continued until exhaustion; †, rates calculated from reported metabolite data; #, total for hexose monophosphates, individual values not specified; §, exercise at 95% $\dot{V}O_{2peak}$ was preceded by 20 min exercise at 33, then 66% $\dot{V}O_{2peak}$; ‡, for all metabolites, wet weight values in paper converted to dry weight by multiplying by 4.3; Isokin., isokinetic; Tm, treadmill; I, type I muscle fibres; II, type II muscle fibres; ES, electrical stimulation; occ. circ, occluded circulation; B2, bout 2 of 3 bouts separated by 4 min rest; B3, bout 3 of 3.

maximal exercise of even briefer duration (e.g. Boobis *et al.*, 1982; Gaitanos *et al.*, 1993).

The breakdown of glycogen is regulated by the activity of GP, which exists as the more active GP*a* and less active GP*b*, and it is thought that the combined activation of the two forms occurs during intense exercise (Connett & Sahlin, 1996). Putative regulators of GP include free AMP (AMP_f), IMP, calcium (Ca^{2+}), pH, inorganic phosphate (P_i), G 6-P, and the catecholamines. The rate of muscle glycogenolysis is closely related to the rate of ATP turnover, and when the rate of ADP rephosphorylation is slowed during intense contraction, the increase in AMP_f that occurs activates GP*a* (Ren & Hultman, 1990). AMP_f may also allosterically activate GP*b* during intense exercise, although the latter is inhibited by accumulation of G 6-P (Connett & Sahlin, 1996). During the later stages of intense exercise, IMP content may rise sufficiently to activate GP*b* which has a Km of ~5 mM (Connett & Sahlin, 1996).

GP*a* did not differ significantly whether the quadriceps femoris was stimulated intermittently at 15 or 50 Hz, despite a nearly 2-fold higher ATP turnover rate in the latter (Ren & Hultman, 1990). Similarly, GP*a* did not differ in intense dynamic exercise at 35, 65 or 90% $\dot{V}O_{2peak}$ (although a lower GP*a* was found after 10 min at 90% $\dot{V}O_{2peak}$) (Howlett *et al.*, 1998). Greater rates of glycogenolysis evident at higher submaximal intensities must therefore be partly controlled post-transformationally. AMP_f rose significantly with exercise at 90% $\dot{V}O_{2peak}$, however GP*a* was reduced; a change suggested to be due to increasing muscle acidosis (Howlett *et al.*, 1998).

Cycling to exhaustion (4-4.5 min) resulted in an increase in the fraction of GP*a* from 22% at rest to 34% after 30 s, then a fall to 17% by exhaustion; AMP and P_i were suggested to be of importance in activating GP (Chasiotis, 1983). In recent work, GP*a* increased from 12% at rest to 47% after only 6 s of a maximal 30-s cycle bout, probably due to increased Ca^{2+} release from the sarcoplasmic reticulum; and glycogenolysis proceeded at 4 mmol·kg dw·s^{-1} (Parolin *et al.*, 1999). Both the fraction of GP*a* and the glycogenolytic rate were maintained through to 15 s, but in

the final 15 s of the sprint, GP*a* fell to basal levels, the rate of glycogenolysis slowed to 0.6 mmol·kg dw·s^{-1}, and the muscle [H^+] had risen 3-fold above resting values (Parolin *et al.*, 1999). In the third, 30-s bout GP*a* remained at resting values, glycogen degradation was negligible, rates of glycogenolysis were very low (Table 2.16), attaining rates similar to the final 15 s of the first bout, and [H^+] remained ~4-fold above rest throughout (Parolin *et al.*, 1999). Although AMP_f was high, G 6-P was also high and may have accounted for the apparently minimal activation of GP*b*; and the high [H^+] may have inhibited conversion from GP*b* to GP*a* (Parolin *et al.*, 1999). Thus in maximal exercise, both transformational and post-transformational factors must interact in determining flux through GP (Parolin *et al.*, 1999). The inability to restimulate glycogenolysis and glycolysis during repeated bouts of maximal exercise places greater importance on other sources of ATP, i.e. PCr degradation and aerobic metabolism (see section 2.20).

2.18.7 Enzyme binding and glycolytic flux

Glycolytic enzymes may bind reversibly to each other or to part of the cytoskeleton, i.e. membranous or contractile elements (e.g. F-actin), which increases their activity and their proximity to each other, thus providing a further mechanism for regulating the rate of glycolytic flux and possibly providing a local pool of ATP for the contractile elements (Parkhouse, 1992). Insulin acts within minutes to stimulate the binding of PFK to muscle cytoskeleton (perhaps by phosphorylation of PFK), and synchronously, to stimulate mitochondrial HK in rats (Livnat *et al.*, 1993).

2.18.8 Effect of type 1 DM on glycogenolysis during exercise

No studies have examined the effect of high intensity exercise on muscle metabolism in subjects with type 1 DM.

In rats, electrical stimulation of the sciatic nerve, leading to intense isometric contraction of ~33% of the perfused hindquarter, increased the glycolytic rate 11-fold to ~0.4 μmol·min^{-1}·g w.w.$^{-1}$ (Berger *et al.*, 1976). Neither starvation for 48 hours nor STZ-diabetes for 72 hours affected the glycolytic rate during electrical stimulation in rats (Berger *et al.*, 1976).

2.18.9 Contribution of glycolysis to total anaerobic and total ATP generation during repeated bouts of maximal exercise

During the first of ten 6-s maximal sprints, glycolysis provided 44% of the total anaerobic ATP production, whereas in the final sprint absolute ATP production from anaerobic metabolism was ~66% lower, with only 16% provided by glycolysis (Gaitanos *et al.*, 1993). In the first 6 s of an exhausting 30-s cycle sprint 43% of the ATP was provided by glycolysis, which declined to ~26% in the final 15 s (Parolin *et al.*, 1999). In the first 6 s of the third bout ~13% of ATP turnover was derived from glycolysis and in the final 15 s ~20% was from glycolysis (Parolin *et al.*, 1999). Averaged over the duration of an exhausting 30-s cycle sprint, 78% of total anaerobic and 55% of total ATP production was derived from glycolysis (Putman *et al.*, 1995a). A 41% reduction in anaerobic ATP production and an 18% reduction in power output was evident in the second 30-s sprint in one study (Bogdanis *et al.*, 1996), however, in another (Putman *et al.*, 1995a), the relative contribution to ATP generation changed little from the first bout. In contrast, in the third bout, glycolysis contributed 58 and 21% to total anaerobic and total ATP generation, respectively (Putman *et al.*, 1995a).

2.18.10 Sprint training, glycogen content at rest, and glycogen phosphorylase activity

Muscle glycogen content at rest was either unchanged (Boobis *et al.*, 1987; Nevill *et al.*, 1989) or significantly increased (Boobis *et al.*, 1983) by 8 weeks of sprint training, and was doubled in young athletes following an 8-month intensified sprint training programme, consistent with the finding of increased glycogen synthase (GS) activity (Cadefau *et al.*, 1990). Maximal *in vitro* GP activity was also markedly increased following 8 months of intensified sprint training in adolescent athletes (Cadefau *et al.*, 1990), however was not significantly increased after training programmes of 7-8 weeks duration (Sharp *et al.*, 1986; MacDougall *et al.*, 1998). Thus, a protracted period of sprint training may be required to induce significant change in resting glycogen content and/or GP activity. However, during a single bout of high intensity exercise neither high nor low resting muscle glycogen content influences glycogenolysis (Symons & Jacobs, 1989; Bangsbo *et al.*, 1992b), although may do so in subsequent bouts (Putman *et al.*, 1995a; Parolin *et al.*, 1999). Additionally, since children have a lower glycolytic capacity than adults (Eriksson *et al.*, 1973; Inbar & Bar-Or, 1986), it is also possible that the increase in enzyme

activities in the adolescent athletes was at least partly attributable to maturation over the course of 8 months.

2.18.11 Sprint training, performance and the glycolytic rate

Sprint training has been demonstrated to improve performance during single or repeated bouts of brief high intensity exercise (Sharp *et al.*, 1986; Boobis *et al.*, 1987; Bell & Wenger, 1988; Nevill *et al.*, 1989; Stathis *et al.*, 1994; McKenna *et al.*, 1997b; MacDougall *et al.*, 1998). Improved performance has been linked with an enhanced glycolytic rate in the exercising skeletal muscle (Sharp *et al.*, 1986; Nevill *et al.*, 1989; Linossier *et al.*, 1993). Glycolytic rate, calculated from the accumulation of intermediates and products, has been reported to be 20% higher (Nevill *et al.*, 1989) after 30 s of intense exhausting exercise following 8 weeks of sprint training. Average power was not increased, although peak power was 12% higher after training (Nevill *et al.*, 1989). Another study reported improved performance during a 30-s bout and associated it with increased energy production from glycolysis; the latter assumed from higher muscle Lac^- and resting PFK activity (Linossier *et al.*, 1993) (see below for a more extended review of the effects of sprint training on muscle Lac^- regulation). However, another study reported an unchanged glycolytic rate during 30 s exhausting exercise after 8 weeks of sprint training, but an 8% higher average power during the bout, leading the authors to conclude that a larger muscle mass must have been recruited after training (Boobis *et al.*, 1983). Similarly Stathis *et al.* (1994) demonstrated increases in both peak and mean power during an exhausting 30-s exercise bout after 7 weeks of sprint training, however muscle Lac^- at fatigue was similar after training. In contrast, peak and mean power was not enhanced in the first of four, 30-s sprint bouts, even though an acceleration of glycolytic flux may have occurred after sprint training (resting PFK activity was increased), however peak and mean power was increased after training in the 2^{nd} to 4^{th} 30-s bouts (MacDougall *et al.*, 1998). This result supported an earlier study in which neither peak nor mean power during a single 30-s exercise bout was increased after 6 weeks of sprint training, despite evidence of enhanced glycogenolysis/ glycolysis (Jacobs *et al.*, 1987). Thus the relationship between the rate of glycogenolysis/glycolysis and work performed during maximal exhausting exercise after sprint training remains unclear.

Only one study (Nevill *et al.*, 1989) included a test following sprint training in which pre-training work was matched, and measured muscle metabolites from which the rates of glycogenolysis and glycolysis could be calculated. Based on their data, the glycogenolytic and glycolytic rates during a non-exhausting 2-min treadmill run at 110% pre-training $\dot{V}O_{2peak}$ were quite low (0.2 and 0.1 mmol·kg d.m.$^{-1}$·s^{-1}), and were very similar after training. However, as previously mentioned, the run was not exhausting and elicited only a minor metabolic perturbation, and hence may not have allowed discrimination of a training effect. Thus the effect of sprint training on the rates of glycolysis and glycogenolysis during matched-work exercise also requires further investigation.

2.18.12 The effect of sprint training upon PFK activity, glycolytic intermediates, and muscle lactate accumulation

2.18.12.1 PFK activity and glycolytic intermediates

PFK is acknowledged to be the major rate-limiting enzyme in glycolysis, and hence an upregulation of PFK activity may be expected to result in a higher glycolytic rate, providing that maximum capacity was taxed during exercise prior to training and that the activity of glycogen phosphorylase exceeded that of PFK. PFK activity at rest was increased by 16-49% after 6-8 weeks (Sharp *et al.*, 1986; Jacobs *et al.*, 1987; Linossier *et al.*, 1993; MacDougall *et al.*, 1998) and by 42% after 8 months (Cadefau *et al.*, 1990) of sprint training. In contrast, 8 weeks of one-legged interval training (Pilegaard *et al.*, 1999), and a 15-week combined endurance and sprint training programme (Simoneau *et al.*, 1986) did not effect a significant increase in PFK activity. In support, in a cross-sectional study, highly trained anaerobic athletes had similar PFK activity to untrained subjects (Sahlin & Henriksson, 1984). Studies reporting higher PFK activity generally utilized 5-30 s sprint repeats during training, whilst those finding no change in PFK used longer sprint intervals (60-90 s); perhaps contributing to the contrasting findings. However, whilst resting PFK activity may be increased after sprint training, the effect of an acute bout of intense exercise (either before or after training) on *in vitro* PFK activity has rarely been reported.

In vivo, the accumulation of G 6-P during a 30-s exhausting exercise bout was 19% greater ($P<0.051$) after 8 weeks of sprint training; and more work was accomplished during the bout than before training (Nevill *et al.*, 1989). Muscle Lac^- was similarly increased (20%) after an exhausting bout after training, hence the ratio of G 6-P to Lac^- remained constant (Nevill *et al.*, 1989), suggesting that PFK activity at exhaustion was similar after training. However, another 8-week sprint training study from the same group (described in a brief report), resulted in greater post-exercise F 6-P and G 6-P content after a 30-s sprint, however, similar Lac^- to the pre-training test (Boobis *et al.*, 1983); the latter finding was supported by a recent study (Stathis *et al.*, 1994). In further contrast, a study (Linossier *et al.*, 1993) which employed repeated 5-s sprints during training (in contrast to the longer efforts in Nevill *et al.*, 1989), demonstrated that G 6-P accumulation was not significantly higher after 2 series of 8 sprints in which more work was performed after 7 weeks of sprint training, whereas Lac^- accumulation was higher; consistent with the measured increase in resting PFK activity that may have been maintained during exercise. In another study, PFK activity at rest was enhanced and blood [Lac^-] was increased after a 30-s sprint, however performance during the sprint was not improved after 6 weeks of sprint training (Jacobs *et al.*, 1987). Seven weeks of one-legged sprint training, using repeated 20-s sprint intervals, resulted in improved performance in each of the trained and untrained legs (evident to a greater degree in the trained leg), but higher blood [Lac^-] after 60-s one-legged maximal exercise in the trained leg (Bell & Wenger, 1988). Following 8 weeks of one-legged high intensity interval training, PFK activity at rest was not significantly increased (although 14% higher) and muscle Lac^- content during one-legged knee-extensor exercise was lower in the trained leg, despite a longer exercise time at the same power output, and hence more work being performed, before exhaustion (Pilegaard *et al.*, 1999).

The only study that examined muscle metabolism during intense matched-work exercise (110% pre-training $\dot{V}O_{2peak}$, not conducted to fatigue) before (Table 2.16) and after sprint training, reported no change in post-exercise G 6-P (although 13% lower) or lactate contents after training (Nevill *et al.*, 1989). However, the calculated glycolytic rate during the test was relatively low (Table 2.16), and exercise did not cause a significant fall in muscle pH. After 4 weeks of one-legged sprint training, in a

60-min submaximal two-legged exercise test in which the work performed by the sprint-trained and untrained legs did not differ significantly, glycogen degradation, muscle Lac^- release and lactate content were higher in the untrained leg (Saltin *et al.*, 1976).

2.18.12.2 Effects of sprint training on muscle lactate regulation

Net muscle lactate release (indicated by a negative arteriovenous difference for [Lac^-]), arterial and femoral venous blood [Lac^-], and femoral venous plasma [Lac^-], were unchanged during an exhausting 30-s exercise bout after 7 weeks of sprint training in men (McKenna *et al.*, 1997a; McKenna *et al.*, 1997b). The authors concluded that muscle glycolysis was unlikely to have been substantially greater after training, and the greater power output after training was instead suggested to be consistent with enhanced aerobic metabolism, supported by a higher peak and recovery $\dot{V}O_2$ (McKenna *et al.*, 1997b). However, it was suggested that total muscle Lac^- release may have been greater after sprint training, based upon an inferred increase in maximal muscle blood flow (McKenna *et al.*, 1997b).

After 8 weeks of one-legged high intensity interval training in habitually active men, the rate of Lac^-/H^+ transport in sarcolemmal giant vesicles (SGVs - obtained from muscle biopsies at rest) was 12% higher in the trained leg than in the untrained leg (Pilegaard *et al.*, 1999). Supporting this result was a 33-70% greater content of the monocarboxylate Lac^- transporters (MCT) MCT1 and MCT4 after training (Pilegaard *et al.*, 1999). However, Lac^- release (product of blood flow and arteriovenous difference) did not differ significantly between trained and untrained legs (although 17% higher in the trained leg) during exercise to exhaustion at a set power output (Pilegaard *et al.*, 1999). Lower muscle Lac^- and higher arterial and venous [Lac^-], i.e. a reduced gradient, in the trained leg, coupled with similar Lac^- release rates, was suggested to be explained by enhanced sarcolemmal Lac^- transport as a consequence of training (Pilegaard *et al.*, 1999). Higher Lac^- transport capacity (as determined in SGVs) in competitive cyclists (track and road cyclists who undertook frequent high intensity training sessions) than in either recreationally trained or untrained subjects (Pilegaard *et al.*, 1994) appears to support the results from longitudinal training studies.

2.18.13 Sprint training and muscle metabolism in subjects with type 1 DM

No studies have examined the effect of sprint training on muscle metabolism during intense exercise in subjects with type 1 DM.

In summary, the onset of muscle glycogenolysis during maximal exercise is very rapid and is probably mediated by increased calcium release. A number of transformational and post-transformational regulators acutely up- or down-regulate glycogenolytic flux during maximal exercise via effects on GPa and GPb, with the rate of glycogenolysis being closely related to ATP turnover. The glycogenolytic and glycolytic rates are markedly attenuated during repeated bouts of maximal exercise. In subjects with type 1 DM, few studies have examined muscle metabolites at rest, and no studies have examined the metabolic response of muscle to maximal exercise.

The effects of sprint training on the rates of glycolysis and glycogenolysis and their relationship to the work performed during exhausting and matched-work exercise remain unclear. In vitro PFK activity at rest may be increased following training, particularly in programmes that utilize briefer sprints, however the in vivo effect on glycolytic rate and glycolytic intermediate accumulation during intense exercise is less clear.

Skeletal muscle may be capable of higher rates of Lac^- removal following sprint training. Enhanced Lac^- transport during intense exercise unites findings of similar or lower muscle Lac^- but higher blood or plasma [Lac^-] at exhaustion after sprint training. However, in addition to enhanced Lac^- removal via membrane transporters, increased active pyruvate dehydrogenase (PDHa) following sprint training would permit a higher proportion of pyruvate to be oxidized, and hence would be anticipated to reduce cellular accumulation of Lac^-. The affect of sprint training on PDH activity has not been examined (see below). No sprint training studies have included subjects with type 1 DM.

2.19 The pyruvate dehydrogenase complex

2.19.1 Pyruvate dehydrogenase reaction

The chemical reaction in which the oxidative decarboxylation of pyruvate occurs:

$$\text{pyruvate} + NAD^+ + CoA \rightarrow \text{acetyl CoA} + NADH + H^+ + CO_2$$

(where NAD^+ is nicotinamide adenine dinucleotide, CoA is coenzyme A, and NADH is reduced NAD^+) is catalyzed by the activated pyruvate dehydrogenase (PDH) complex (Newsholme & Leech, 1983; Randle, 1986; Randle *et al.*, 1988; Randle *et al.*, 1994), and is considered to represent a flux-generating step since it is likely that it is saturated with pyruvate (Newsholme & Leech, 1983), with a K_m of 25 μM (Denton & Halestrap, 1979). The PDH reaction is pivotal in the regulation of the relative proportions of carbohydrate and fat entering oxidative phosphorylation as muscular fuel.

The PDH complex is located on the inner mitochondrial membrane in skeletal muscle and other tissues and is constituted by three enzymes: E1 - pyruvate dehydrogenase, E2 – dihydrolipoamide (or dihydrolipoate) acetlytransferase, and E3 - dihydrolipoamide reductase (or dihydrolipoyl dehydrogenase) (Newsholme & Leech, 1983; Randle, 1986; Randle *et al.*, 1988); and a binding protein (protein X) which links E3 to the complex, as recently reviewed (Randle *et al.*, 1994). The reactions catalyzed by E2 and E3 are freely reversible, whereas the reaction catalyzed by E1 is essentially irreversible, and therefore confers a non-equilibrium status upon the complex (Denton & Halestrap, 1979; Newsholme & Leech, 1983; Randle, 1986; Randle *et al.*, 1988; Randle *et al.*, 1994). The PDH enzyme complex also contains an intrinsic PDH kinase (PDK), the enzyme responsible for the interconversion of PDH (E1) from the dephosphorylated active form (PDH*a*) to the phosphorylated inactive form (PDH*b*) (Randle, 1986; Randle *et al.*, 1988; Randle *et al.*, 1994). Four PDK isoforms have been characterised in mammalian tissue, with the lower-specific activity pyruvate-sensitive isoform PDK2, and the higher-specific activity relatively pyruvate-insensitive PDK4, being the major isoforms in skeletal muscle (Bowker-Kinley *et al.*, 1998; Wu *et al.*, 1999; Holness *et al.*, 2000). (A kinase activator protein which responds to nutritional and hormonal changes in a very similar manner to PDK,

may actually be PDK not bound to the PDH complex (Kerbey & Randle, 1982).) Associated with, but not intrinsic to the PDH complex is PDH phosphatase (PDHP), which activates PDH by dephosphorylation. PDH*a* activity provides a measure of the maximum possible flux of pyruvate to acetyl CoA (Cooney *et al.*, 1993). End-product inhibition of the PDH reaction is thought to be important in rapid adjustments of flux (Randle *et al.*, 1994), however reversible phosphorylation is the major mechanism by which the PDH complex is regulated (Linn *et al.*, 1969a; Linn *et al.*, 1969b; Fuller & Randle, 1984), with reactions summarised thus [from (Newsholme & Leech, 1983)]:

$$PDHb + H_2O \rightarrow PDHa + P_i \qquad \text{catalyzed by PDH phosphatase}$$

$$PDHa + ATP \rightarrow PDHb + ADP \qquad \text{catalyzed by PDH kinase}$$

2.19.1.1 Activation of PDH phosphatase

PDH phosphatase is activated by micromolar concentrations of calcium ($[Ca^{2+}]$) with a K_m of 0.1-1.0 μM in the presence of magnesium and thiamin pyrophosphate (Denton *et al.*, 1972; Fuller & Randle, 1984; Tate *et al.*, 1991). At rest, cytosolic $[Ca^{2+}]$ is too low (0.03-0.1 μM) (Cairns *et al.*, 1993; Duty & Allen, 1994; Williams & Klug, 1995) to act as a cofactor (Tate *et al.*, 1991). However, during muscular contraction, the increase in mitochondrial $[Ca^{2+}]$, consequent to increases in cytosolic $[Ca^{2+}]$ to 0.3-10 μM with varying protocols of tetanic stimulation in animals (Cairns *et al.*, 1993; Westerblad *et al.*, 1993; Duty & Allen, 1994; Westerblad & Allen, 1994; Williams & Klug, 1995; Chin & Allen, 1997), activates PDHP, which increases PDH*a* and hence increases flux through the PDH complex. As reviewed below, insulin also acutely increases PDHP activity (Feldhoff *et al.*, 1993), however not by the same mechanism as exercise or muscular contraction (Randle *et al.*, 1994).

2.19.1.2 Activation of PDH kinase

PDK is acutely activated by increased ratios of acetyl CoA/CoA and NADH/NAD^+, and inhibited by pyruvate (an effect potentiated by ADP) and potassium (Kerbey *et al.*, 1977; Denton & Halestrap, 1979; Newsholme & Leech, 1983; Randle *et al.*, 1994). ATP is required to phosphorylate (and hence inactivate) the complex, thus an increased ATP/ADP ratio activates PDK. There are three sites at which the E1

component of the PDH complex can be phosphorylated: inactivation requires only site 1 to be phosphorylated, but multisite phosphorylation or hyperphosphorylation may occur in skeletal muscle (Fuller & Randle, 1984) and may be important in retarding reactivation of the complex (Sale & Randle, 1982; Sugden & Holness, 1994), and maintaining low PDH*a* in starvation and diabetes (Wu *et al.*, 1999). No allosteric activator of PDH*b* has been found, and thus inactivation of the PDH complex by phosphorylation can only be reversed by dephosphorylation catalyzed by PDHP (Randle *et al.*, 1988; Randle *et al.*, 1994). The activation state of the PDH complex is therefore determined by the relative activities of PDK and PDHP (Randle, 1986).

2.19.2 Total PDH – animal studies

Total PDH (PDH*t* = PDH*a* + PDH*b*) was unaffected by 15-60 hours of starvation (Hennig *et al.*, 1975; Hagg *et al.*, 1976; Fuller & Randle, 1984; Denyer *et al.*, 1989; Holness *et al.*, 1989), a high-fat diet (Holness *et al.*, 2000), alloxan- or STZ-diabetes (Hennig *et al.*, 1975; Hagg *et al.*, 1976), or hyperinsulinaemia in rats (Denyer *et al.*, 1989; Cooney *et al.*, 1993; Feldhoff *et al.*, 1993; Nakai *et al.*, 1999).

2.19.3 Effects of fasting and type 1 diabetes mellitus on PDH*a* at rest – animal studies

At rest PDH*a* ranged from 7.5% in soleus to 37.5% in gastrocnemius muscle of rats, and was reduced to 1-12% of PDH*t* after only 15 hr of starvation (Holness *et al.*, 1989). PDH*a* at rest (16-24.5% of PDH*t*) was reduced by starvation for 48-60 hours or by insulin-deficient diabetes in rats by ~40-80% compared to the fed, non-diabetic state (Hennig *et al.*, 1975; Hagg *et al.*, 1976; Caterson *et al.*, 1982; Fuller & Randle, 1984; Feldhoff *et al.*, 1993). PDH*a* in the red quadriceps of 48-hour starved rats was increased from 12.2 to 20.6% 30 min after an injection of insulin, however was not restored to fed (3-hr postprandial) levels (55.9%) (Denyer *et al.*, 1989). Hyperinsulinaemia induced by insulin infusion or injection in non-diabetic rats increased skeletal muscle PDH*a* at rest 1.8- to 2-fold to be 52-68% of PDH*t* (Cooney *et al.*, 1993; Feldhoff *et al.*, 1993).

2.19.4 Effects of fasting and type 1 diabetes mellitus on PDHP and PDK at rest – animal studies

PDHP activity in rat skeletal muscle was unaffected by 48 hours of starvation or alloxan-diabetes (Fuller & Randle, 1984). In contrast, both acute and longer-term regulation of PDK activity is effected by starvation and insulin-deficient diabetes (probably related to increased cyclic AMP) (Randle *et al.*, 1994). PDK activity was 2- to 4-fold higher at rest in skeletal muscle of 48-hr starved rats (Fuller & Randle, 1984; Wu *et al.*, 1999) or alloxan- or STZ-diabetic rats deprived of insulin for 48 hours (Feldhoff *et al.*, 1993; Wu *et al.*, 1999). In rat gastrocnemius, the amount of PDK4 was increased 3.5- and 5.2-fold, and the relative abundance of the mRNA for PDK4 was increased 3.8- and 7.5-fold by starvation and diabetes, respectively, providing a molecular mechanism to explain the latency and 'stable' increase in PDK activity with each condition (Wu *et al.*, 1999). The signal for increased expression of PDK4 may be the elevated level of long-chain fatty acids which occurs in starvation and diabetes (Wu *et al.*, 1999). Consistent with this hypothesis, high-fat feeding led to a stable increase in PDK4 in slow- and fast-twitch rat muscle, and upregulation of PDK4 protein expression (Holness *et al.*, 2000) (see below). In STZ-induced diabetes 48 hours of insulin treatment reduced PDK activity 50%, however it was still ~2-fold above control values (Wu *et al.*, 1999). Sixty min after insulin injection PDH*a* in alloxan-diabetic rats did not differ from controls, and remained similar for another 5 hours; however by 24 hours PDH*a* had returned to the low basal values (Feldhoff *et al.*, 1993). Despite the normalization of PDH*a* shortly after a single insulin injection, PDK activity remained 2-fold higher than in controls, suggesting that increased PDHP activity may have mediated the increase in PDH*a* acutely; however, after continued insulin therapy (48-72 hours) both PDK activity and PDH*a* were normalized (Feldhoff *et al.*, 1993). Thus the acute and chronic effects of insulin to increase PDH*a* are mediated via different mechanisms.

2.19.5 Effect of lipids or their metabolites on the PDH complex at rest- animal studies

Lipid oxidation provides a rich source of acetyl CoA and NADH, and thus acts to reduce flux through the PDH reaction by increasing PDK activity. A 28-day high-fat diet reduced PDH*a* in soleus muscle from 38% to 15% of PDH*t* (Holness *et al.*,

2000). PDK activity was increased 2.1-fold, the amount of PDK4 protein expressed was similarly increased (Holness *et al.*, 2000). Fatty acids or their metabolites may lead to the upregulation of PDK4 and impair PDH complex activation by the stable increase in PDK activity (Holness *et al.*, 2000). Perfusion of muscle (from fed rats) with acetoacetate, or incubation of isolated mitochondria with tricarboxylic acid cycle (TCA) intermediates reduced PDH*a* at rest by 53-72% (Hagg *et al.*, 1976; Feldhoff *et al.*, 1993). The addition of pyruvate or dichloroacetate [DCA - a derivative of acetate which binds at the pyruvate-binding site and mimics the action of pyruvate in the PDH reaction and thus increases flux (Newsholme & Leech, 1983)] to the TCA intermediate incubation medium fully restored PDH*a* (Feldhoff *et al.*, 1993).

2.19.6 PDH*a* at rest and the effect of nutritional state - human studies

At rest in human skeletal muscle, PDH*a* comprises 12-40% of PDH*t* (Tables 2.17, 2.18), although a resting value of 56% has been reported (Mandarino *et al.*, 1987). Mean activity of PDH*a* at rest (Tables 2.17, 2.18) was 0.45 $mmol \cdot min^{-1} \cdot kg\ ww^{-1}$, with a range of 0.09 to 0.90 $mmol \cdot min^{-1} \cdot kg\ ww^{-1}$. Variation in absolute values of PDH activity may be accounted for by the use of different assay techniques (Constantin-Teodosiu *et al.*, 1991b) and different nutritional states.

Resting PDH*a* was reduced 43% by a 20-min sodium acetate infusion (Putman *et al.*, 1995b). Acute elevation (30 min) of plasma [FFA] achieved by a lipid infusion, had no affect on resting PDH*a*, consistent with the lack of effect on muscle acetyl CoA and acetylcarnitine contents (Dyck *et al.*, 1993), however at odds with lower muscle pyruvate and malonyl-CoA contents and higher NADH (Odland *et al.*, 2000). In the latter study (Odland *et al.*, 2000), subjects consumed a high carbohydrate breakfast and snack 1-2 hours before testing which probably increased plasma insulin concentration and may have counteracted the expected reduction in PDH*a* by stimulating PDHP. Reduced malonyl-CoA relieves inhibition of carnitine palmitoyltransferase I (CPT-I), the rate-limiting transporting enzyme which facilitates oxidation of fatty acids and hence increases PDK activity via increased acetyl CoA and NADH (Sugden & Holness, 1994).

Table 2.17 *Resting values and the acute effect of exercise on activity of human skeletal muscle pyruvate dehydrogenase (PDH): cross-sectional studies*

Study	Subjects *n*, gender	Fasted/fed	Muscle	Exercise/Condition	Total PDH*		PDH*a*		Effect of Exercise/condition		
					Rest	Ex	Rest	Ex	PDH*t*	PDH*a*	PDH*a*/PDH*t*
(Kruszynska *et al.*, 1986)	1F, 5M	Fasted overnight	VL	rest	0.82		0.12				
(Mandarino *et al.*, 1987)	2F, 6M	Fasted overnight	VL	rest	0.50†		0.28†				
(Mandarino *et al.*, 1990)	2F, 6M	Fasted overnight	VL	rest	0.33†		0.09†				
(Constantin-Teodosiu *et al.*, 1991b)	11	NR	VL	rest	1.69		0.44				
(Peters *et al.*, 1998)	2F, 9M	Fed	VL	Mixed breakfast	4.6		~0.63				~14%
				6 days LCD then high fat breakfast	4.8		~0.18		↔	↓~70%	~4%
(Timmons *et al.*, 1998a)	9M	NR	VL	rest	-	-	0.4				
(Putman *et al.*, 1999)	7M	HCHO meal	Deltoid inactive	3 x 30s max cycle	-	-	0.71	1.30	-	↑180%	-
							-	-			
							-	1.83		↑260%	
(Ward *et al.*, 1982)	7M	Fasted, 12 – 16 h	VL	Isometric 50% MVC to fatigue, quadriceps	0.30	~0.30	~0.11	~0.12	↔	↔	38%
(Parolin *et al.*, 2000)	6 trained M	NR, HCD 2/7 pre	VL	Cycling, 15 min @ 55% $\dot{V}O_{2peak}$	-	-	~0.9	~3.2	-	↑~360%	-
(Howlett *et al.*, 1999a)	2F, 7M	2 h PP	VL	10min cycle 65% $\dot{V}O_{2peak}$	-	-	0.9	~3.3	-	↑~370%	-

Table 2.17 (cont.): *Resting values and the acute effect of exercise on activity of human skeletal muscle pyruvate dehydrogenase (PDH): cross-sectional studies*

Study	Subjects *n*, gender	Fasted/fed	Muscle	Exercise/Condition	Total PDH*			PDH*a*		Effect of Exercise/condition		
					Rest	Ex		Rest	Ex	PDH*t*	PDH*a*	PDH*a*/PDH*t*
(Odland *et al.*, 2000)	7M	1-2 h PP HCHO; 30 min saline or lipid infusion	VL	cycle 40%, 0-1 min	-	-	S	~0.75	2.07	-	↑280%	-
				1-10 min					~2.0		↑270%	
				10 min 65% $\dot{V}O_{2peak}$					~2.7		↑360%	
							Li	~0.7	1.33		↑190%	
									~1.6		↑230%	
									~2.4		↑340%	
(St. Amand *et al.*, 2000)	7M	2-3 h PP; 6 day MxD or LCD	VL	30min cycle 65% $\dot{V}O_{2peak}$	-	-	M	0.63	~2.7	-	↑~420%	-
							L	0.18	~3.0	-	↑1670%	-
(Putman *et al.*, 1993)	5 trained M	NR, HCD 3/7 pre	VL	75 % $\dot{V}O_{2peak}$ cycle to fatigue	1.85	2.87		0.69	3.11	↑	↑450%	108%
		NR, LCD 3/7 pre			1.5	2.37		0.20	1.88	↑	↑940%	79%
(Putman *et al.*, 1995b)	1F, 7M	NR, MxD 2 days pre	VL	5 min 40% $\dot{V}O_{2peak}$	~2.6	~2.7		0.32	1.28	↔	↑400%	47%
				15 min 80% $\dot{V}O_{2peak}$		~2.7			2.41		↑750%	90%
(Dyck *et al.*, 1993)	6M	2-4 h PP HCHO meal	VL	15 min 85% $\dot{V}O_{2peak}$	-	-		0.49	2.42	-	↑490%	-
(Constantin-Teodosiu *et al.*, 1991a)	8M	NR	VL	3-4min cycle 30% $\dot{V}O_{2peak}$	-	-		0.37	0.8	-	↑220%	-
				60% $\dot{V}O_{2peak}$					1.28		↑350%	
				90% $\dot{V}O_{2peak}$					1.25		↑340%	

Table 2.17 (cont.): *Resting values and the acute effect of exercise on activity of human skeletal muscle pyruvate dehydrogenase (PDH): cross-sectional studies*

Study	Subjects *n*, gender	Fasted/fed	Muscle	Exercise/Condition	Total PDH*		PDH*a*		Effect of Exercise/condition		
					Rest	Ex	Rest	Ex	PDH*t*	PDH*a*	PDH*a*/PDH*t*
(Howlett *et al.*, 1998)	3F, 4M	2-3 h PP	VL	10min cycle 35% $\dot{V}O_{2peak}$	-	-	~0.5	~1.6	-	↑320%	-
				65% $\dot{V}O_{2peak}$			0.65	~3.1		↑480%	
				90% $\dot{V}O_{2peak}$			0.40	4.45		↑1110%	
(Gibala *et al.*, 1998)	6M	Fasted overnight	VL	10min knee ext @ 60% then 100% to exhaustion	-	-	0.45	2.07 3.60	-	↑460% ↑800%	- -
(Gibala & Saltin, 1999)	2F, 4M	NR	VL	15min knee ext @ 70% then 100% to exhaustion	-	-	0.64	~2.2 ~3.2	-	↑310% ↑460%	- -
(Ward *et al.*, 1982)	5M	Fasted, 12 – 16 hr	VL	Incr. cycle to fatigue	0.30	~0.30	~0.12	~0.26	↔	↑220%	87%
	6M			140 % $\dot{V}O_{2peak}$, 1 min cycle bouts	~0.32	~0.33	~0.12	~0.20	↔	↑160%	60%
(Parolin *et al.*, 1999)	6M	NR, HCD 2 days pre	VL	3 x 30s max cycle	3.74	3.74	0.53 - 1.56	3.71 - 3.46	↔	↑700% - ↑220%	99% - 93%
(Howlett *et al.*, 1999b)	3F, 7M	NR	VL	10s max cycle	-	-	0.74	~2.4	-	↑320%	-

Ex, exercise; *, units for PDH activity are mmol·min^{-1}·kg wet weight^{-1}; PDH*a*, active PDH form; PDH*t*, total PDH; M, males; F, females; VL, vastus lateralis; †, recalculated from nmol·min^{-1}·mg protein^{-1} using a protein content of 140 mg·g ww^{-1} (Constantin-Teodosiu *et al.*, 1991b); NR, not reported; LCD, low-carbohydrate diet; MVC, maximum voluntary contraction; ext, extension; HCHO, high carbohydrate; S, saline infusion; Li, lipid infusion; Mx, mixed diet; L, LCD; PP, postprandial; MD, normal mixed diet; Incr., incremental; HCD, high carbohydrate diet.

The sensitivity of both PDK and CPT-I to their negative effectors is greater in the fed state, and under conditions of high circulating insulin the primary fate of fatty acids is esterification (Sugden & Holness, 1994). DCA administration in humans increased PDH*a* 3- to 4.8-fold at rest (Timmons *et al.*, 1998a; Timmons *et al.*, 1998b; Gibala & Saltin, 1999; Howlett *et al.*, 1999a; Howlett *et al.*, 1999b).

The effects of the longer-term regulators in human muscle are less well known than in animals. PDH*a* was generally, though not invariably, lower after an overnight fast than when subjects were fed (Tables 2.17, 2.18). A high-fat, low-carbohydrate diet (LCD) consumed for 3-6 days increased muscle acetylcarnitine content and the acetyl CoA/ CoA ratio at rest, and resulted in a lower resting PDH*a* than a 3-day high-carbohydrate diet (Putman *et al.*, 1993) or a normal mixed diet (St. Amand *et al.*, 2000). Three and 6 days after institution of a high-fat LCD, PDK activity had increased 3- to 5-fold; and concomitantly at 6 days both plasma insulin and PDH*a* were reduced ~70%, the latter to ~0.18 $mmol \cdot min^{-1} \cdot kg$ wet $weight^{-1}$ which represented ~4% of PDH*t* (Peters *et al.*, 1998). Increased PDK activity is therefore effected by a high-fat diet in humans, similar to results in rats.

2.19.7 Effects of type 1 DM on the PDH complex at rest - human studies

Only one human study has reported upon the effect of type 1 DM on muscle PDH. Basal PDH*t* and percent PDH*a* in patients with type 1 DM of ~13 years' duration with an HbA_{1c} of 11% who had fasted overnight whilst on an intravenous insulin infusion (to ensure euglycaemia - 4.4 $mmol \cdot l^{-1}$), was not significantly different (although PDH*a* was 34% lower) to non-diabetic subjects (Kruszynska *et al.*, 1986).

2.19.8 Effects of insulin on the PDH complex at rest in subjects with and without type 1 DM

Insulin infusion for 2-5 hours during a euglycaemic clamp did not affect PDH*t* in non-diabetic subjects (Mandarino *et al.*, 1987; Mandarino *et al.*, 1990; Kelley *et al.*, 1993) or in patients with type 1 DM (Kruszynska *et al.*, 1986). PDH*t* was not related to insulin sensitivity, which was lower in the patients, leading the authors to tentatively conclude that PDH synthesis is not disturbed in patients with type 1 diabetes who are maintained on regular insulin dosages (Kruszynska *et al.*, 1986).

During a hyperinsulinaemic clamp, PDH*a* rose from 14.1 to 16.1% in a non-diabetic group, and from 9.9 to 16.8% in patients with type 1 DM, with no differences between groups (Kruszynska *et al.*, 1986). A maximal effect of insulin was evident at concentrations of 40-60 $\mu U \cdot ml^{-1}$ in non-diabetics, in whom PDH*a* rose to 80% (from 56%) (Mandarino *et al.*, 1987). Insulin-induced activation of PDH was correlated with the activation of glucose oxidation and negatively correlated with fat oxidation over the physiological range of insulin concentrations (Mandarino *et al.*, 1987).

2.19.9 Effects of exercise on the PDH complex

The transformation of PDH*b* to PDH*a* increased as a function of increasing power output (Howlett et al., 1998; Putman et al., 1998) and was closely matched to pyruvate flux (Howlett et al., 1998); although one study found no further increase in PDHa when exercise intensity was increased from 60 to 90% $\dot{V}O_{2max}$ (Constantin-Teodosiu et al., 1991a) (Table 2.17). PDH*a* flux during an exhausting 30-s cycle bout was exceeded by glycolytic flux by 13-fold (Putman et al., 1995a), and during exhausting one-legged exercise was 90% of the estimated flux through the tricarboxylic acid cycle (Gibala et al., 1998).

The conversion of PDH*b* to PDH*a* occurs rapidly in intense exercise, evinced by a 3.2- to 3.4-fold rise in PDH*a* after only 6-10 s of maximal cycling (Howlett et al., 1999b; Parolin et al., 1999). Further, after 6 s in the first of three 30-s maximal cycle sprints, PDH*a* had risen from 14 to 48% of PDH*t*, and by 15 s to 95% of PDH*t* (Parolin et al., 1999). Thus full activation of the PDH complex was rapidly achieved, and was maintained for the remainder of the maximal bout, with ~99% activation evident at 30 s (Putman et al., 1995a; Parolin et al., 1999). Fatiguing exercise conducted at intensities from 100 to 140% $\dot{V}O_{2max}$ resulted in rises in PDH*a* of 1.6- to 8-fold, whilst after non-fatiguing exercise at 30 to 90% $\dot{V}O_{2max}$ PDH*a* increased from rest by 1.9- to 16.7-fold (Table 2.17). During the first and second 4-min recovery periods between 30-s sprints, PDH*a* fell but remained 2.4- to 2.9-fold above resting values (Putman et al., 1995a; Parolin et al., 1999).

2.19.10 Pyruvate and the PDH complex during intense exercise

It is likely that increased PDH*a* during intense exercise is effected by increases in pyruvate (which inhibits PDK activity and is a substrate for the PDH reaction) and mitochondrial Ca^{2+} concentration (which increases PDHP activity) rather than by changes in the ratio of acetyl CoA/CoA which does not consistently change so as to favour inhibition of PDK activity (Putman et al., 1993; Putman et al., 1995a; Howlett et al., 1998; Putman et al., 1998; Parolin et al., 1999). Acidosis has been reported to increase PDH*a* in perfused rat heart, however the role of increased muscle $[H^+]$ in intensely contracting skeletal muscle is uncertain and it has been suggested that $[H^+]$ may be of more importance in promoting the oxidation of lactate in recovery from intense exercise (Putman et al., 1995a). The role of pyruvate (and possibly $[H^+]$) as an effector of physiologically significant increases in PDH*a* was highlighted by the finding of a 2.6-fold increase in PDH*a* in inactive deltoid muscle after maximal leg cycling, contemporaneous with a 2.3-fold rise in pyruvate in the deltoid, and in the absence of changes in other regulators (Putman et al., 1999). However in less intense exercise (40-65% $\dot{V}O_{2peak}$) pyruvate was not a dominant factor in determining PDH*a* activity (Odland et al., 2000) (see below).

The diminished (probably overridden) effect of the acetyl CoA/CoA ratio during exercise as opposed to resting, fasting or postprandial conditions may be further illustrated by findings in human muscle. Dichloroacetate increased acetylcarnitine (Timmons *et al.*, 1998a; Howlett *et al.*, 1999b), and thence acetyl CoA content and PDH*a* at rest (Howlett *et al.*, 1999b), however had no affect upon accumulation of either glycolytic (Howlett *et al.*, 1999b) or TCA intermediates (Gibala & Saltin, 1999) during intense exhausting exercise of 10 s to 4 min duration. Similarly, acetate, which also increased acetylcarnitine at rest, was without effect on skeletal muscle metabolism during a 10-s cycle sprint (Howlett *et al.*, 1999b) or during 15 min cycling at 80% $\dot{V}O_{2peak}$ (Putman *et al.*, 1995b).

2.19.11 Effects of dietary manipulation on the PDH complex when exercising in the fed state

An acute infusion of lipid did not alter PDH*a* at rest, but attenuated the rise in PDH*a* activity during moderate intensity exercise (Odland *et al.*, 2000) (Table 2.17). During

the first minute of exercise, lower PDH*a* (in comparison to a saline control condition) was accompanied by higher NADH (and lower NAD^+/NADH ratio), but also by higher pyruvate (Odland *et al.*, 2000). It was concluded that NADH was the primary effector of lower PDH*a* in the early phase of exercise (Odland *et al.*, 2000). Later in exercise PDH*a* remained lower in the lipid condition despite higher muscle pyruvate content and similar NAD^+/NADH and ATP/ADP ratios; however malonyl-CoA was lower which may have allowed greater fat oxidation and reduced PDH*a* (Odland *et al.*, 2000).

A 6-day high-fat, LCD, followed by a high-fat breakfast, reduced PDH*a* by ~70% compared to a mixed diet and breakfast (St. Amand *et al.*, 2000). However during non-exhausting moderate exercise, both muscle pyruvate content and PDH*a* rose similarly to when a mixed diet had preceded the exercise, even though the ratio of acetyl CoA/ CoA was lower in the LCD condition (St. Amand *et al.*, 2000). It was concluded that the elevation of pyruvate during exercise overrode the factors that were dominant at rest (St. Amand *et al.*, 2000). These results are somewhat unexpected given that a 6- or 28-day high-fat diet induced a stable upregulation of PDK activity (Peters *et al.*, 1998; Holness *et al.*, 2000), via increased PDK4 expression, which resulted in a marked loss of sensitivity of PDK to acute inhibition by pyruvate (Holness *et al.*, 2000). (However, it is possible that the more-pyruvate-sensitive PDK2 may have been inhibited by exercise, which may have then allowed rapid flux through PDH*a*; however this has never been tested.) The pyruvate concentration required for 50% PDH*a* was increased to ~2 $mmol \cdot l^{-1}$ (Holness *et al.*, 2000); which is likely to be of physiological relevance since a 30-min exercise bout increased muscle pyruvate to ~0.5 $mmol \cdot kg\ d.m.^{-1}$ (St. Amand *et al.*, 2000), and a 30-s maximal exercise bout resulted in a muscle pyruvate content of 3.8 $mmol \cdot kg\ d.m.^{-1}$ (Nevill *et al.*, 1989), although it may be difficult to equate *in vivo* pyruvate contents with an incubation media concentration.

2.19.12 The effect of fasting on the PDH complex during exercise

No human studies have examined the effect of exercising in the fasted versus the fed state on PDH*a* or PDK activities.

In support of Holness *et al.* (2000), following 10-15 min electrical stimulation of the perfused hindlimb or the gastrocnemius in 48-60 hr fasted rats, PDH*a* was ~2- to 3-fold lower than in the fed condition (Hennig *et al.*, 1975; Hagg *et al.*, 1976). Sixty min voluntary treadmill running in fasted rats (with similar resting glycogen content to fed rats) also markedly attenuated the rise in PDH*a* in gastrocnemius muscle in comparison to fed rats (Brozinick *et al.*, 1988). Fasting thus appears to result in a stable increase in PDK activity that persists during exercise. This is likely to be related to hyperphosphorylation of PDH*b*, which is thought to be important in retarding reactivation of the complex by PDHP (Sale & Randle, 1982; Sugden & Holness, 1994), and hence maintaining low PDH*a* after fasting (Wu *et al.*, 1999).

2.19.13 Effects of exercise on the PDH complex in type 1 DM

No human studies have examined the effect of exercise on the PDH complex in patients with type 1 DM. Thus the effect of hypo-, normo-, or hyperinsulinaemia and a fixed insulin concentration on PDH*a* during and after intense exercise (indeed any exercise intensity) is unknown.

In rats, electrical stimulation of the gastrocnemius muscle or the hindquarter (via the sciatic nerve) for 10-15 min at 5-10 Hz did not alter PDH*t*, but increased PDH*a* by 2.2- to 3.6-fold in control rats and by only 1.6- to 1.8-fold in diabetic rats (Hennig *et al.*, 1975; Hagg *et al.*, 1976) thus likely highlighting the effect of a stable increase in PDK activity with insulin deficiency. Fuller & Randle (1984) suggested that the stable mechanism of increased PDK activity, that is independent of NADH and acetyl CoA, provided an explanation for the effect of both DM and starvation to lower PDH*a* in both resting and contracting skeletal muscle.

2.19.14 Effects of sprint training on the PDH complex

No sprint training studies have reported upon PDH activity, and few studies have examined the effect of other forms of exercise training on the PDH complex.

2.19.15 Effects of other forms of exercise training on the PDH complex

2.19.15.1 PDHt- animal studies

PDH*t* was unchanged after 1-8 weeks of endurance training (Brozinick *et al.*, 1988; (Nakai *et al.*, 1999) in rats. An interesting and perplexing issue raised in one study (Brozinick *et al.*, 1988) was the lack of change in PDH*t* despite evidence of increased mitochondrial density after training. Endurance training may therefore reduce PDH*t* concentration, or alternatively, may alter PDH such that full activation is not evident when standard assay conditions for total enzyme activity are applied (Brozinick *et al.*, 1988).

2.19.15.2 PDHt- human studies

PDH*t* was not altered by 7 days of endurance training (Putman *et al.*, 1998) or 5 months of "sprint" strength training (Ward *et al.*, 1986), and did not differ between elite athletes and untrained subjects (Ward *et al.*, 1982) (Table 2.18).

2.19.15.3 PDHa and PDK at rest – animal studies

PDH*a* was increased in rested rats after 1 and 4 weeks of endurance training (Brozinick *et al.*, 1988). In contrast, after 8 weeks of endurance training (voluntary running) in rats, PDH*a* in the gastrocnemius was 44% lower (7.5% of PDH*t*) in the rested, fed state compared to sedentary rats (15% of PDH*t*) (Nakai *et al.*, 1999). The activities of PDK and 3-hydroxyacyl CoA dehydrogenase (HAD) were 25% and 37% higher, respectively; acetyl CoA and CoA were 43% and 28% higher (however the ratio of acetyl CoA/CoA was unchanged); and pyruvate was 39% lower in the muscle of the trained rats than in the sedentary rats (Nakai *et al.*, 1999). It was suggested that greater β–oxidation in the trained muscle contributed to the elevation of PDK activity, which probably accounted for the lower PDH*a* (Nakai *et al.*, 1999). In contrast to the effect when fed, starvation for 24 hours reduced PDH*a* equally in sedentary rats and those trained for 8 weeks by voluntary running (Nakai *et al.*, 1999). The difference

between the fasted and fed state is probably linked with changes in insulin sensitivity and possibly the acute effect of fasting overriding an effect of training. The sensitivity of PDK to its negative effectors is greater in the fed state, and under conditions of high circulating insulin the primary fate of fatty acids is esterification rather than oxidation (Sugden & Holness, 1994).

The reason for the marked contrast between the results of the two studies (Brozinick *et al.*, 1988; Nakai *et al.*, 1999) is not clear, particularly since the same muscle was examined in each study. Food was permitted *ad libitum* during training in the earlier study (Brozinick *et al.*, 1988) but the nutritional state prior to testing was not detailed. It is possible that differing nutritional conditions prevailed after endurance training, which may explain the unexpected finding of higher resting PDH*a*. The authors (Brozinick *et al.*, 1988) acknowledged a higher resting PDH*a* to be opposite to their hypothesis, and in conflict with the *in vivo* findings. Greater training volume (voluntary running, averaging 5 km per day for 8 weeks) in one study (Nakai *et al.*, 1999) may have resulted in greater adaptation in terms of fat metabolism than the other study (120 min consecutive treadmill running per day, 6 days per week for 4 weeks, i.e. 3 km per session) (Brozinick *et al.*, 1988); however this seems unlikely to explain the study differences.

2.19.15.4 PDHa at rest - human studies

PDH*a* at rest was higher in elite athletes than the untrained (Ward *et al.*, 1982) and was increased after 5 months of strength training (Ward *et al.*, 1986), but was not changed by 7 days of endurance training (Putman *et al.*, 1998) (Table 2.18). In trained subjects, a 3-day high-fat, LCD reduced PDH*a* at rest compared with a 3-day high carbohydrate diet (Putman *et al.*, 1993) (Table 2.17). The ratio of acetyl CoA to CoA was higher in the LCD condition, consistent with a lower PDH*a* (Putman *et al.*, 1993).

2.19.16 Exercise training and the effect of insulin on the PDH complex at rest

2.19.16.1 Animal studies

Hyperinsulinaemia (euglycaemic) at rest increased PDH*a* ~3-fold in sedentary rats after 24 hours starvation, but increased PDH*a* ~5.5-fold in rats that had been trained by voluntary running for 8 weeks (Nakai *et al.*, 1999). It was suggested that endurance training may have increased muscle glucose uptake and glycolytic flow to a greater extent than in sedentary rats or may have increased PDHP (Nakai *et al.*, 1999). However, it is also possible that the amount of PDHP was unchanged, but its sensitivity to the activating effect of insulin was increased after training. Enhanced sensitivity of PDK to activating/deactivating factors and/or PDHP to activating factors may explain or compensate for the apparent lack of training effect upon PDH*t*.

2.19.16.2 Human studies

The effect of exercise training on the response of the PDH complex to a hyperinsulinaemic euglycaemic clamp has not been examined in human subjects.

2.19.17 The effect of exercise on the PDH complex after exercise training

Two longitudinal studies have examined the effect of exercise training on PDH*a* during exercise at submaximal workloads. Despite metabolic changes that supported an increased fat and reduced CHO oxidation during exercise after training, the nearly complete conversion of PDH*b* to PDH*a* evident during cycling at 75% $\dot{V}O_{2peak}$ was not significantly different after 7 days of endurance training when exercising at the same absolute workload (Putman *et al.*, 1998) (Table 2.18). Similarly, complete conversion of PDH*b* to PDH*a* was evident after 15 min exercise at 75% maximum heart rate and did not differ after 5 months of strength training (Ward *et al.*, 1986).

However, when trained subjects consumed a high-fat, LCD for 3 days prior to exercise testing, the conversion of PDH*b* to PDH*a* during an identical fatiguing exercise bout at 75% $\dot{V}O_{2max}$ was markedly lower than when a 3-day high carbohydrate diet (HCD) preceded the exercise test (Putman *et al.*, 1993) (Table 2.17). During exercise, the acetyl CoA/ CoA ratio increased in the HCD condition,

Table 2.18 *Studies reporting the effect of exercise training on human skeletal muscle pyruvate dehydrogenase (PDH) activity at rest and during exercise*

Study	Subjects	Intervention	Mus.	Test	Fasted/ fed	Pre-intervention				Post-intervention				Effect of intervention	
						Total PDH*		PDH*a*		Total PDH		PDH*a*			
						Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex	PDH*t*	PDH*a*
(Ward *et al.*, 1982)	4 controls, 4 athletes	Cross-sectional comparison	VL	Rest	Fasted, 12–16h	0.30	-	0.12	-	0.31	-	0.22	-	Same	↑Ath.
(Ward *et al.*, 1986)	15 (Rest) 6 (Ex) M	5/12 strength 'sprint' training	T br.	Arm cranking 15min @ 75% max HR	NR	0.30	0.30	0.11	0.28	0.30	0.30	0.19	0.30	↔	rest↑
(Putman *et al.*, 1998)	7M	1/52 endurance training at 60% pre-training $\dot{V}O_{2max}$	VL	Cycling, 15 min 30% p-t $\dot{V}O_{2peak}$	Fed, 1-2hPP	3.22	-	0.87	1.81	3.52	-	0.84	1.65	↔	R,E↔
				65% p-t $\dot{V}O_{2peak}$					3.58				2.60		
				75% p-t $\dot{V}O_{2peak}$					3.89				3.78		

Mus., muscle; *, units for PDH activity are mmol·min^{-1}·kg wet weight^{-1}; PDH*a*, active PDH form; Ex, exercise; PDH*t*, total PDH; M, male; VL, vastus lateralis; Ath. Athletes; 5/52, 5 weeks; T br, triceps brachii; NR, not reported; R,E, rest, exercise; 5/12, 5 months; 1/52, 1 week; p-t, pre-training; PP, postprandial.

but decreased in the LCD condition, despite the changes evident in PDH*a* (Putman *et al.*, 1993). It was suggested that other factors, such as calcium, insulin and pyruvate concentrations, may have assumed greater importance than the acetyl CoA/CoA ratio during exercise (Putman *et al.*, 1993). Lower pyruvate accumulation in the LCD state (Putman *et al.*, 1993) is likely to have reduced inhibition of PDK, and would also have provided less substrate for the PDH reaction, both consistent with lower PDH*a*. Additionally, the considerably lower conversion of PDH*b* to PDH*a* during an identical exercise bout after the LCD in the trained subjects (Putman *et al.*, 1993) may indicate that a chronic effect of increased fat metabolism at rest persisted to an extent during exercise, possibly due to a stable increase in PDK activity (similar to the diabetic state).

2.19.18 Effects of training on the PDH complex during exercise in subjects with type 1 DM

No studies have examined the effect of exercise training on the PDH complex during intense exercise in type 1 DM.

In summary, the reaction catalyzed by the PDH complex is central in the determination of the rate of pyruvate oxidation (which is reflected by PDHa), and hence the relative contribution of carbohydrate-derived fuels to oxidative metabolism at rest and during exercise. PDHt is unchanged by dietary, hormonal, exercise, or exercise training manipulations. Intense exercise rapidly increases the proportion of PDHa, with full activation evident within 6-15 s of commencing a maximal 30-s sprint. Starvation and insulin deficiency reduce PDHa via a stable increase in PDK activity, associated with increased protein expression and probably also linked with the hyperphosphorylation of PDHb. The rise in PDHa with exercise may also be attenuated by starvation and diabetes in animals, however this has not been investigated in humans. One study has reported PDHa at rest in type 1 DM, however, no studies have examined the PDHa response to exercise. Also, no studies have examined the effect of sprint training on PDHa at rest or during maximal exercise. A recent study in rodents demonstrated lower muscle pyruvate, lower PDHa and higher PDK activity at rest, but greater PDHa increase in response to insulin after 8 weeks of endurance training; however the effect of

exercise was not examined. The effect of exercise training on PDHa in subjects with type 1 diabetes mellitus is unknown.

2.20 Muscle oxidative metabolism

2.20.1 Contribution of oxidative metabolism to total ATP generation during repeated bouts of maximal exercise

In the final sprint of ten, 6-s maximal sprints, power output had declined only 27%, whilst absolute ATP production from anaerobic metabolism was ~66% lower than in the first sprint (Gaitanos *et al.*, 1993). It was suggested that a significant shift to aerobic metabolism occurred during the later 6-s sprints (Gaitanos *et al.*, 1993). In the first 6 s of an exhausting 30-s cycle sprint 9% of ATP was generated via oxidative phosphorylation, and by the final 15 s of the sprint 60% of ATP was produced oxidatively (Parolin *et al.*, 1999). Averaged over the duration of the exhausting 30-s cycle sprint, 28-29% of total ATP was generated by oxidative metabolism (Withers *et al.*, 1991; Putman *et al.*, 1995a). In a second 30-s sprint, $\dot{V}O_2$ was 18% higher, and power output 18% lower (Bogdanis *et al.*, 1996). In the first 6 s of the third 30-s bout ~24% of ATP turnover was derived from oxidative metabolism, and in the final 15 s ~80% was produced oxidatively (Parolin *et al.*, 1999). Averaged over the duration of the third 30-s sprint bout, oxidative metabolism accounted for 63% of total ATP generation (Putman *et al.*, 1995a).

Thus, a single maximal sprint, and repeated maximal sprints, even if of very brief duration, become progressively oxidative in nature. This shift in ATP source is imposed upon the muscle by the inability to maintain or restimulate glycogenolysis/ glycolysis. Sprint training is therefore likely to induce both oxidative and non-oxidative metabolic adaptations.

2.20.2 Effects of sprint training on the contribution of oxidative metabolism to total ATP generation during maximal exercise

No studies have specifically examined the effects of sprint training on muscle oxidative metabolism. However, following 7 weeks of sprint training, which entailed

repeated 30-s sprint bouts, peak $\dot{V}O_2$, which occurred in early recovery from a maximal exhausting 30-s cycle sprint, tended to be higher (McKenna *et al.*, 1997b) (also see section 2.3.4). The small increase in $\dot{V}O_2$ was suggested to be due to an increase in muscle blood flow, and suggested to be consistent with greater oxidative metabolism during maximal exercise following sprint training (McKenna *et al.*, 1997b). However, in another study (Nevill *et al.*, 1989), $\dot{V}O_2$ during a maximal 30-s treadmill sprint to exhaustion was unchanged after sprint training.

Two studies in which maximal work was matched before and after sprint training found either no change in $\dot{V}O_2$ during a 2-min run at 110% pre-training $\dot{V}O_{2peak}$ after 8 weeks of training (Nevill *et al.*, 1989), or a 3% lower $\dot{V}O_2$ averaged across 12-24, 60-s trials at 120% $\dot{V}O_{2peak}$ after 3 days of training (Green *et al.*, 1987a).

Thus effects of sprint training on indirect assessments of muscle oxidative metabolism during either exhausting or matched-work exercise remain equivocal, and direct estimates have not been reported.

2.20.3 Maximal exercise and flux through the tricarboxylic acid (Krebs) cycle

Recent work demonstrated that flux through the TCA cycle was increased ~70-fold during 10 min of knee extensor exercise at 60% of maximum capacity, and was further increased to be 100-fold greater than rest after exercise to exhaustion at 100% of maximum capacity (Gibala *et al.*, 1998). Despite the marked increase in TCA flux (estimated from muscle $\dot{V}O_2$), the total concentration of seven TCA intermediates was only increased 1-fold during the submaximal exercise and 3-fold during maximal exercise (Gibala *et al.*, 1998). The concentration of 2-oxoglutarate decreased during exercise, whilst concentrations of each of the other intermediates increased, with the major contributions being a 6-fold increase in malate, and smaller rises in succinate and fumarate, i.e. the intermediates in the second span of the TCA (Gibala *et al.*, 1998). The increase in the intermediates was suggested to reflect an imbalance between the rate of pyruvate production and its rate of oxidation. Coincident with the

changes at exhaustion was a 7.1-fold rise in the activity of PDH*a*; the flux through which represented ~90% of the estimated TCA flux at exhaustion (Gibala *et al.*, 1998). This small difference may have been due to small measurement inaccuracies or to a small portion of acetyl-CoA being derived from intramuscular triglycerides (or amino acids) rather than via glycolysis during maximal exercise (Gibala *et al.*, 1998).

It has been suggested that a more rapid activation of oxidative energy delivery, and hence lower ADP accumulation, may minimise ATP regeneration from substrate level phosphorylation during the rest-to-work transition in intense exercise (Greenhaff & Timmons, 1998). PDH activation via an inhibitor of its kinase, dichloroacetate, may achieve a more rapid onset of oxidative metabolism (Greenhaff & Timmons, 1998). Infusion with dichloroacetate (DCA) elevated PDH*a* by 4-fold and reduced the total concentration of five measured TCA intermediates by 50% at rest (Gibala & Saltin, 1999). However, following maximal exhausting knee extensor exercise (~4 min) total TCA intermediate concentration, PDH*a*, and muscle metabolite contents did not differ compared to that during a saline infusion, nor did time to exhaustion differ between trials (Gibala & Saltin, 1999). Similarly, in maximal cycling exercise of 10 s duration, DCA infusion increased resting PDH*a*, however did not result in a greater increase in oxidative ATP provision during exercise compared with a saline trial (Howlett *et al.*, 1999b). Post-exercise, PDH*a* was higher in the DCA trial, as was acetylcarnitine, leading the authors to suggest that a limitation to oxidative metabolism (or in O_2 availability) may reside in a site distal to PDH in exercise of very brief duration (Howlett *et al.*, 1999b).

2.20.4 Effects of sprint training on flux through the TCA cycle during maximal exercise

The effect of sprint training on flux through the TCA cycle during maximal exercise is unknown.

2.20.5 Effects of sprint training on enzymes associated with carbohydrate and/or fat oxidative metabolism

Sprint training has been reported to increase muscle mitochondrial enzymes. Citrate synthase (CS) was increased 12-36% following 6-7 weeks of sprint training (Jacobs *et al.*, 1987; MacDougall *et al.*, 1998). A non-significant 14-18% increase in CS was

found after 7-8 weeks of sprint training (Linossier *et al.*, 1993; Pilegaard *et al.*, 1999). Eight weeks of sprint training had no affect on succinate dehydrogenase (SDH) in one study (Sharp *et al.*, 1986), however, SDH was increased 55-65% in others (Cadefau *et al.*, 1990; MacDougall *et al.*, 1998), and malate dehydrogenase was also increased by 29% (MacDougall *et al.*, 1998).

The activity of an enzyme in fatty acid oxidation, β-hydroxyacyl-CoA dehydrogenase (HAD), was increased by 20% following 8 weeks of one-legged high-intensity knee-extensor training, which entailed repeated 30-s or 60-s efforts (Pilegaard *et al.*, 1999). Seven weeks of two-legged sprint training (either repeated 5 or 30-s cycling bouts) increased HAD by 14-39%, however the change was not significant (Linossier *et al.*, 1993; MacDougall *et al.*, 1998).

Sprint training may thus enhance the capacity to oxidize both carbohydrates and fatty acids in skeletal muscle.

2.20.6 Fat oxidation during maximal exercise

Few studies have examined intramuscular fat metabolism during maximal exercise. Brief, intermittent exercise (15 s work: 15 s rest) at 100% $\dot{V}O_{2peak}$ reduced intramuscular triglycerides by ~20% within 5 mins of commencing exercise (Essén *et al.*, 1977; Essén, 1978). Plasma glycerol was increased immediately after the first of four 30-s maximal cycling bouts, and continued to increase, peaking after the final bout ~5-fold higher than rest, whereas plasma FFA was unchanged from rest (McCartney *et al.*, 1986). It was suggested that intramuscular triglycerides may have served as an important fuel in repeated maximal exercise (McCartney *et al.*, 1986). A previous study, in which FFA tracers were used during heavy exercise (70% maximum power), yielded similar results and conclusions (Jones *et al.*, 1980).

The relative contribution of fat oxidation to muscle ATP generation during a single bout or repeated bouts of maximal exercise is unknown. The effect of type 1 DM on intramuscular fat utilization during maximal exercise is similarly unknown.

2.20.7 Effects of sprint training on fat metabolism during maximal exercise

No studies have examined the effect of sprint training on intramuscular fat metabolism during maximal exercise.

2.20.8 The 'glucose-fatty acid cycle' and muscle metabolism

In 1963, Randle *et al.* (Randle *et al.*, 1963) demonstrated that plasma fatty acids and ketone bodies reduced glucose uptake in rat heart and diaphragm muscle, resulted in intracellular accumulation of glucose and glucose 6-phosphate (G 6-P), and markedly impaired pyruvate oxidation; thus resembling the metabolic state of diabetic animals. Conversely, it was proposed that glucose uptake by adipose tissue would inhibit fatty acid release, which would reduce the formation of ketone bodies in the liver; thus the relationship between glucose and fatty acid metabolism was reciprocal, and was termed the 'glucose fatty-acid cycle' (Randle *et al.*, 1963). A number of studies have supported, but others refuted the operation of the cycle in humans (briefly reviewed in (Randle *et al.*, 1988; Randle *et al.*, 1994; Rasmussen & Wolfe, 1999); results appear to depend on experimental conditions (Ferrannini *et al.*, 1983a; Nuutila *et al.*, 1992; Saloranta *et al.*, 1993) and muscles examined (Jenkins *et al.*, 1988; Sidossis & Wolfe, 1996).

It has recently been proposed that the intracellular availability of glucose, rather than FFA, determines substrate oxidation in human skeletal muscle (Sidossis & Wolfe, 1996). During an hyperinsulinaemic-hyperglycaemic clamp, combined with a lipid and heparin infusion to maintain fatty acid availability, long-chain fatty acid oxidation was inhibited in skeletal muscle of resting humans (Sidossis *et al.*, 1996; Sidossis & Wolfe, 1996). The authors concluded that high glucose and/or insulin concentrations regulated fatty acid oxidation by directly or indirectly increasing the formation of malonyl-CoA (Sidossis *et al.*, 1996). Malonyl-CoA inhibits the activity of carnitine palmitoyl-transferase 1 (CPT 1), the rate-limiting enzyme which regulates the transport of fatty acyl-CoA into the mitochondrial matrix, and thus modulates the rate of long-chain fatty acid uptake (Sidossis *et al.*, 1996). Conditions of high insulin concentration, secondary to, and coupled with hyperglycaemia, exist during recovery from intense exercise in non-diabetics, thus it may be speculated that both lipolysis and intramuscular FFA oxidation may be suppressed during early recovery. In

contrast, hyperglycaemia with a fixed insulin concentration will prevail in recovery from maximal exercise in subjects with type 1 DM. Intramuscular FFA oxidation may therefore be somewhat less inhibited in early recovery in these subjects, however this has not been studied.

In summary, a single bout and repeated bouts of maximal exercise, even of very brief duration become progressively oxidative. It has been suggested that a more rapid activation of oxidative energy delivery, and hence lower ADP accumulation, may minimise ATP regeneration from substrate level phosphorylation during the rest-to-work transition in intense exercise. The site/s of limitation may lie distal to PDH. The contribution of intramuscular triglyceride oxidation to ATP generation during maximal exercise has been little investigated. Sprint training has been reported to up-regulate mitochondrial enzyme activity. Sprint training may be anticipated to enhance both oxidative and non-oxidative metabolism.

3. STUDY 1

3.1 Introduction

Study 1 examined the effects of sprint training upon metabolic, ionic, and cardiorespiratory responses to maximal constant load exercise in untrained men, under two different conditions:

1. Exercise to exhaustion
2. Matched-work exercise, in which pre- and post-training work was the same

3.2 Aims and Hypotheses

3.2.1 Aims:

1. To investigate the effects of a 7-week programme of sprint training programme on integrated respiratory, metabolic and ionic responses to maximal exercise in young, untrained subjects.
2. To compare the effects of sprint training upon respiratory, metabolic and ionic responses during maximal exercise to exhaustion and during maximal exercise in which post-training work matched pre-training work. As a consequence of this comparison Study 1 aimed to clarify some discordant findings in the sprint training literature.

3.2.2 Experimental Hypotheses:

3.2.2.1 Metabolism

1. Greater work will be performed during maximal exercise to exhaustion after sprint training, however the degradation of glycogen and PCr, rise in $[H^+]$ and accumulation of Lac^- within the muscle will be similar to that evident at exhaustion prior to training.
2. The net degradation of ATP and accumulation of IMP will be reduced during maximal exercise to exhaustion after sprint training, even though greater work will be performed.
3. Associated with improved cellular energy balance, the rate of anaerobic ATP production will be attenuated and ATP generation will be accomplished more oxidatively during maximal exercise to exhaustion after sprint training.

4. After training, during matched-work maximal exercise (identical exercise conditions), the degradation of glycogen and ATP, the rise in muscle [H^+] and accumulation of Lac^- and IMP will be attenuated.
5. After training, during matched-work maximal exercise (identical exercise conditions), the rate of anaerobic ATP production will be attenuated and ATP generation will be accomplished more oxidatively.
6. Greater work will be performed during maximal exercise to exhaustion after sprint training, and therefore peak plasma [NAdr] and [Adr] will be higher than in the exhausting test prior to training.

3.2.2.2 Ion regulation

1. Greater work will be performed during maximal exercise to exhaustion after sprint training, however peak values for plasma [K^+], [H^+], and [Lac^-] will be similar to that evident at exhaustion prior to training.
2. After training, during matched-work maximal exercise (identical exercise conditions), peak plasma [K^+] will be lower, i.e. a reduction in exercise-induced hyperkalaemia will be evident, and [H^+] and [Lac^-] will be reduced.

3.2.2.3 Cardiorespiratory variables

1. Greater work will be performed during maximal exercise to exhaustion after sprint training, however peak cardiorespiratory responses will be similar to those evident during exhausting exercise prior to training.
2. When normalised for the duration of exercise, 'mean' HR, $\dot{V}_E$, and $\dot{V}CO_2$ will be lower, whilst $\dot{V}O_2$ will be higher when exercising to exhaustion after training.

3.3 Methods

3.3.1 Subjects

Seven healthy, recreationally active male subjects, gave informed consent to participate in Study 1, which was approved by The University of Sydney Human Research Ethics Committee. Prior to inclusion in the study each subject was informed in writing of the purpose of the study, was verbally apprised of all experimental procedures, completed a medical screening questionnaire and provided signed informed consent. Subjects abstained from caffeine and alcohol consumption, refrained from strenuous exercise for the 24 hours prior to each exercise test, and on each occasion presented at the laboratory 2 to 3 hours postprandial.

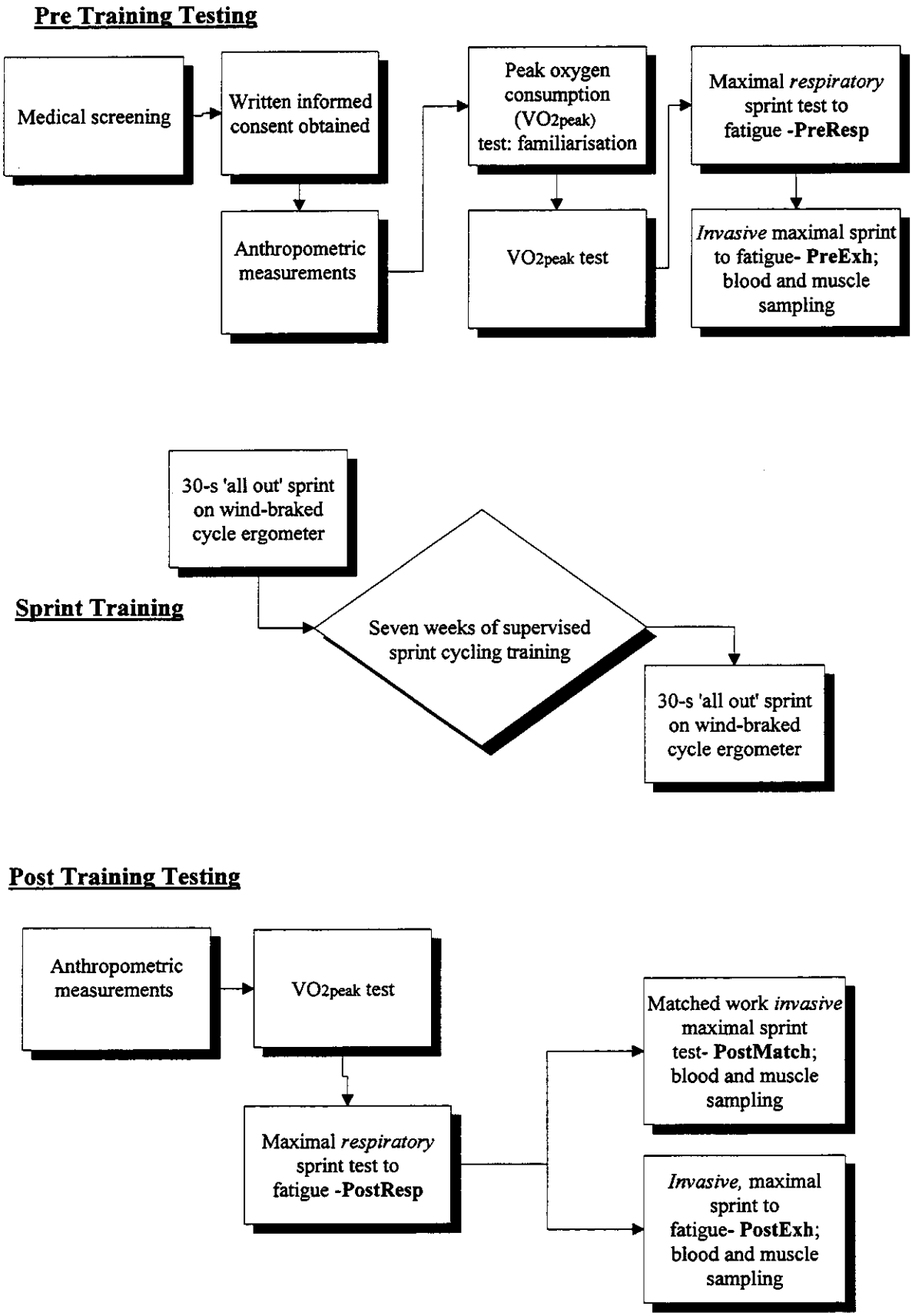
3.3.2 Research Design and Experimental Overview

Figure 3.1 illustrates the research design and experimental overview.

In the 2 weeks prior to training, following anthropometric measurements on the dominant leg of each subject, two incremental tests to assess peak oxygen consumption ($\dot{V}O_{2peak}$) were completed, and two maximal sprints were conducted to fatigue at a power output equivalent to 130% $\dot{V}O_{2peak}$. Expired ventilation was measured, expired gases analysed for O_2 and CO_2 fractions, and derived variables, e.g. $\dot{V}O_2$, determined at rest, during exercise and in recovery in the first maximal test (designated 'PreResp'). Arterialised venous blood was sampled at rest, immediately after fatiguing exercise, and in recovery in the second of the maximal tests (designated 'PreExh'), and analysed for plasma ion concentrations, pH, blood gases and catecholamine concentrations. In the same test, muscle biopsies were taken at rest and immediately after fatiguing exercise and analysed for pH and metabolites.

Subjects then participated in 7 weeks of supervised cycle sprint training conducted in the laboratory. The first 30-s sprint bout of each of the first and final training sessions was conducted on an air-braked cycle ergometer which was instrumented to provide total work and peak power.

Figure 3.1 Study 1 Research Design and Experimental Overview



In the 8 days following the last training session anthropometric measurements were made, one incremental test to reassess $\dot{V}O_{2peak}$ was performed and three maximal tests at 130% $\dot{V}O_{2peak}$ were conducted. Each of the maximal tests was conducted at the same power output as in PreExh. In the first maximal test (designated 'PostResp'), which was conducted to fatigue, expired ventilation was measured and expired gases were analysed. The remaining two invasive maximal tests were conducted in random order. One maximal test (designated 'PostMatch') was time-matched with the pre-training exhaustion time, rather than extending until post-training exhaustion; whereas the other test was continued until exhaustion (designated 'PostExh'). Muscle biopsies were performed and arterialised venous blood was sampled during each of the invasive maximal tests (PostMatch, PostExh), with sampling times matched as closely as possible with those of the pre-training test (PreExh).

3.3.3 Training

Subjects participated in a supervised, progressive high intensity intermittent cycling training programme, conducted in the laboratory, 3 times per week for 7 consecutive weeks, as described by McKenna et al. (McKenna *et al.*, 1993). Each training session consisted of four to ten 30-s 'all out' sprints on a mechanically braked cycle ergometer (Monark 668, Varberg, Sweden), with each sprint separated by a 3 to 4 min passive rest interval. Each subject completed 21 training sessions. The flywheel tension was kept constant for the duration of the training programme at 0.075 $kp \cdot kg^{-1}$ body mass. Training overload was imposed by progressively increasing the number of 30-s sprint bouts from four per session in week 1, to six per session in week 2, eight per session in week 3, through to ten per session in weeks 4 - 7, and by reducing the rest interval from 4 to 3 min in weeks 5 - 7 of the training period.

Subjects warmed up with 5 min of continuous cycling at the start of each training session. Just prior to each 30-s bout the subject was instructed to pedal as quickly as possible, after which the tension was rapidly applied to the flywheel by the experimenter and the sprint commenced. Subjects were verbally encouraged to produce a maximum effort during each bout. A 5 - 10 min recovery period, consisting of slow continuous cycling at a minimal load completed each session. Heart rate and

ECG were monitored for the first two training sessions, during which no anomalies were observed, and were thus not monitored thereafter. Water was taken *ad libitum* during training sessions, and a fan applied upon request.

3.3.4 Test Procedures

3.3.4.1 Anthropometric measurements

Bare-footed standing height was measured to the nearest 0.5 cm with a stadiometer (Holtain Ltd, Crymmych, Pembs., U.K.) and body mass was measured to the nearest 0.01 kg (Wedderburn Scales, Australia). Body mass index (BMI) was calculated as weight·height^{-2}, and expressed as kg·m^{-2}. Total leg volume of the dominant leg was estimated at rest before and after the training programme, using the method of Jones & Pearson (1969). Briefly, this involves the partitioning of the leg into six segments that are considered to be truncated cones. The height above the floor and the circumference of the leg at seven sites was measured and served to delineate the six truncated cones. The volume of each cone was calculated according to the following formula - $1/3\ h\ [a + \sqrt{(ab)} + b]$, where h is height, and a and b are the areas of two parallel surfaces derived from circumference measurements (Jones & Pearson, 1969).

Harpenden calipers (John Bull, British Indicators Ltd, England) were used to obtain skin-fold thicknesses at four sites, i.e. anterior and posterior thigh, and medial and lateral calf. Caliper readings were corrected to account for the double thickness of skin and fat necessarily measured by the technique.

Muscle plus bone volume (inner cone) was calculated after subtracting the summed, corrected thigh and calf fat readings from their respective diameters. Subcutaneous fat volume was estimated to be the difference between total leg volume and muscle plus bone volume. Muscle volume was calculated assuming bone volume to constitute 11% of the inner cone (Jones, 1970).

3.3.4.2 Gas Exchange and Ventilation

Gas exchange and ventilation were measured during the incremental tests and non-invasive maximal tests using open-circuit spirometry. Subjects breathed through a low-resistance Hans Rudolph valve (# 2700) supported by a head-piece; a nose-clip occluded nasal airflow. Expired volume ($\dot{V}E$, $l \cdot min^{-1}$, BTPS) was determined using a Hans Rudolph flow transducer and pneumotachometer (model 3813), and mixed expired fractions of O_2 (F_EO_2) and CO_2 (F_ECO_2) were determined in dried gas by a fast response, zirconium cell oxygen analyser (S-3A/1, Ametek, Thermox Instruments, PA) and an infra-red carbon dioxide analyser (CD-3A, Ametek). The gas analysers and pneumotach were calibrated just before, and immediately after each test; the former using a tank calibration gas (β-grade, BOC gases) of known composition (determined using an α-grade standard, BOC) and room air, and the latter with a manual 3 litre syringe (Vacumed Inc., U.S.A.). Voltage outputs from the cycle ergometer, ECG, pneumotach, and gas analysers were transmitted to a computer via a 12-bit analogue to digital board. Barometric pressure (P_B, mmHg), ambient temperature (°C), and anthropometric data were manually entered into the integrated Deltacom computer system using custom-written software (ExerStress, T.J. Turner, Sydney, Australia) which displayed the following raw and derived variables (averaged over 10-s periods): cycle power output, HR, F_EO_2, F_ECO_2, minute ventilation ($\dot{V}E$ $l \cdot min^{-1}$, STPD and BTPS), oxygen uptake ($\dot{V}O_2$ $l \cdot min^{-1}$, $ml \cdot kg^{-1} \cdot min^{-1}$, STPD), carbon dioxide output ($\dot{V}CO_2$ $l \cdot min^{-1}$, STPD), respiratory exchange ratio (RER), and ventilatory equivalents for O_2 ($\dot{V}E \cdot \dot{V}O_2^{-1}$) and CO_2 ($\dot{V}E \cdot \dot{V}CO_2^{-1}$).

3.3.4.3 Incremental tests to determine $\dot{V}O_{2peak}$

Pre-Training. Subjects completed two incremental tests to determine peak oxygen consumption ($\dot{V}O_{2peak}$) on an electronically braked cycle ergometer (Ergoline 800s, Mijnhardt, Netherlands) prior to training. The first test served to familiarise each subject with the cycling protocol and the expired gas collection equipment and procedures. The test was repeated two days later and submaximal and maximal values for $\dot{V}O_2$ were obtained. The cycle ergometer was calibrated with precision weights prior to each test.

Subjects were weighed, wearing a pair of shorts, to the nearest 0.01 kg (Wedderburn Scales, Australia), then had ECG electrodes attached in the CM5 configuration. Electrocardiograms were displayed (LifeScope 6, Medtel, Australia) and monitored throughout the test. Subjects were seated at a self-selected saddle height (previously determined) on the cycle ergometer and had their feet secured to the pedals by toe-clips. A 5 min warm up at 20 W at a comfortable, self-selected cadence preceded the incremental test.

Subjects then cycled for 4 minutes at each of four work rates, i.e. 60, 90, 120, and 150 W, to obtain steady state oxygen uptake. This was immediately followed by a 25 $W \cdot min^{-1}$ incremental test (commencing at 150 W) to volitional fatigue. $\dot{V}O_{2peak}$ was defined as the mean of the three highest, consecutive $\dot{V}O_2$ s. After the completion of the incremental test the power was reduced to 20 W, and subjects cycled for 10 min as an active form of recovery.

Post-Training. The test procedure to to reassess submaximal $\dot{V}O_2$ and $\dot{V}O_{2peak}$ post-training was identical to that of the pre-training incremental test, and was conducted 2.6 ± 0.2 days after the final training session.

3.3.4.4 Calculation of the power output required to elicit 130% $\dot{V}O_{2peak}$

Oxygen consumption for each of the four steady state power outputs was calculated from the mean $\dot{V}O_2$ for the final minute at each load. A linear regression was applied to steady state $\dot{V}O_2$ and power output data for each subject, and in conjunction with the measured $\dot{V}O_{2peak}$, used to determine the power output for the subsequent maximal test, which was conducted at 130% $\dot{V}O_{2peak}$. The cycle ergometer was designed to allow power increments of 5 W, therefore the power output calculated to elicit 130% $\dot{V}O_{2peak}$ was rounded off to the nearest 5 W.

3.3.4.5 Constant power tests conducted at 130% $\dot{V}O_{2peak}$

Pre-Training. Prior to training, two maximal 'sprint' tests to fatigue were conducted on different days, at a power output calculated to elicit 130% $\dot{V}O_{2peak}$. The first was a non-invasive respiratory test, whilst the second was invasive and involved collection of blood and muscle samples. The respiratory test was conducted separately to the invasive sprint, both for practical reasons and to minimise the stressful effects of complex testing procedures on subjects.

The first maximal test (PreResp) served both to familiarise subjects and to determine peak and mean values for $\dot{V}E$, HR and derived respiratory variables. Subjects were weighed to the nearest 0.01 kg and ECG electrodes were placed in the CM5 configuration. The cycle saddle was set to the predetermined height, the subject seated, and resting HR and BP was recorded after 10 - 15 min of quiet sitting. Respiratory gas exchange analysis was performed at rest, during exercise, and for 20 min of recovery. A 5 min warm-up at 20 W was conducted on the cycle ergometer (Ergoline 800s) at a comfortable cadence. The subject was then instructed to increase the cadence to 110 revs·min^{-1}, and the load that was calculated to produce 130% $\dot{V}O_{2peak}$ was applied. The subject was instructed to maintain the cadence until exhaustion. The time to exhaustion was timed manually, commencing at the moment the required power output was set, and ending when the subject could no longer maintain a cadence ≥ 80 rev·min^{-1} despite verbal encouragement to do so. Peak values for cardiorespiratory variables were calculated as the two highest consecutive 10-s values during the exercise period. The reported values for the accumulated VO_2 and the accumulated oxygen deficit are based on 10-s collection periods, or part thereof (when exercise did not end evenly on a 10-s period). The oxygen deficit was calculated as the difference between the oxygen consumption theoretically required to produce 130% $\dot{V}O_{2peak}$, and the measured $\dot{V}O_2$ for the duration of the maximal sprint, according to the method of Medbø & Tabata (Medbø & Tabata, 1989). Each of the accumulated cardiorespiratory variables was divided by the exercise time, and expressed in l·min^{-1} to generate a mean value.

In the second maximal test (PreExh), procedures were similar to those described for PreResp, however blood and muscle samples were taken (as detailed below in 3.3.5 and 3.3.6, respectively). The post-exercise biopsies were taken whilst still on the cycle, and after collection of the one and two minute post-exercise blood samples, the subject was assisted to the bed and remained supine for the remainder of the blood sampling. Time to exhaustion was also recorded for this test.

Post-training. Three constant load maximal sprint tests were performed on different days after training. Each test was conducted at the same power output that was used for the pre-training maximal tests, i.e. the power output required to elicit 130% pre-training $\dot{V}O_{2peak}$. The first post-training maximal test was non-invasive and was conducted to volitional exhaustion 1.9 ± 0.3 days after the final training session. This test (PostResp) was used to determine peak and mean cardiorespiratory values and time to exhaustion. The other two maximal tests, which were both invasive, were conducted in random order for each subject. In one test (PostExh) subjects cycled to exhaustion as previously defined. Time to exhaustion was recorded, venous blood sampled, and muscle biopsies taken as per the PreExh test. In addition, another maximal sprint test (PostMatch) was performed in which both the power output and the exercise time were identical to the PreExh test, i.e. the subject did not cycle until exhaustion. The first of the invasive post-training maximal tests occurred 5.4 ± 0.2 days after the final training session, and the second 3 days later.

3.3.4.6. 'All out' air-braked cycle sprint test

After familiarisation, on a different day, the 'all out' test was performed as the first sprint in each of the first and last training sessions.

Subjects were connected to an ECG via skin electrodes and the electrocardiogram was continuously monitored. Subjects were seated on a forward-entry Repco air-braked cycle ergometer. The ergometer was instrumented with an Exertech work monitor unit (Repco, Melbourne, Australia) to enable determination of peak power, which was proportional to the cube of pedal frequency, and total work during cycling. The operating principles of the air-braked cycle ergometer have been described and

validated (Maxwell *et al.*, 1998). The subject's feet were secured in the toe-clips, and further strapping applied to prevent the shoes coming out of the clips during the maximal effort. A 5 min warm up was performed prior to the 30-s test. The subject adopted a standing position on the pedals (with preferred leg forward), and at the command to begin, pedalled as quickly and as hard as possible, i.e. 'all out' until instructed to stop after 30 s. Peak power and total work performed were recorded. Mean power was calculated by dividing total work (J) by the sprint time, i.e. 30 s.

3.3.5 Blood sampling, handling and analyses

3.3.5.1 Subject preparation

Subjects sat with a hand and forearm immersed in warm water (43°C) for 10 min, then lay supine covered with a blanket. A 22G flexible Jelco catheter (Critikon, Johnson & Johnson) was inserted using aseptic technique into a dorsal hand vein and secured with rigid cloth tape. A three-way stopcock valve was connected to the catheter to enable rapid sampling. The hand was placed inside a perspex box (except for the period during the 130% $\dot{V}O_{2peak}$ tests when the subject was located on the cycle when a heating fan was applied over the dorsal aspect of the catheterised hand), with an aperture for attachment of a heating fan, and heating was continuously applied to maintain arterialization of the dorsal hand circulation (McLellan & Gass, 1989). The catheter was kept patent by administration of sterile isotonic saline after each blood sample.

3.3.5.2 Blood sampling times

After training, blood sampling times were matched as closely as possible to those recorded for the pre-training tests.

Rest. A resting blood sample was taken when the subject was supine. This sample was always obtained prior to the muscle biopsy procedure.

Exercise. A sample was collected in the final seconds of the sprint at volitional fatigue, or at the end of exercise in the case of the matched-time test.

Recovery. Blood was sampled at 1, 2, 5, 10, and 20 min of recovery from the exercise tests. The 5, 10, and 20 min samples were collected in a supine position, so as

to minimise the likelihood of syncope, and to facilitate the maintenance of manual pressure over the biopsy site.

3.3.5.3 Blood handling and analyses

Figure 3.2 depicts the schema for aliquoting blood samples.

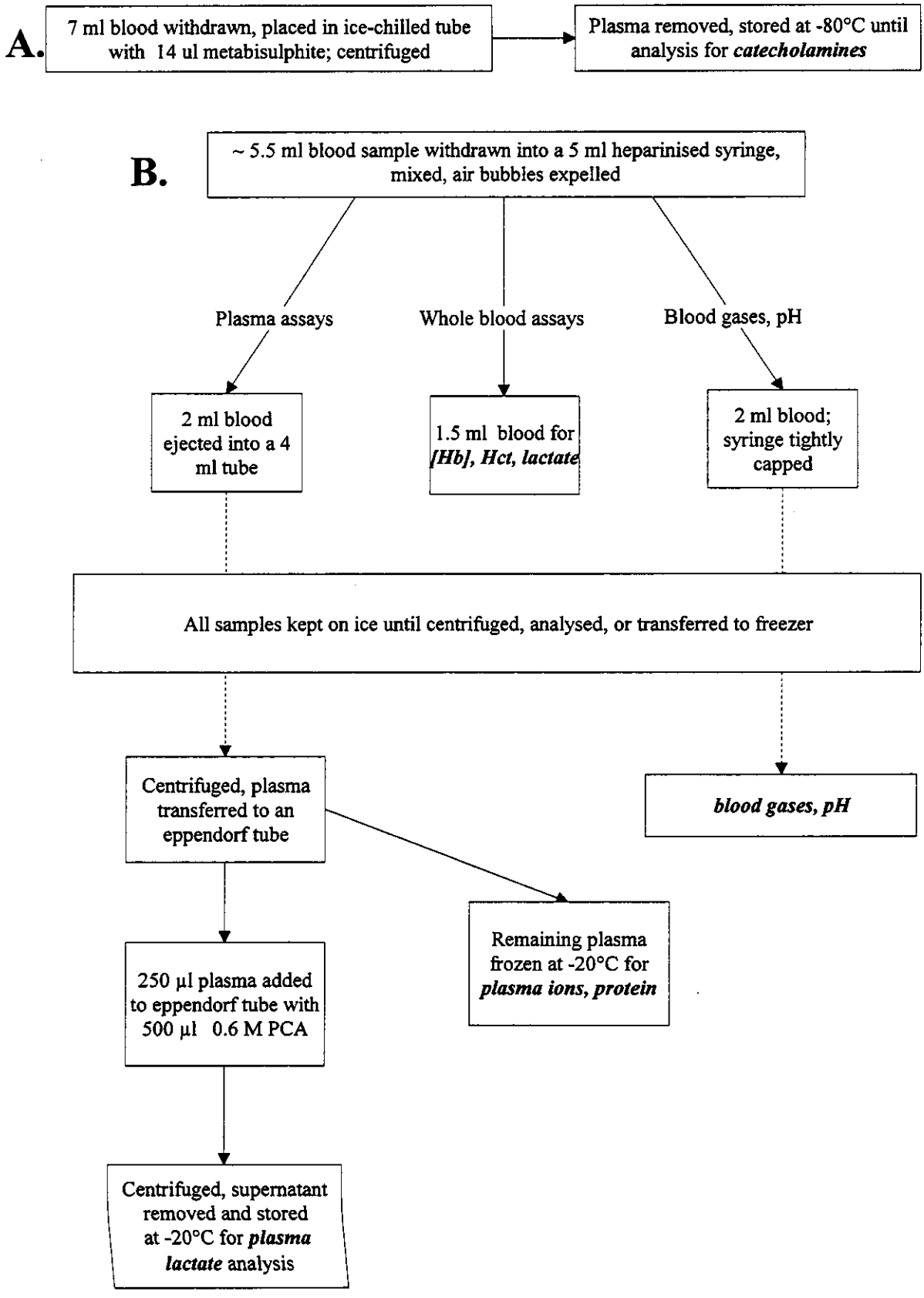
(i) Handling

To account for the effect of deadspace, 1 to 1.5 ml of blood were withdrawn and discarded prior to sample collection. Two samples were then withdrawn. The first blood sample was always used for catecholamine analysis and the second for ions, metabolites, blood gases, etc.

A 7 ml blood sample for determination of plasma catecholamine concentrations was taken at each sampling point. The blood was immediately transferred into ice-chilled heparinised tubes containing 14 µl sodium metabisulphite ($5g \cdot dl^{-1}$), gently mixed, and then kept on ice (<30 min) until centrifuged at 4°C and 1,300 g for 10 min. The plasma was removed using glass pipettes, transferred to a plastic tube and immediately placed in a -80°C freezer, where it remained until analysis.

A separate 5.5 ml blood sample was withdrawn into a syringe coated with lithium heparin. Air bubbles were expelled from the syringe, the blood gently mixed and two aliquots removed. The first aliquot (2 ml) was used for separation of plasma for subsequent metabolite, ion, and protein analyses. 250 µl of plasma was added to 500 µl of chilled 0.6 M perchloric acid, and centrifuged for 10 min at 20,000 g. The supernatant was removed and stored at -20°C until analysis for lactate concentration. The remaining plasma was placed in a plastic tube and stored at -20°C until analysis for potassium ($[K^+]$), sodium ($[Na^+]$), chloride ($[Cl^-]$), and protein ($[PPr^-]$) concentrations. The second aliquot (1.5 ml) of whole blood, used to determine haematocrit (Hct) and haemoglobin concentration ([Hb]), was placed in an eppendorf tube and stored at -20°C until analysis. The syringe was tightly capped and the remaining blood placed on ice until determination of pH, P_{O_2} and P_{CO_2} (BMS3 MK2 Blood gas analyser, Radiometer, Denmark).

Figure 3.2 Study 1 Blood Aliquoting Schema



(ii) Analyses

The blood for catecholamine analysis was assayed using high performance liquid chromatography (HPLC), performed by Ms P.A. Ruell (School of Exercise and Sport Science, The University of Sydney). A half ml of plasma was adsorbed onto activated alumina (Anton & Sayre, 1962) in 0.5 ml Tris buffer (0.5M), pH 8.6, containing 1% sodium EDTA. After addition of 25 µl 3,4 dihydroxybenzylamine (200 nM) and 25 µl sodium metabisulphite (0.5 $mg \cdot ml^{-1}$), the solution was mixed for 10 min. The catecholamines were eluted with 125 µl of 0.1 M PCA containing 400 µM metabisulphite, after washing with two 1 ml aliquots of chilled MilliQ water. Aliquots (100 µl) of the eluted solution were injected onto a 15 cm Novapak column (Waters, Millipore Corp., USA). The mobile phase consisted of 23.4 g NaH_2PO_4, $2H_2O$, 0.5 g Na_2EDTA, 1.171 g Na octylsulphonic acid, 1 ml orthophosphoric acid, and 45 ml of methanol, per litre of MilliQ water. Pump flow rate was 1.2 $ml \cdot min^{-1}$. The Waters HPLC system (Waters, Millipore Corp., USA) was comprised of a 510 pump, 712 Wisp autosampler with refrigeration, a 460 electrochemical detector (with electrode potential set to 0.65 V), and baseline 810 software for pump control and data analysis. Twelve assay runs were required to complete sample analysis. High and low concentration quality control standards were included in each assay, and recovery was always close to 100%. The percent recovery when standards were added to a plasma sample was 96.6%. The intra- and inter-assay coefficients of variation for noradrenaline ([NAdr]) were 2.3 and 14.8%, respectively, and for adrenaline ([Adr]) were 4.3 and 65% respectively. The 65% inter-assay CV was as a consequence of a very low Adr value (about 0.4 nM) which the assay cannot reliably detect. The CV for the high and low QC respectively, was 5% and 9.5% for NAdr, and 6.6% and 18.5% for Adr.

Plasma lactate concentration ($[Lac^-]$) was determined in triplicate using a standard enzymatic technique modified for use on a spectrophotometer (Annan, 1975). The intra-assay coefficient of variation was 1%, and the inter-assay coefficient of variation was 1.13 % for plasma Lac^-. Plasma $[K^+]$ and $[Na^+]$ analyses were performed in triplicate using an auto-injection flame photometer (IL 943, Instrumentation Laboratory, Italy). The intra-assay coefficients of variation for $[K^+]$ and $[Na^+]$ using abnormal serum were 0.5 and 1.1%, and when using the respective standards were 0.3

and 0.3%, respectively. The inter-assay coefficients of variation for plasma [K^+] and [Na^+] samples were 0.58 and 0.69%, respectively. Plasma chloride concentration was determined by titration in triplicate (CMT10 Chloride Titrator, Radiometer, Denmark). The strong ion difference (SID), the difference between the sum of the strong cations and anions, was calculated using measured values of Lac^-, K^+, Na^+, and Cl^-. Plasma protein concentration ([PPr^-]) was determined according to the method of Markwell *et al.* (1978). The total concentration of plasma weak acids (A_{tot}) was calculated using the following formula: 2.45 * measured [PPr^-] (Rossing *et al.*, 1986). Haematocrit was measured in duplicate after centrifugation. Haemoglobin concentration was analysed in triplicate using the cyanmethemoglobin method. The intra- and inter-assay coefficients of variation for [Hb] were respectively 0.95%, and 0.76%. The percentage change in plasma volume (ΔPV), relative to rest, was calculated from measured Hct and [Hb] according to the method of Harrison (Harrison, 1985). pH, P_{O_2} and P_{CO_2} were measured in triplicate using a blood gas analyser (BMS3 MK2, Radiometer, Denmark). pH was converted to [H^+] using the equation: $pH = -\log_{10}([H^+])$. Bicarbonate concentration ([HCO_3^-]) was calculated from the Henderson equation: $[H^+] = 24 * P_{CO_2}/[HCO_3^-]$.

Excepting pH and blood gas analysis, all pre- and post-training blood samples for a particular assay were analysed together on the same day for each subject. When instrumentation was used to analyse samples, calibration was performed immediately prior to, and periodically throughout the analysis with both quality control assayed serum, and high and low precision standards.

3.3.6 Muscle Biopsies – sampling, handling and analyses

Muscle biopsies were performed in the 130% $\dot{V}O_{2peak}$ tests both before and after the training programme.

3.3.6.1 Subject preparation

The skin overlying a selected area of vastus lateralis was shaved and then swabbed with Betadine antiseptic. Anaesthesia of the skin and superficial subcutaneous tissues overlying the vastus lateralis at the lateral mid-thigh region was achieved using a

subcutaneous injection of 2% xylocaine (without adrenaline) (Figure 3.3). After assessment of the level of anaesthesia via a needle point, a single small incision (~0.8 mm) was made in the skin and underlying adipose tissue.

Percutaneous biopsy of the vastus lateralis muscle (Figure 3.4) was performed with 6mm biopsy needles (AB Stille-Werner, Stockholm, Sweden) using aseptic technique (Bergström, 1962), modified for suction. Fresh muscle samples were immmediately immersed, whilst still in the biopsy needle, in liquid nitrogen (N_2). Samples were carefully prised free of the needle under liquid N_2 and then stored in cryotubes in liquid N_2 until subsequent analysis.

The skin incision was closed with steristrips. Pressure was applied manually to the biopsy site for 30 min after the exercise biopsy, and was maintained at a lower level for several days post-biopsy via an elastic wrap bandage (over a sterile dressing) to minimise bleeding and bruising. Subjects were also advised to abstain from vigorous exercise for several days. No complications arose from any muscle biopsy, and visual inspection of the site was carried out on at least two occasions (on separate days) following the biopsy by the experimenters.

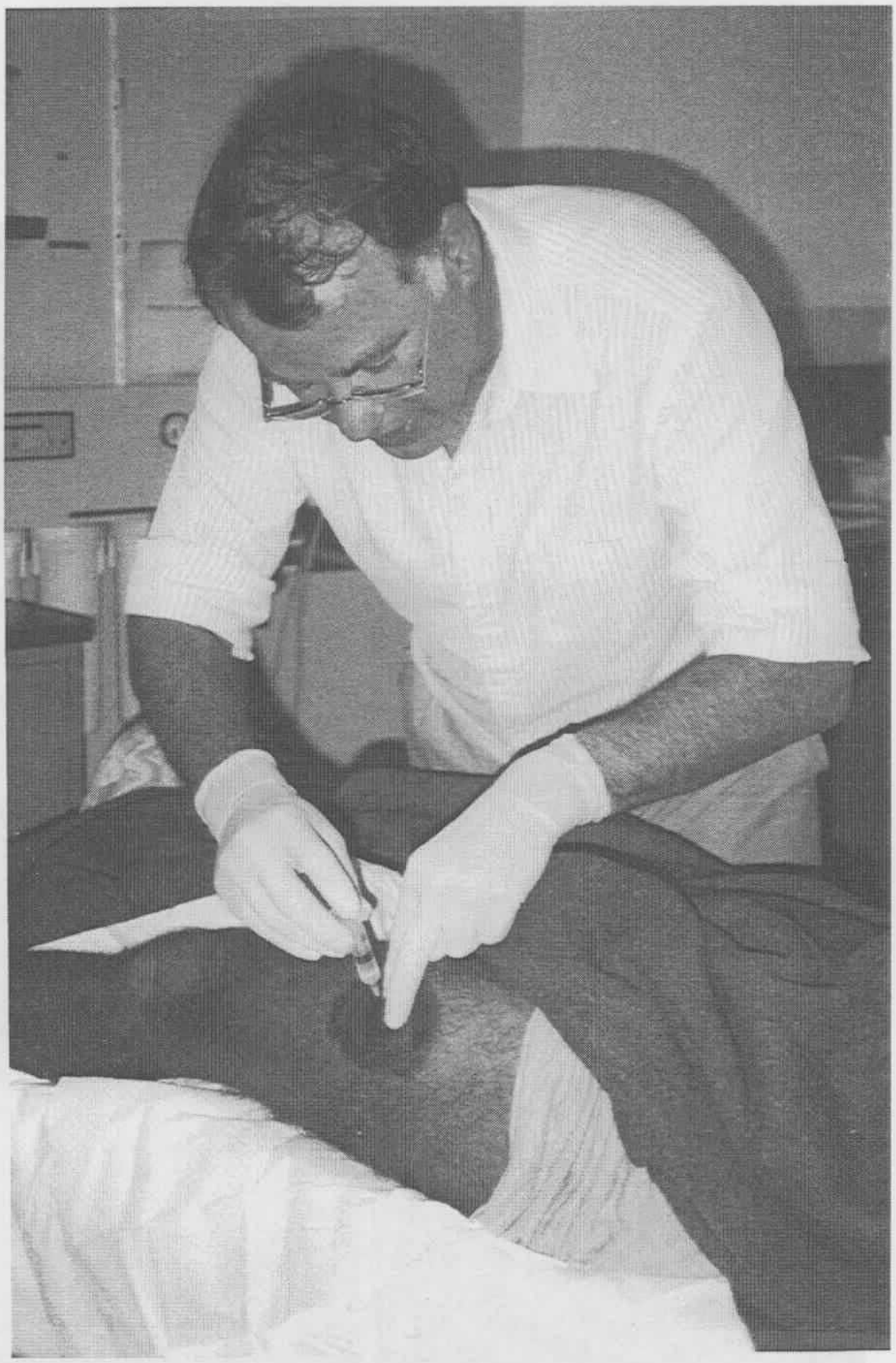


Figure 3.3 A subcutaneous injection of 2% xylocaine (without adrenaline) to achieve anaesthesia of the skin and superficial subcutaneous tissues overlying the vastus lateralis at the lateral mid-thigh region.

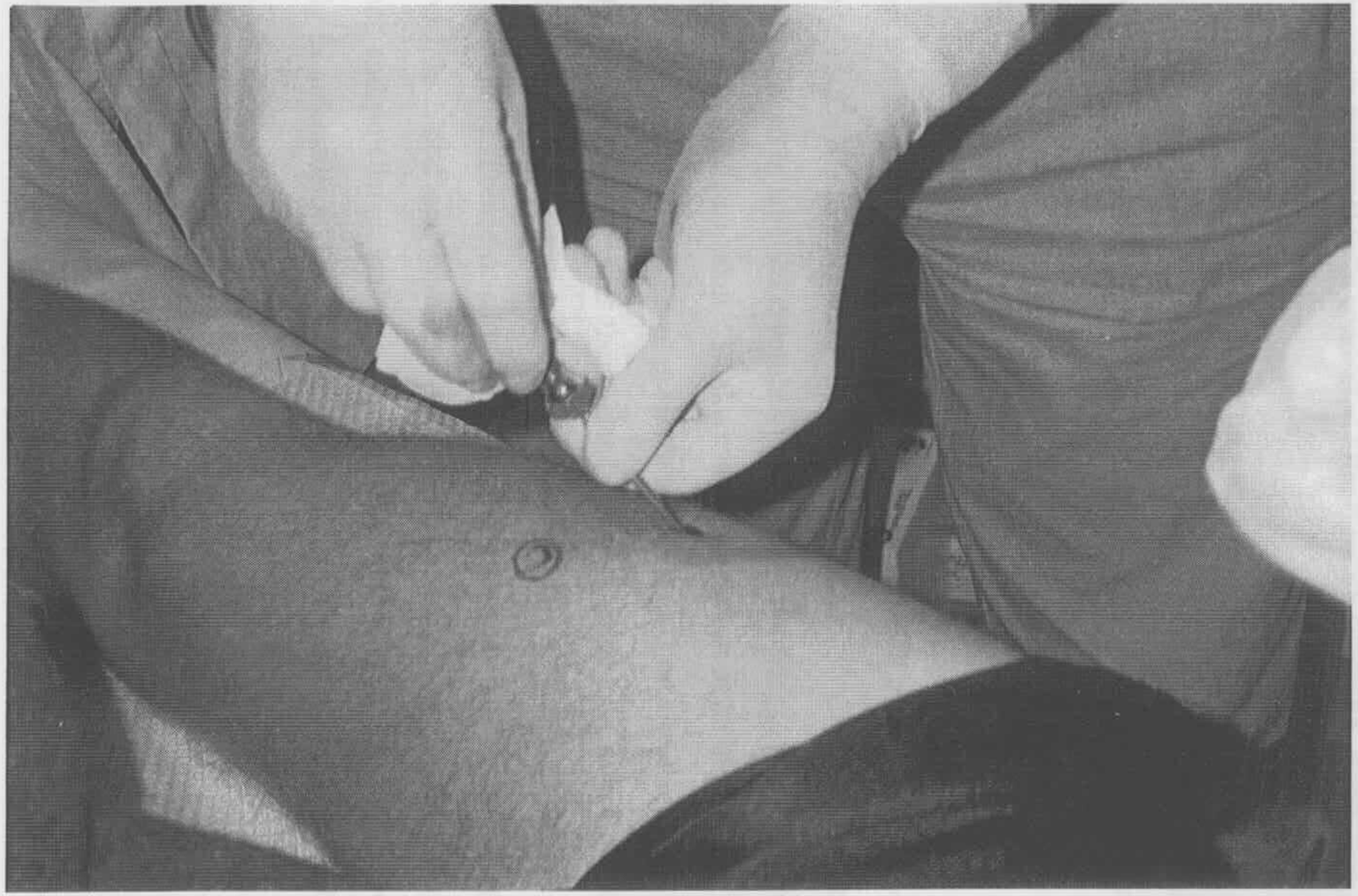


Figure 3.4 A muscle biopsy of the vastus lateralis performed prior to exercise

3.3.6.2 Muscle sampling times

Prior to training two muscle samples were obtained in the PreExh test. The first sample was taken at rest with the subject supine, and the second immediately at the cessation of exercise, whilst the subject was still seated, supported in a semi-reclined position on the cycle ergometer. Identical sampling procedures were adopted in the two invasive maximal tests conducted after training (PostExh, PostMatch).

3.3.6.3 Muscle Handling and Analyses

All muscle samples were stored in cryotubes immersed in liquid nitrogen until required for analysis. Figure 3.5 illustrates the muscle handling and analysis procedures.

(i) Freeze-drying procedure

Frozen wet muscle was weighed to the nearest 0.0001 g on precision scales (Ohaus Galaxy, 160D; ICI Instruments) in a room cooled to –30°C. Weighed, frozen muscle was then placed in labelled cryotubes (with punctured lids) in a small amount of liquid nitrogen in a chilled beaker. Beakers were placed in a vacuum bottle situated in a well, cooled to -30°C. Vacuum pressure to less than 3 mbar was applied for 36 hours. The freeze-dried muscle was then placed in a dessicator for 1 hour (to allow muscle to equilibrate to room temperature), then immediately re-weighed, and the wet to dry ratio calculated. Freeze-dried muscle was then viewed under a dissecting microscope and visible blood and connective tissue removed. The cleaned sample was then crushed and powdered, and 2 to 4 mg was transferred into eppendorf tubes and stored briefly in a dessicator. The weighed, cleaned muscle was then extracted on the same day.

(ii) Extraction procedures

Metabolites

Muscle was extracted according to the method of Harris *et al.* (1974). Chemical volumes described pertain to a muscle weight of 4 mg; where only 2 mg were extracted, the volumes were halved. Eppendorf tubes containing weighed, cleaned and crushed muscle were placed on ice, and 500 µl of pre-cooled 0.5 M PCA/ 1 mM EDTA was added to each tube. Tubes were repeatedly vortexed, tapped, and replaced

on ice for a 10 min period. The eppendorfs were then centrifuged (Heraeus Sepatech; Biofuge 28RS) at 28,000 rpm and 0°C for 2 min. Eppendorfs were replaced on ice and the supernatant removed. 400 µl of supernatant was transferred into a fresh eppendorf tube (on ice) and 100 µl of pre-cooled 2.1 M $KHCO_3$ was added. The tubes were vortexed, placed on ice for 5 min, vortexed, and then centrifuged at 28,000 rpm and 0°C for 2 min. The supernatant was transferred into cryotubes and stored at -80°C. Subsequent analyses for adenine nucleotides and their degradation products (by HPLC); ATP, PCr, creatine and lactate were carried out using this supernatant.

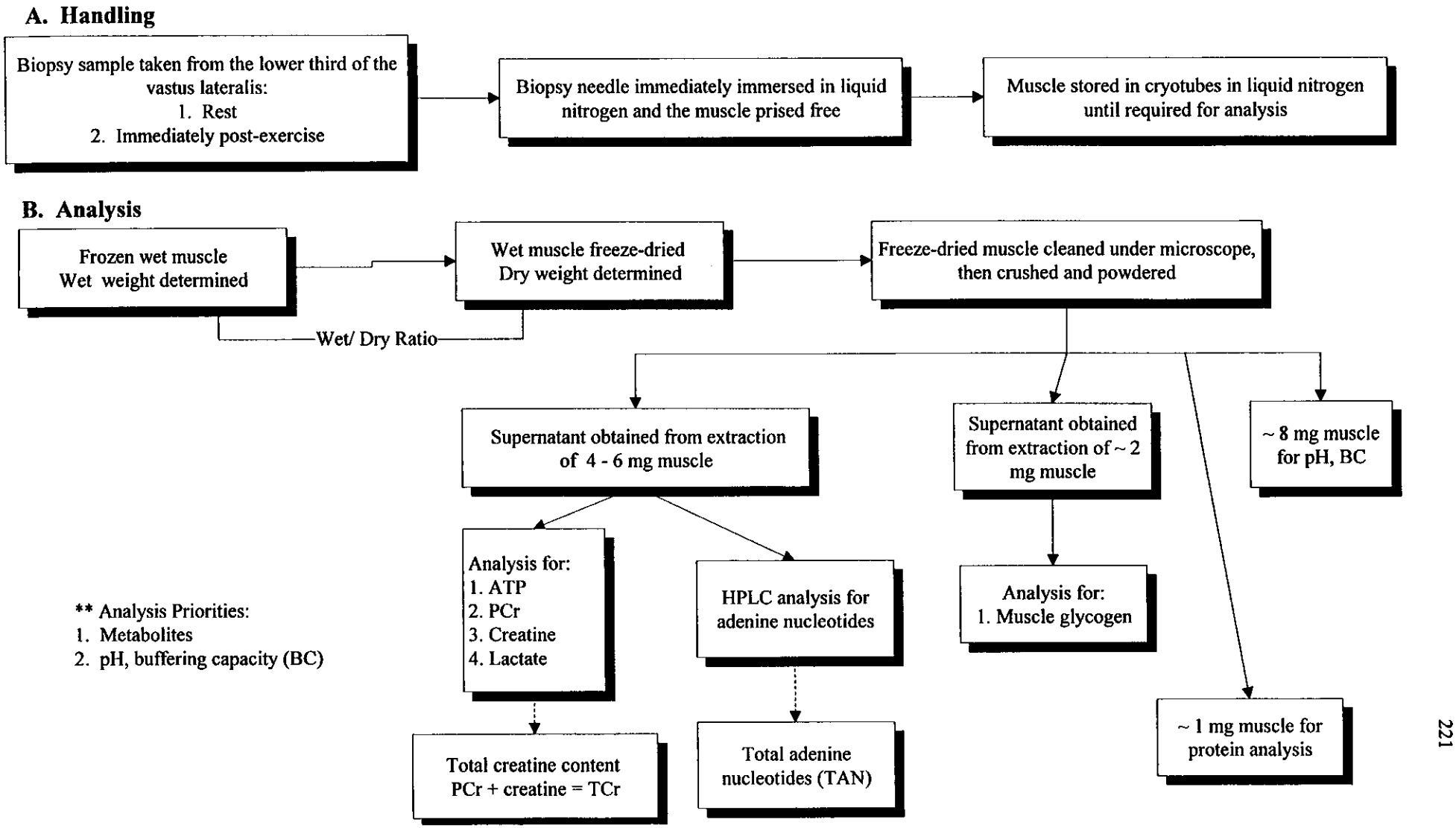
Glycogen

2 mg of muscle was extracted. 500 µl of 1 M HCl was added to each resting sample, which was vortexed, then weighed. Post-exercise samples were treated slightly differently, so as to destroy free glucose and the hexose monophosphates. 250 µl of 2 M KOH was added to each tube. Samples were heated to 50°C for 10 min, cooled, 250 µl of 3 M HCl added, and the tubes weighed. Both resting and post-exercise samples were then placed in a boiling water bath for 1 hour. The tubes were re-weighed, and if evaporation had occurred during boiling, MilliQ water added to replace the lost volume. After cooling, 90 µl of 5 M KOH was added, the tube vortexed and centrifuged, and the supernatant removed. The supernatant was stored in cryotubes at -80°C until analysis for glucose concentration was performed.

(iii) Assay Procedures

Dried muscle was dissected free from visible blood and connective tissue, powdered, extracted and analysed for the adenine nucleotides (ATP, ADP, AMP) and IMP by high performance liquid chromatography (HPLC) (Wynants & Van Belle, 1985), and for ATP, PCr, creatine, lactate, and glycogen by standard enzymatic, fluorimetric methods(Lowry & Passonneau, 1972). For each subject, muscle metabolites (except glycogen and Lac^-) in pre- and post-training samples were corrected to the peak total creatine content obtained before and after training, respectively, and expressed as $mmol \cdot kg^{-1}$ dry mass (dm).

Figure 3.5 Study 1 Muscle Handling and Analysis Schema



Muscle metabolites

Each metabolite assay was conducted according to the standard procedures outlined in Lowry & Passoneau (1972). A Turner 112 Digital Filter Fluorometer was used in the analysis, with readings conducted at 365 nm absorption and 455 nm emission. NADH standards were read on the spectrophotometer (Cecil CE 2020) at 340 nm to obtain 'exact' concentrations, then included as standards in each assay (using the fluorometer) as an instrument calibration check.

Enzymatic fluorimetric assays to determine muscle ATP and PCr were performed on the same day as the muscle was extracted. Briefly, the reagent consisted of 0.05 M Tris buffer, pH 8.1, 1 mM magnesium chloride ($MgCl_2$), 0.5 mM dithiothreitol (DTT), 100 µM glucose, 50 µM $NADP^+$, and G-6-P dehydrogenase (G-6-P DH). 10 µl of muscle extract, or MilliQ water, or ATP (50, 100, 200 µM), or PCr (125, 250, 500 µM), or NADH (50, 100, 200, 400 µM) standards was added to 1 ml reagent, and the initial reading performed. 25 µl dilute hexokinase (HK) was added to each tube, which was vortexed and incubated in the dark for 30 min prior to the second reading. 20 µl dilute ADP/ creatine kinase (CK) was added to each tube which was incubated for 60 min before the final reading was performed.

Muscle creatine was determined on the day following ATP/ PCr analysis. The reagent comprised 0.05 M imidazole, pH 7.5, 5 mM $MgCl_2$, 30 mM potassium chloride (KCl), 0.1 mM phosphoenolpyruvate (PEP), 0.2 mM ATP, 1 $\mu g \cdot ml^{-1}$ lactate dehydrogenase (LDH), 5 $\mu g \cdot ml^{-1}$ pyruvate kinase (PK), and 0.03 mM NADH. 30 µl of muscle extract, or MilliQ water, or creatine standard (125, 250, 500 µM) was added to 1 ml of reagent, vortexed, and stored in the dark for 15 min before the first reading. 20 µl of a 10 mg creatine kinase/ ml 0.05% bovine serum albumin (BSA) mixture was added to each tube, which was vortexed, then incubated for 60 min. A second reading was performed, after which a further 15 min incubation was allowed, and a final reading performed.

Muscle Lac^- was determined within a week of extraction. After an initial reading of the reagent cocktail (comprising 0.1 M hydrazine, 0.1 M glycine, 0.5 mM NAD^+, and

LDH), 10 µl of extract, or MilliQ water, or lactate (125, 250, 500 µM), or NADH standard was added, the tube vortexed, then incubated in the dark for 60 min, after which the final reading was performed.

HPLC for metabolites

The HPLC technique was based on the method of Wynants & van Belle (1985). Briefly, mobile phase A, which was filtered and degassed, consisted of 0.15 mM ammonium phosphate buffer and 10 $ml \cdot l^{-1}$ orthophosphoric acid, pH 6. Mobile phase B consisted of a 50% acetonitrile/ 50% methanol solution. Flow rate was set at 1 $ml \cdot min^{-1}$, and UV λ at 254 nm. Three standards were included in each run. The HPLC system (ICI Instruments) was composed of a refrigerated circulator (Model 4860; BioRad), an autosampler (LC 1610), a Jun-Air compressor minor, a systems organiser (LC 1440), an UV/ VIS detector (LC 1200), pump (LC 1150), with a DI 510 interface and DP 900 software.

Muscle Protein

Protein concentration was determined according to the method of Markwell *et al.* (1978). Boehringer Preciset protein standards (1, 0.5, 0.25, 0.125, 0.0625, 0.03125 $g \cdot dl^{-1}$) were included in each assay and were treated in the same manner as the homogenate. Cuvettes were read on the spectrophotometer at 660 nm. The intra-assay co-efficient of variation was 8.26%.

Glycogen

The supernatant for glycogen analysis was diluted 1:3 with MilliQ water to enable the samples to be read on a fluorimeter. 10 µl of extract, or MilliQ water, or glucose (125, 250, 500 µM), or NADH standard was added to 1 ml of reagent which was comprised of 0.05 M Tris, pH 8.1, 1 mM $MgCl_2$, 0.5 mM DTT, 0.3 mM ATP (pH 7.0), 50 µM $NADP^+$, and G 6-P DH. The tubes were vortexed and read on the fluorimeter before 25 µl of dilute HK was added to each tube. After 60 min incubation in the dark, the second reading was performed.

Muscle anaerobic ATP production

Muscle anaerobic ATP production was calculated as $(2(\Delta ATP)) + (1.5(\Delta Lac^{-})) + (\Delta PCr)$ (Spriet, 1995) and expressed as $mmol \cdot kg^{-1}$ dm. The rate of muscle anaerobic ATP production was expressed as $mmol \cdot kg^{-1}$ $dm \cdot s^{-1}$.

Muscle pH

Approximately 8-10 mg freeze-dried, cleaned, powdered muscle was used to determine muscle pH and buffering capacity. For determination of pH, freeze-dried muscle was rehydrated for 60 s, then homogenised on ice for 3 x 15 s bursts at 60% maximum speed (10 s intervals between bursts) using a hand-held electric homogeniser (Omni 2000: Omni International, USA), in a buffer ($100\ \mu l \cdot mg^{-1}$) comprised of 145 mM KCl, 10 mM NaCl, and 5 mM sodium iodoacetate, pH 7. A magnetic stirring bar was then inserted, the Kimble tube containing the homogenate placed in a water bath maintained at 37°C, and a 4 mm pH probe (AEP341; Activon, Australia), connected to a pH meter (PHM62; Radiometer, Copenhagen), inserted. Continuous stirring was applied and the pH read to the nearest 0.01 units, and recorded after 45, 60, 75, and 90 s (Spriet *et al.*, 1986). The mean value for the final three readings was recorded as the muscle pH. Muscle pH was determined in duplicate from a single homogenate, with the mean difference for duplicate measures of the starting pH being 0.02 ± 0.00 pH units. Muscle pH was converted to muscle $[H^{+}]$. The change in muscle $[H^{+}]$ (exercise minus rest) relative to the work performed during exercise ($\Delta[H^{+}] \cdot work^{-1}$) was calculated.

In vitro buffering capacity ($\beta_{in\ vitro}$)

Immediately following the pH measurement, 250 to 400 µl of homogenate was placed in an 8 x 70 mm glass culture tube (Duran; Schott) for determination of $\beta_{in\ vitro}$. The culture tube containing the homogenate was placed in a water bath maintained at 37°C, the pH probe inserted, and the homogenate allowed to equilibrate for 5 min while being stirred continuously with a small magnetic bar. The starting pH was recorded and the homogenate then titrated with 0.1 M NaOH (or occasionally with 0.1 M HCl) to a pH of approximately 7.1. 10 µl aliquots of 0.01 M HCl were then added to the homogenate until the pH approximated 6.1; usually requiring 5 additions of

0.01 M HCl. Continuous stirring was maintained throughout the measurement procedure, which was performed in duplicate. Dilute (0.01 M) HCl and NaOH titration solutions were made up freshly each day from 0.1 M stock. $\beta_{in\ vitro}$ is expressed as μmol $HCl \cdot g^{-1}$ $dm \cdot pH^{-1}$. The mean difference for duplicate measures of $\beta_{in\ vitro}$ was 0.07 ± 1.14 μmol $HCl \cdot g^{-1}$ $dm \cdot pH^{-1}$.

In vivo buffering capacity ($\beta_{in\ vivo}$)

$\beta_{in\ vivo}$ was calculated from the rise in muscle Lac^- divided by the decline in muscle pH, and is expressed as μmol $Lac^- \cdot g^{-1}$ $dm \cdot pH^{-1}$.

3.3.7 Statistical Procedures

Two-tailed, paired *t*-tests were used to compare subject characteristics and other single pre- and post-training values, e.g. peak respiratory data. Blood data were analysed using two-way (sample time, training status) ANOVA for repeated measures, with Newman-Keuls tests used when a significant F ratio was found for the main effects. Paired *t*-tests were used to locate pre- and post-training differences at a particular sample time when a significant interaction effect was detected. One-way repeated measures ANOVA was used to determine if test order influenced resting muscle metabolite values in PreExh, PostMatch or PostExh, and an ANOVA contrast technique used to locate differences. Comparisons between the sprint trials for muscle data were made using repeated measures two-by-two (sampling time i.e. rest vs exercise; training status) ANOVA with orthogonal contrasts (Winer *et al.*, 1991). A significant interaction effect in this two-by-two analysis indicates a difference between the respective pre- and post-training delta values (rest minus exercise, or vice-versa), and hence a difference in degradation or accumulation. Significance was accepted at $P<0.05$. Results are reported as the mean ± S.E.M., unless otherwise indicated.

Due to the intense nature of the exercise and occasional difficulties obtaining blood, some blood samples were missed, or delayed. When either situation arose, the subject's blood results for both pre- and post-training for that particular time were omitted, and a group mean used instead. Any reduction in the *n* for a particular time is indicated in the corresponding text, table or graph. To account for the effect of

missing values, a more conservative *P* value was used. The latter was obtained by using the Huynh-Feldt adjustment for the degrees of freedom when determining the F ratio.

In two subjects, the muscle sample obtained immediately following the cessation of exercise in the PostExh test (in which both subjects exercised for 26-29% longer at the same power output before exhaustion) yielded metabolite values that approximated those of rest. Since the values for all metabolites were similarly low and differed dramatically from the values obtained at exhaustion prior to training (PreExh test), it was concluded that a problem had occurred with the muscle and that the results were not representative of real values and hence would unfairly affect statistical analysis. It cannot be determined whether the problem occurred during muscle sampling, handling or storage. The data are included in the appendices, however have been excluded from mean data and statistical analysis. Thus, whenever rested and exercised muscle samples were compared (excepting total creatine, wet/dry ratio and percentage water content for which an *n* of 7 was used for each test, enabling a three-way comparison), an *n* of five was used for comparing PreExh with PostExh, whilst an *n* of seven was used for the PreExh *vs* PostMatch comparison. This approach has been used (rather than a three-way comparison with an *n* of 5) to enable the use of as much paired data as possible.

3.4 Results

3.4.1 Subject Characteristics

The physical characteristics of the seven male subjects who participated in Study 1 are summarised in Table 3.1. Body mass, and hence body mass index (BMI), did not change after the seven weeks of the training programme. Individual data appear in Appendix C1.

Table 3.1 *Physical characteristics of Study 1 subjects*

	Age (yr)	Height (cm)	Mass (kg)	BMI ($kg \cdot m^{-2}$)
Pre-training	22 ± 2	180.0 ± 5.1	76.1 ± 3.0	23.5 ± 1.2
Post-training	-	-	76.2 ± 4.6	23.6 ± 1.4

Mean ± S.D.; n = 7. BMI, body mass index.

3.4.1.1 Leg volumes

Total leg volume (TLV) and calculated fat volume (FV) were not altered as a consequence of training. Muscle plus bone volume (M+B)V and muscle volume (MV) were increased 3.3% after training, however failed to reach statistical significance (P=0.08; Table 3.2). See Appendix C2 for individual data.

Table 3.2 *Calculated leg volumes pre- and post-training.*

	TLV	FV	(M+B)V	MV
Pre-training	8.85 ± 0.18	1.79 ± 0.18	7.06 ± 0.11	6.28 ± 0.10
Post-training	8.99 ± 0.16	1.69 ± 0.16	7.29 ± 0.12	6.49 ± 0.11

Mean ± S.E.M.; n = 7. All volumes are expressed in litres. TLV, total leg volume; FV, fat volume; (M+B)V, muscle plus bone volume; MV, muscle volume.

3.4.2 Performance

Performance was improved after training in each of the three types (i.e. incremental, 130% $\dot{V}O_{2peak}$, and 30-s 'all out') of tests.

(i) Incremental tests

Maximum power attained in the incremental tests to fatigue was increased 7.5% after training (332 ± 13 vs 357 ± 9 W, pre- vs post-training, respectively; $P<0.02$).

(ii) 130% $\dot{V}O_{2peak}$ tests

Time to exhaustion in the 130% pre-training $\dot{V}O_{2peak}$ respiratory test (PreResp) was 78 ± 11 s, which tended to be less than during the pre-training invasive test (PreExh, 83 ± 11 s; $P=0.051$). In the post-training tests, time to exhaustion in the respiratory (PostResp, 100 ± 12 s) and in the invasive tests (PostExh, 100 ± 12 s) did not differ.

There was a significant ($P<0.001$) increase in the average time to exhaustion during both the respiratory (PreResp vs PostResp, 29%) and invasive (PreExh vs PostExh, 21%) 130% $\dot{V}O_{2peak}$ tests following training. Therefore the work output was also increased significantly ($P<0.001$) after training, being 43 ± 3 kJ in both PostExh and PostResp (vs 35 ± 3 kJ, in PreExh, and 33 ± 3 kJ in PreResp; Figure 3.6). In the PreExh and PostMatch tests, duration of exercise was matched and thus work output (35 ± 3 vs 35 ± 3 kJ, respectively) did not differ.

(iii) 30-s maximal sprints on the air-braked ergometer

The maximal 30-s sprint performance was increased after training, with a 9.4% increase in peak power ($P<0.05$; Table 3.3) and a 10.8% increase in total work ($P<0.05$). Mean power was therefore also significantly higher ($P<0.05$), being 838 ± 36 and 927 ± 18 W, first and last training sessions, respectively.

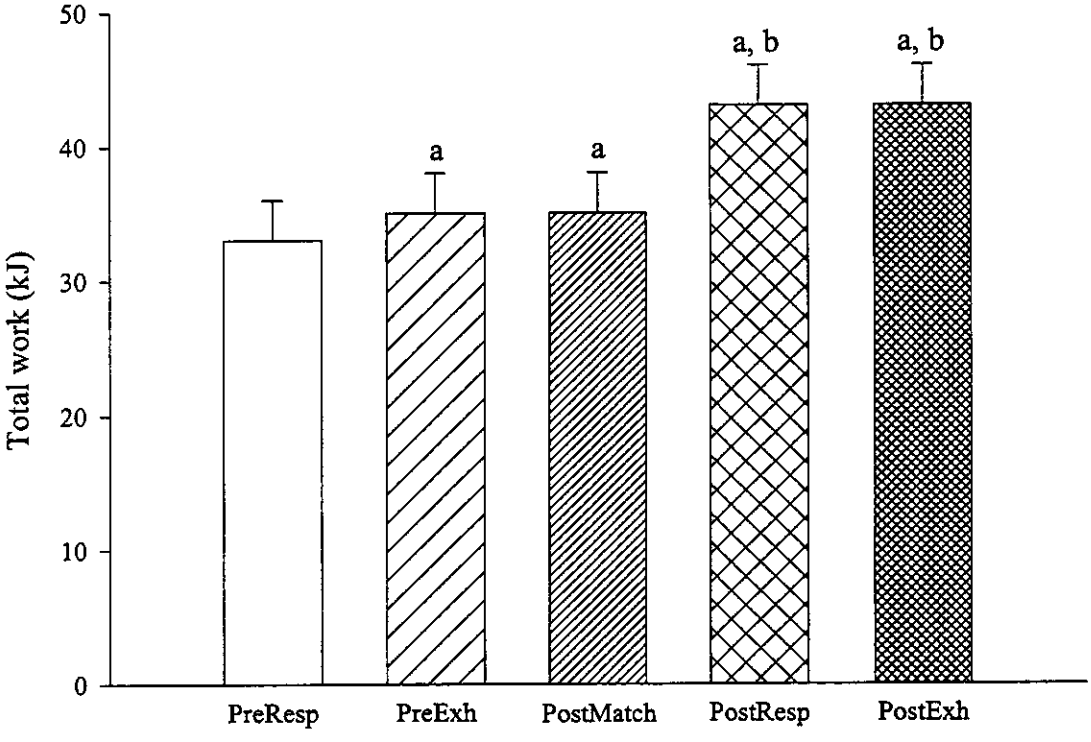


Figure 3.6 Total work (mean ± S.E.M.) completed during the pre- and post-training respiratory tests (PreResp, PostResp) and invasive tests (PreExh, PostMatch, PostExh). Each test was conducted at the same absolute power output, i.e. that calculated to elicit 130% pre-training $\dot{V}O_{2peak}$. a, > PreResp; b, > PreExh, PostMatch.

Table 3.3 *Peak power and total work in a 30-s maximal exercise bout on an air-braked cycle ergometer, first vs last training session.*

	Peak power (W) *	Total work (kJ) *
1st training session	1158 ± 56	25 ± 1
21st training session	1267 ± 38	28 ± 1

Values are means ± S.E.M.; $n = 7$. * $P<0.05$, 21st > 1st training session.

3.4.3 Cardiorespiratory responses to exercise

(i) Incremental tests

Peak oxygen consumption ($\dot{V}O_{2peak}$) tended to be higher (7%) following training, however just failed to reach statistical significance (P=0.07). Training did not significantly alter the peak values (Table 3.4) attained during exercise for heart rate (HR_{peak}), expired ventilation ($\dot{V}_{E\,peak}$), carbon dioxide output ($\dot{V}CO_{2\,peak}$), or the respiratory exchange ratio (RER).

Table 3.4 *Peak cardiorespiratory responses to incremental exercise.*

Variable	Pre-training	Post-training
HR_{peak} (beats·min^{-1})	196 ± 3	192 ± 2
$\dot{V}_{E\,peak}$ (l·min^{-1})	153.7 ± 7.7	161.4 ± 6.2
$\dot{V}O_{2\,peak}$ (l·min^{-1})	3.79 ± 0.16	4.05 ± 0.15
$\dot{V}CO_{2\,peak}$ (l·min^{-1})	4.66 ± 0.20	4.78 ± 0.16
RER	1.24 ± 0.02	1.19 ± 0.03

Values are means ± S.E.M.; $n = 7$. HR_{peak}, peak heart rate; $\dot{V}_{E\,peak}$, peak expired ventilation, BTPS; $\dot{V}O_{2\,peak}$, peak oxygen uptake, STPD; $\dot{V}CO_{2\,peak}$, peak carbon dioxide output, STPD; RER, respiratory exchange ratio at peak workrate.

Individual cardiorespiratory responses measured during the submaximal and maximal sections of the pre- and post-training incremental tests are detailed within Appendix C (C3-5).

(ii) Constant power tests at 130% pre-training $\dot{V}O_{2peak}$

Relative intensity

The power output that was calculated to elicit 130% $\dot{V}O_{2peak}$ prior to training represented 122 ± 0.03% $\dot{V}O_{2peak}$ after training (P=0.06). The relative exercise intensity was therefore slightly lower after training, whilst the absolute stress was the same.

Relative aerobic contribution to ATP generation

Prior to training, 58.3 ± 3.8% of the ATP generation during exercise at 130% $\dot{V}O_{2peak}$ was accomplished aerobically (non-invasive assessment). After sprint training 61.7 ± 3.8% of ATP was produced aerobically, however there was no statistical difference (P=0.12) from the pre-training value.

Peak values

Peak expired ventilation ($\dot{V}E_{peak130}$) was higher during exercise in the 130% $\dot{V}O_{2peak}$ respiratory test after training (PostResp, P<0.05; Table 3.5). Training did not affect the peak values reached during the 130% $\dot{V}O_{2peak}$ respiratory tests for HR, $\dot{V}O_2$, $\dot{V}CO_2$, or RER.

The peak values attained during exercise in the pre-training 130% $\dot{V}O_{2peak}$ respiratory test (PreResp) for HR, $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were 89, 90, 99, 107, and 110% of the values attained in the pre-training incremental test, respectively. In PostResp, the corresponding values relative to the post-training incremental test were 93, 94, 96, 107, and 113%, respectively.

Table 3.5 *Peak cardiorespiratory responses when exercising to fatigue at 130% $\dot{V}O_{2\,peak}$, pre- (PreResp) vs post-training (PostResp).*

Variable	PreResp	PostResp
$HR_{peak130}$ (beats·min^{-1})	176 ± 5	173 ± 4
$\dot{V}E_{peak130}$ (l·min^{-1})	138.0 ± 7.5	152.2 ± 8.1 *
$\dot{V}O_{2peak130}$ (l·min^{-1})	3.76 ± 0.10	3.87 ± 0.13
$\dot{V}CO_{2\,peak130}$ (l·min^{-1})	4.99 ± 0.36	5.11 ± 0.18
RER	1.36 ± 0.07	1.35 ± 0.04

Values are means ± S.E.M.; n = 7. $HR_{peak130}$, peak heart rate in 130% respiratory tests; $\dot{V}E_{peak130}$, peak expired ventilation in 130% respiratory tests, BTPS; $\dot{V}O_{2peak130}$, peak oxygen uptake in 130% respiratory tests, STPD; $\dot{V}CO_{2\,peak130}$, peak carbon dioxide output in 130% respiratory tests, STPD; RER, peak respiratory exchange ratio during exercise. * P<0.05, Post > Pre-training.

Accumulated and normalised values

The accumulated $\dot{V}O_2$ (mmol·kg^{-1}) was 35% higher in the post-training respiratory test than in the pre-training respiratory test, partially reflecting the 29% longer test duration (P<0.01; Table 3.6). The oxygen uptake in each of the PreResp and PostResp tests was normalised for the duration of exercise (expressed in l·min^{-1}), and the resulting $\dot{V}O_2$ did not differ significantly (P=0.13; Table 3.6).

The accumulated oxygen deficit (mmol·kg^{-1}) was 19% higher in PostResp than in PreResp (P<0.05; Table 3.6). The oxygen deficit normalised for the duration of exercise (l·min^{-1}) did not differ significantly after training (P=0.13).

The accumulated V_E was 42% higher (P<0.02; Table 3.6), and the accumulated $\dot{V}CO_2$ 32% higher (P<0.05) in PostResp than in PreResp. When averaged over the duration of exercise, $\dot{V}E$ was 10% higher (P=0.05), whilst neither mean $\dot{V}CO_2$ nor mean HR were different after training. Individual data are contained within Appendix C (C6-7).

Table 3.6 *Accumulated and normalised respiratory data, and mean heart rate during the pre- (PreResp) and post-training (PostResp) tests to fatigue at 130% $\dot{V}O_{2\,peak}$*

Variable	PreResp	PostResp
Mean HR (beats·min^{-1})	157 ± 6	153 ± 5
Accumulated V_E (litres)	121.1 ± 20.8	171.9 ± 27.6 *
$\dot{V}_E$ (l·min^{-1})	90.7 ± 5.2	99.5 ± 6.7
Accumulated VO_2 (litres)	3.76 ± 0.64	5.06 ± 0.67 **
Accumulated VO_2 (mmol·kg^{-1})	2.23 ± 0.41	3.00 ± 0.43 **
$\dot{V}O_2$ (l·min^{-1})	2.83 ± 0.11	2.99 ± 0.10
Accumulated oxygen deficit (litres)	2.54 ± 0.31	3.01 ± 0.36 *
Accumulated oxygen deficit (mmol·kg^{-1})	1.50 ± 0.20	1.79 ± 0.25 *
Oxygen deficit (l·min^{-1})	2.09 ± 0.27	1.92 ± 0.25
Accumulated VCO_2 (litres)	4.45 ± 0.87	5.87 ± 0.91 *
$\dot{V}CO_2$ (l·min^{-1})	3.27 ± 0.23	3.40 ± 0.18

Values are means ± S.E.M.; $n = 7$. * $P<0.05$, ** $P<0.01$, PostResp>PreResp.

In summary, peak power was increased (P<0.02), and $\dot{V}O_{2peak}$ tended to be higher (P=0.07) after training during the incremental tests. Each of peak HR, $\dot{V}_E$, and peak $\dot{V}CO_2$ was unchanged with training.

Time to fatigue (P<0.001) and hence total work (P<0.001) was increased after training in the exhaustive 130% $\dot{V}O_{2peak}$ tests. The relative intensity of the 130% pre-training $\dot{V}O_{2peak}$ tests tended to be reduced after training (P=0.06). Peak $\dot{V}_E$ during the 130% $\dot{V}O_{2peak}$ tests was increased after training (P<0.05), however the respective peaks values of HR, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were unchanged.

Increased accumulated values of oxygen uptake ($P<0.01$), oxygen deficit ($P<0.05$), ventilation ($P<0.02$), and carbon dioxide output ($P<0.05$) were evident after training in the 130% $\dot{V}O_{2peak}$ test to fatigue. When each of these variables was normalised for the time of exercise, i.e. expressed as $l \cdot min^{-1}$, $\dot{V}E$ was higher ($P=0.05$), whilst $\dot{V}O_2$, oxygen deficit, and $\dot{V}CO_2$ were not significantly altered by training.

Peak power ($P<0.05$) and total work ($P<0.05$) were significantly increased after training when exercising maximally for 30 s on the air-braked cycle ergometer. Ventilation and gas exchange were not assessed in these tests.

3.4.4 Plasma Biochemistry and Haematology

Individual blood data for each variable appear in Appendix C (C8-13).

3.4.4.1 Catecholamines - Noradrenaline and Adrenaline

PreExh vs PostMatch

Prior to training, in PreExh, plasma noradrenaline concentration ([NAdr]) rose significantly with exercise, peaked at 1 min post exercise, and remained above resting concentrations after 10 min of recovery ($P<0.01$). Following training, in PostMatch, plasma [NAdr] also rose significantly with exercise, however both the peak at 1 min and the final sample at 10 min post-exercise were significantly lower than in PreExh ($P<0.05$; Figure 3.7A).

In PreExh, plasma adrenaline ([Adr]) rose during exercise from a resting value of 0.48 ± 0.16 $nmol \cdot l^{-1}$, peaked at 5.74 ± 0.93 $nmol \cdot l^{-1}$ after 1 min of recovery, and returned to approximate resting values after 5 min of recovery. Training did not significantly affect the plasma [Adr] response during exercise or recovery(Figure 3.7B).

PreExh vs PostExh

Both plasma [NAdr] and [Adr] rose sharply with exercise, returned to be similar to resting values by 10 min and 5 min of recovery, respectively, and were higher after training in PostExh ($P<0.05$; Figures 3.8A, B) when exercise was continued until exhaustion.

3.4.4.2 Plasma Electrolytes

(i) Plasma lactate

PreExh vs PostMatch

Plasma lactate concentration ($[Lac^-]$) rose sharply with exercise, continued to rise until peaking at 5 min of recovery, and thereafter slowly declined to remain above resting values at all measurement times in both PreExh and PostMatch ($P<0.001$). Following training, in PostMatch, a significant 2.36 ± 0.41 $mmol \cdot l^{-1}$ reduction ($P<0.01$; mean difference) was evident in plasma $[Lac^-]$. Significantly less $[Lac^-]$

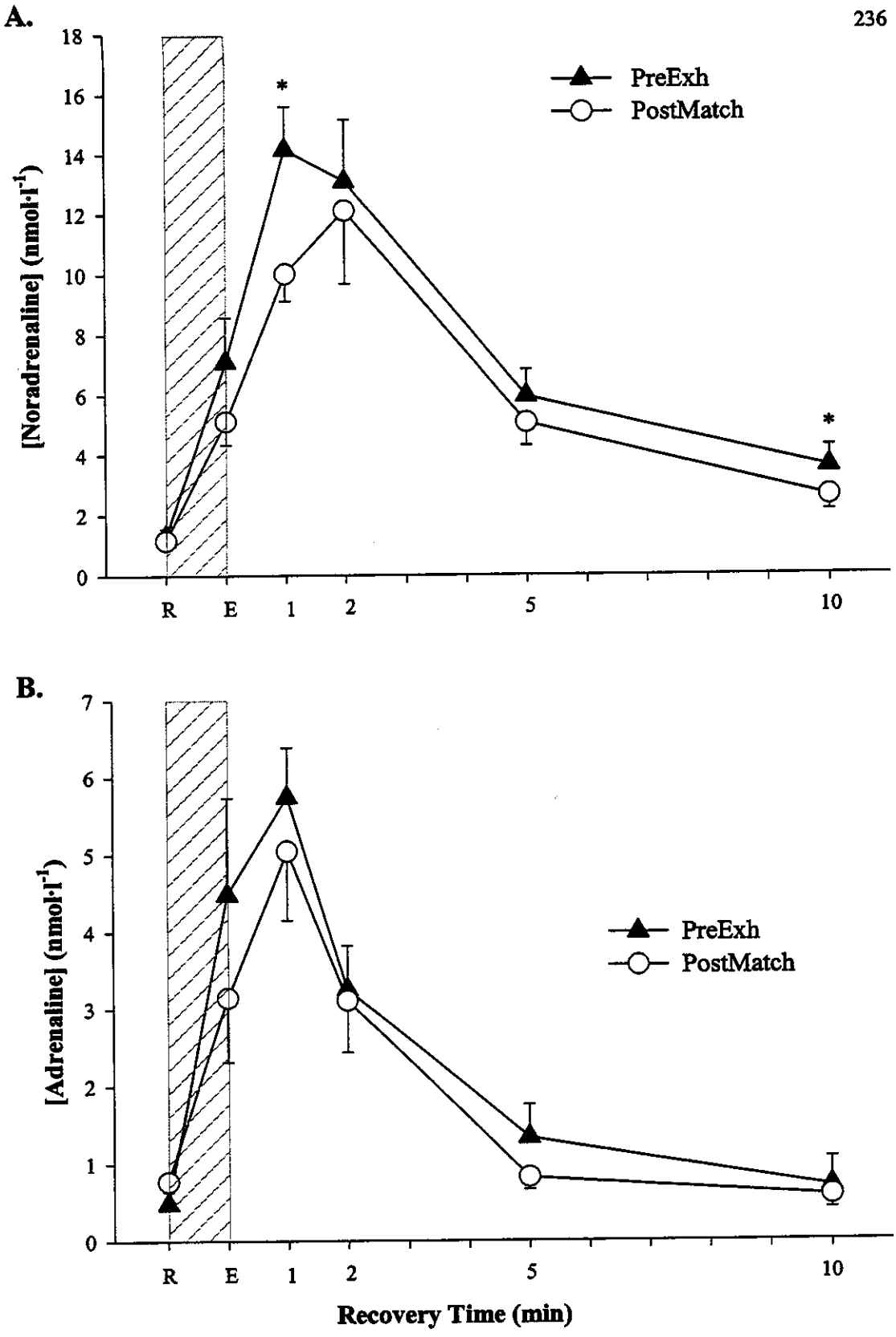


Figure 3.7 A,B Plasma catecholamine concentrations (mean ± S.E.M.) at rest (R), immediately after matched-work exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. Noradrenaline. $n = 7$, except E ($n = 6$) and 1 ($n = 5$). Main effects for time ($P<0.001$) and training ($P<0.05$) were found. The interaction effect was also significant; $*P<0.05$, PreExh > PostMatch. B. Adrenaline. $n = 7$, except E ($n = 6$) and 1 ($n = 5$). A main effect for time ($P<0.001$) was found.

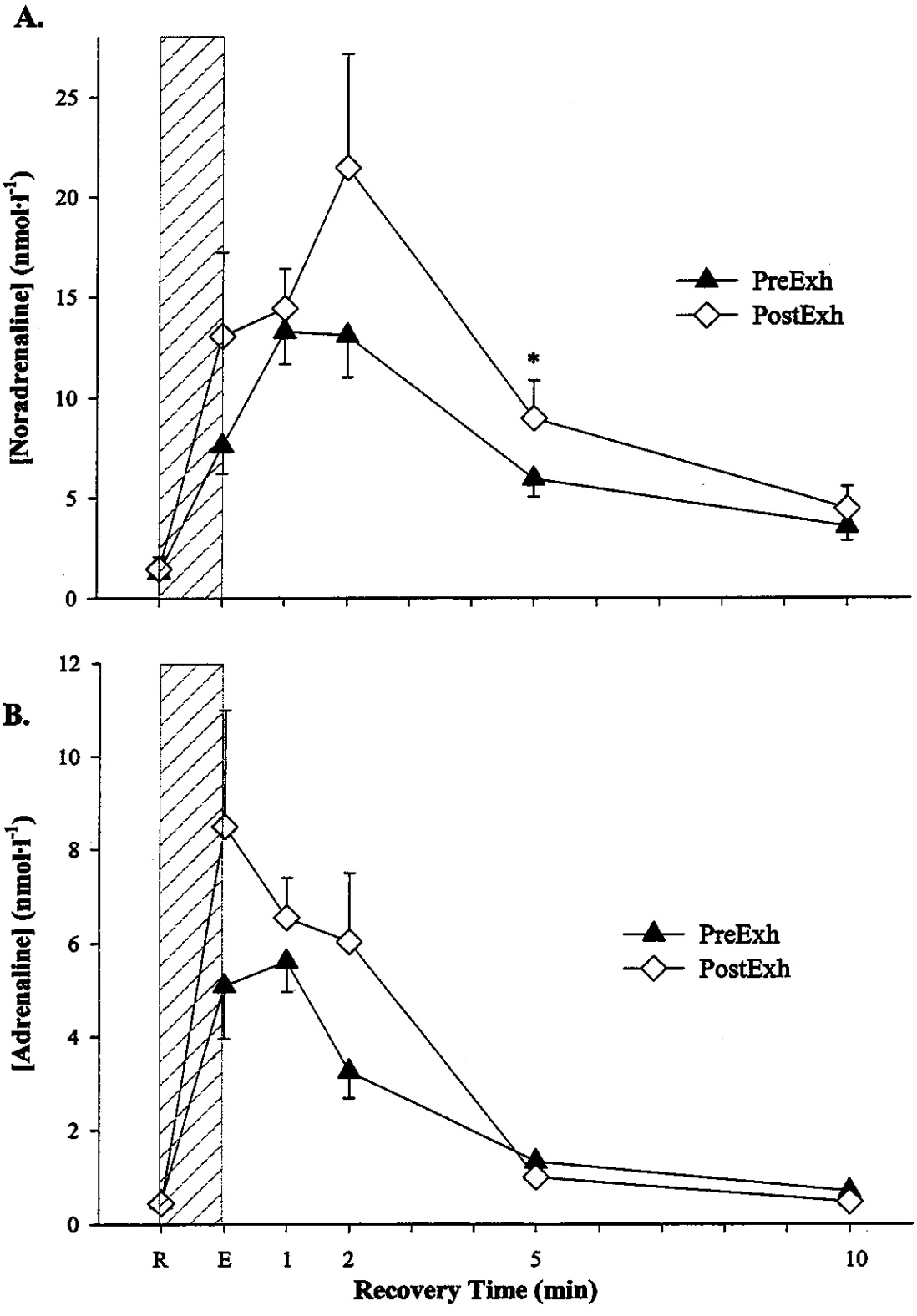


Figure 3.8 A,B Plasma catecholamine concentrations (mean ± S.E.M.) at rest (R), immediately after exhausting exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise.
A. Noradrenaline. $n = 7$, except E ($n = 5$) and 1 ($n = 6$). A main effect for time ($P < 0.001$) was found. The interaction effect was significant; * $P<0.05$, PostExh> PreExh.
B. Adrenaline. $n = 7$, except E ($n = 5$) and 1 ($n = 6$). A main effect for time ($P < 0.001$) was found. The interaction effect was significant; $P < 0.05$.

accumulated during exercise, peak concentration was lower, and a more rapid recovery of [Lac^-] was evident in PostMatch ($P<0.01$; Figure 3.9A).

PreExh vs PostExh

Plasma [Lac^-] rose significantly with exercise, and after peaking 5 min post-exercise both pre- and post-training, slowly declined for the remainder of recovery, however exceeded resting [Lac^-] at all times ($P<0.001$). After training, in PostExh, plasma [Lac^-] was 1.40 ± 0.36 $mmol \cdot l^{-1}$ higher ($P<0.05$; mean difference; Figure 3.9B).

Pooling pre- and post-training data, higher peak plasma [Lac^-] was significantly associated with the production of greater work when exercising to exhaustion ($r=0.76$; $P<0.01$), and with higher plasma [NAdr] and [Adr] ($r=0.56$; $r=0.57$, respectively; $P<0.05$).

(ii) Plasma potassium

PreExh vs PostMatch

Plasma potassium concentration ([K^+]) rose significantly with exercise, then rapidly declined to undershoot resting values by 5 min post-exercise, and remained lower than resting [K^+] at the final measurement ($P<0.001$). Plasma [K^+] was lower by a mean difference of 0.11 ± 0.05 $mmol \cdot l^{-1}$ after training in PostMatch ($P<0.05$), and peak plasma [K^+] was 11% lower during exercise ($P<0.001$; Figure 3.10A), when compared to the PreExh test. Since arterialised venous blood sampling was employed, rather than direct femoral venous sampling, peak plasma [K^+] for each subject occurred at either the end of exercise or at 1 min post-exercise. Thus, the highest [K^+] (whether at the end of exercise or at 1 min post-exercise) was also compared in PreExh and PostMatch, with the result that peak plasma [K^+] was still significantly lower (8.5%; $P<0.05$) after training in PostMatch.

The ratio of exercise [K^+]/work in PostMatch was reduced ($P<0.05$) to 89 ± 3% of PreExh, and the rise in plasma [K^+] ($\Delta[K^+]$) with exercise (relative to rest) was reduced 31% after training ($P<0.05$) when compared to PreExh. Similarly significant results were evident when the highest plasma [K^+] was used.

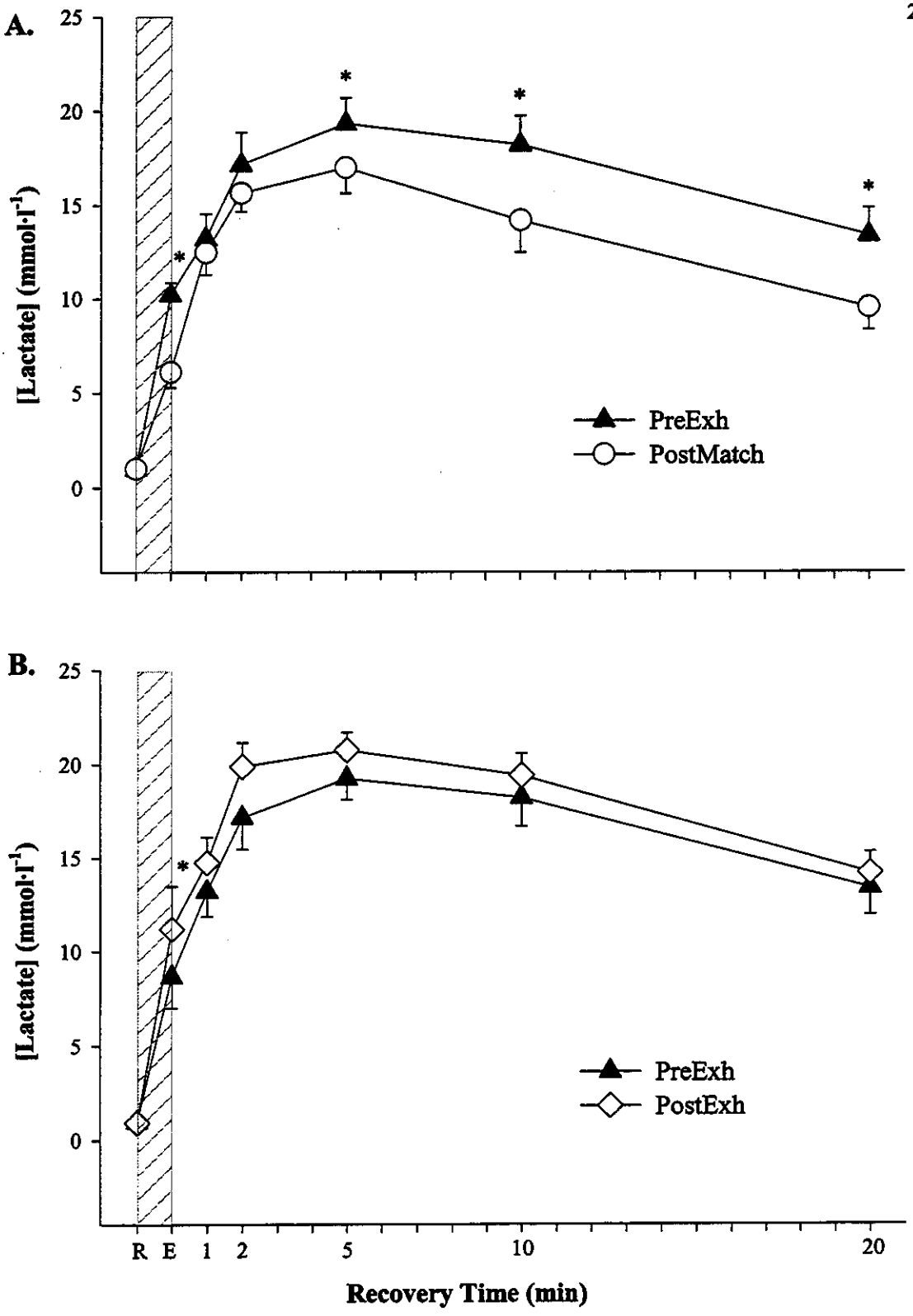


Figure 3.9 A,B Plasma lactate concentrations (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E and 1 ($n = 5$), and 5 ($n = 6$). Main effects for time ($P<0.001$) and training status ($P<0.01$) were found. The interaction effect was also significant ($P<0.01$). *$P<0.05$, PreExh>PostMatch. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). Main effects for time ($P<0.001$) and training status ($P<0.05$) were found. *$P=0.05$.

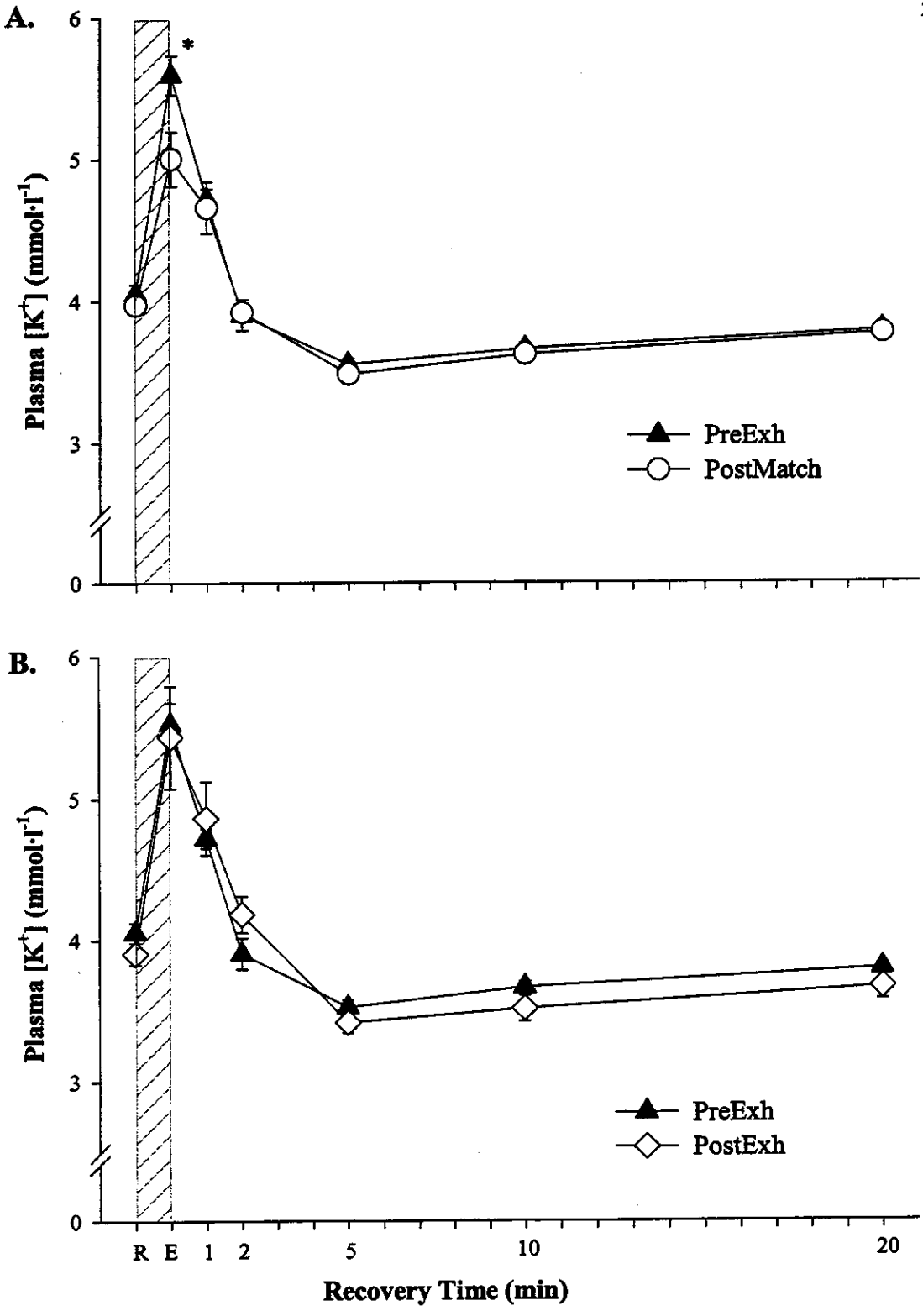


Figure 3.10 A,B Plasma potassium concentrations (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E, 1, and 5 ($n = 6$). Main effects for time ($P<0.001$) and training status ($P<0.05$) were found. The interaction effect was also significant ($P<0.001$). * $P<0.05$, PreExh>PostMatch. B. PreExh vs PostExh. $n = 7$, except E ($n = 5$) and 1 ($n = 6$). A main effect for time ($P<0.001$) was found.

PreExh vs PostExh

In PostExh, plasma [K^+] peaked immediately after exercise, rapidly fell to resting values by 2 min of recovery, then continued to fall, remaining below resting concentration at the final measurement time ($P<0.001$), a pattern not significantly different to that of PreExh (Figure 3.10B), despite cycling for 21% longer. A comparison of the highest plasma [K^+] (whether at the end of exercise or at 1 min post) similarly yielded no difference ($P=0.8$) between PreExh and PostExh.

The ratio of [K^+]/work tended to be reduced in PostExh (18%; $P=0.052$; $n=5$), although there was no correlation between work produced in cycling to exhaustion and peak plasma [K^+] ($r=0.015$; $P=0.97$); and Δ[K^+]/work was not different.

(iii) Plasma sodium

PreExh vs PostMatch

Plasma sodium concentration ([Na^+]) rose significantly with exercise and remained above resting values until 10 min of recovery in both PreExh and PostMatch ($P<0.001$). No effect of training was evident for plasma [Na^+] (Table 3.7).

PreExh vs PostExh

Peak [Na^+] occurred after 1 min of recovery from fatiguing exercise ($P<0.001$), declined to resting levels by 10 min of recovery, and was not significantly different after training (Table 3.8).

(iv) Plasma Chloride

PreExh vs PostMatch

Plasma chloride concentration ([Cl^-]) increased 3% immediately after exercise, peaked at 1 min of recovery, and remained above resting values ($P<0.001$; Table 3.7) until 10 min of recovery. No effect of training was observed on the plasma [Cl^-] response to sprint exercise.

PreExh vs PostExh

Prior to training plasma [Cl^-] rose with exercise, peaked after 1 min of recovery, having risen by 3.8 $mmol \cdot l^{-1}$ from resting values ($P<0.001$), and then returned to resting values by 10 min of recovery (Table 3.8). Training did not alter the plasma [Cl^-] response to exhaustive sprint exercise.

3.4.4.3 Haematocrit, haemoglobin, plasma protein, and the percentage change in plasma volume

(i) Haematocrit

PreExh vs PostMatch

Prior to training, the haematocrit (Hct) increased with exercise, peaked at 5 min of recovery, then slowly declined for the remainder of the recovery period; however remained above resting percentage at all times ($P<0.001$). Haematocrit was lower in the PostMatch test (mean value of 47.3 ± 0.4%) than in PreExh (48.5 ± 0.3%; $P<0.05$; Table 3.9).

PreExh vs PostExh

Hct was increased above rest at all times during exercise and recovery in the exhaustion tests before and after training ($P<0.001$; Table 3.10). Whilst Hct tended to be lower after training in the test to fatigue, the difference just failed to attain statistical significance ($P=0.053$).

(ii) Haemoglobin concentration

PreExh vs PostMatch

Haemoglobin concentration ([Hb]) increased during exercise and remained above resting concentrations during recovery ($P<0.001$; Table 3.9). Following training, the rise in [Hb] was greater, but recovery more rapid ($P<0.05$).

PreExh vs PostExh

During the PreExh test [Hb] rose 8% with exercise, peaked after 1 min of recovery, then slowly declined thereafter (Table 3.10). A similar response occurred after training.

Table 3.7 *Plasma sodium ($[Na^+]$) and chloride concentrations ($[Cl^-]$) (mean ± S.E.M.) at rest, immediately after exercise, and in recovery from the matched work tests – PreExh vs PostMatch, conducted at 130% pre-training $\dot{V}O_{2\,peak}$. Numerals followed by addition sign indicate post-exercise recovery time. n = 7, except exercise $[Na^+]$ (n = 6), $[Cl^-]$ (n = 5), and 1+ and 5+ (n = 6). Significant main effects for time are reported in the text.*

Variable	Pre/Post	Rest	Exercise	1+	2+	5+	10+	20+
$[Na^+]$ $mmol \cdot l^{-1}$	PreExh	138.5 ± 0.8	142.3 ± 1.4	143.7 ± 0.6	142.8 ± 0.8	140.9 ± 0.8	138.8 ± 0.6	137.1 ± 0.8
	PostMatch	137.3 ± 0.5	142.1 ± 0.7	142.7 ± 0.8	142.0 ± 1.0	139.7 ± 0.6	137.5 ± 0.4	137.8 ± 0.6
$[Cl^-]$ $mmol \cdot l^{-1}$	PreExh	102.5 ± 0.5	105.3 ± 1.1	106.3 ± 0.9	104.8 ± 0.6	104.6 ± 0.4	102.3 ± 0.6	102.1 ± 0.5
	PostMatch	102.8 ± 0.3	105.7 ± 1.2	106.9 ± 1.2	105.7 ± 1.0	104.0 ± 1.2	102.5 ± 0.8	103.1 ± 0.4

Table 3.8 *Plasma sodium ($[Na^+]$) and chloride concentrations ($[Cl^-]$; mean ± S.E.M.) at rest, immediately after exercise, and in recovery from the tests to exhaustion – PreExh vs PostExh, conducted at 130% pre-training $\dot{V}O_{2peak}$. Numerals followed by the addition sign indicate post-exercise recovery time. n = 7, except exercise (n = 5), and 1+ (n =6). Significant main effects of time are reported in the text.*

Variable	Pre/Post	Rest	Exercise	1+	2+	5+	10+	20+
$[Na^+]$ $mmol \cdot l^{-1}$	PreExh	138.5 ± 0.8	141.6 ± 1.4	143.7 ± 0.6	142.8 ± 0.8	140.8 ± 0.7	138.8 ± 0.6	137.1 ± 0.8
	PostExh	136.4 ± 0.7	141.9 ± 1.3	143.0 ± 0.9	142.7 ± 0.8	140.6 ± 0.7	137.9 ± 0.9	137.1 ± 1.1
$[Cl^-]$ $mmol \cdot l^{-1}$	PreExh	102.5 ± 0.5	104.4 ± 0.9	106.3 ± 0.9	104.8 ± 0.6	104.2 ± 0.5	102.3 ± 0.6	102.1 ± 0.5
	PostExh	103.5 ± 0.6	105.9 ± 1.2	107.2 ± 0.7	106.0 ± 0.5	104.4 ± 0.5	103.0 ± 0.6	103.4 ± 0.4

(iii) Plasma protein concentration

PreExh vs PostMatch

Plasma protein concentration ([PPr^-]) rose with exercise, peaked 15% above resting values at 2 min of recovery, then decreased for the remainder of the recovery period ($P<0.001$). In PostMatch, [PPr^-] was lower by a mean difference of 0.34 ± 0.05 $g \cdot dl^{-1}$ ($P<0.01$; Table 3.9).

PreExh vs PostExh

Similarly, in the PreExh and PostExh comparison, main effects for time ($P<0.001$) and training ($P<0.01$) were found. Plasma protein concentration was lower in PostExh by a mean difference of 0.21 ± 0.04 $g \cdot dl^{-1}$ (Table 3.10).

(iv) Percentage change in plasma volume

PreExh vs PostMatch

Percentage change in plasma volume (ΔPV) reached a nadir after 5 min of recovery from fatiguing exercise in PreExh, then slowly rose to a level $8.1 \pm 1.6\%$ lower than rest by 20 min ($P<0.001$; Table 3.9). After training, in the PostMatch test, plasma volume changes were similar until 20 min of recovery, by which time ΔPV had recovered more rapidly to be only $2.7 \pm 1.5\%$ below that at rest ($P<0.001$).

PreExh vs PostExh

The contraction of plasma volume below resting values was significant at all time points in both PreExh and PostExh tests ($P<0.001$), with no difference after training (Table 3.10).

3.4.4.4 Plasma acid-base variables

(i) Strong Ion Difference

PreExh vs PostMatch

In PreExh, the plasma strong ion difference (SID) fell after exercise from a resting value of 39.1 ± 0.9 $mmol \cdot l^{-1}$ to a nadir of 20.6 ± 1.6 $mmol \cdot l^{-1}$ at 5 min of recovery ($P<0.001$), and remained significantly below rest after 20 min of recovery. After

Table 3.9 *Haematocrit (Hct), haemoglobin ([Hb]), plasma protein concentration ([PPr$^-$]), and percentage change in plasma volume (Δ PV) (mean ± S.E.M.) at rest, immediately after exercise, and in recovery from the matched work tests – PreExh vs PostMatch, conducted at 130% pre-training $\dot{V}O_{2\,peak}$. Numerals followed by addition sign indicate post-exercise recovery time. n = 7, except exercise(n = 5), and 1+ and 5+ (n = 6). Significant interaction and main effects for time and training status are reported in the text.*

Variable	Pre/Post	Rest	Exercise	1+	2+	5+	10+	20+
Hct %	PreExh	45.2 ± 0.4	49.1 ± 0.7	49.1 ± 0.7	50.0 ± 0.7	50.1 ± 0.8	49.3 ± 0.6	47.5 ± 0.7
	PostMatch	44.0 ± 0.7	48.2 ± 0.9	48.3 ± 0.6	48.9 ± 0.7	49.5 ± 0.9	47.8 ± 0.9	45.3 ± 0.7
[Hb] $g \cdot dl^{-1}$	PreExh	15.6 ± 0.2	16.9 ± 0.1	17.0 ± 0.2	17.1 ± 0.2	17.1 ± 0.3	16.7 ± 0.2	16.2 ± 0.2
	PostMatch	15.7 ± 0.2	17.1 ± 0.3	17.0 ± 0.3	17.1 ± 0.4	16.8 ± 0.4	16.4 ± 0.3	15.8 ± 0.4
[PPr$^-$] $g \cdot dl^{-1}$	PreExh	7.28 ± 0.08	8.20 ± 0.17	8.29 ± 0.28	8.39 ± 0.18	8.26 ± 0.17	8.05 ± 0.12	7.72 ± 0.12
	PostMatch	7.08 ± 0.06	7.95 ± 0.11	7.89 ± 0.11	8.03 ± 0.12	7.97 ± 0.16	7.68 ± 0.10	7.24 ± 0.10
ΔPV %	PreExh	0	-13.6 ± 2.0	-15.3 ± 0.8	-16.8 ± 1.1	-16.6 ± 1.3	-13.9 ± 1.3	-8.1 ± 1.6
	PostMatch	0	-14.8 ± 1.6	-16.4 ± 1.8	-16.1 ± 1.6	-15.7 ± 1.5	-10.9 ± 1.7	-2.7 ± 1.5

Table 3.10 *Haematocrit (Hct), haemoglobin ([Hb]), plasma protein concentration([PPr$^-$]), and percentage change in plasma volume (Δ PV; mean ± S.E.M.) at rest, immediately after exercise, and in recovery from the tests to exhaustion – PreExh vs PostExh, conducted at 130% pre-training $\dot{V}O_{2\,peak}$. Numerals followed by plus sign indicate post-exercise recovery time. n = 7, except exercise and 1+ (n = 5). Significant interaction and main effects of time and training are reported in the text.*

Variable	Pre/Post	Rest	Exercise	1+	2+	5+	10+	20+
Hct %	PreExh	45.2 ± 0.4	48.3 ± 0.9	49.0 ± 0.9	50.0 ± 0.7	50.1 ± 0.6	49.3 ± 0.6	47.5 ± 0.7
	PostExh	44.0 ± 0.5	48.2 ± 1.0	48.4 ± 0.9	49.5 ± 0.8	49.5 ± 0.8	48.2 ± 0.8	46.0 ± 0.7
[Hb] $g \cdot dl^{-1}$	PreExh	15.6 ± 0.2	16.8 ± 0.2	17.1 ± 0.3	17.1 ± 0.2	17.1 ± 0.3	16.7 ± 0.2	16.2 ± 0.2
	PostExh	15.7 ± 0.2	16.8 ± 0.6	17.2 ± 0.4	17.4 ± 0.3	17.1 ± 0.3	16.6 ± 0.3	16.0 ± 0.3
[PPr$^-$] $g \cdot dl^{-1}$	PreExh	7.28 ± 0.08	8.11 ± 0.24	8.35 ± 0.33	8.39 ± 0.18	8.24 ± 0.15	8.05 ± 0.12	7.72 ± 0.12
	PostExh	7.12 ± 0.07	7.87 ± 0.16	8.05 ± 0.15	8.26 ± 0.13	8.16 ± 0.12	7.81 ± 0.14	7.37 ± 0.13
ΔPV %	PreExh	0	-11.9 ± 2.1	-14.8 ± 0.9	-16.8 ± 1.1	-16.9 ± 1.2	-13.9 ± 1.3	-8.1 ± 1.6
	PostExh	0	-13.9 ± 3.2	-16.2 ± 2.4	-18.6 ± 2.0	-17.3 ± 1.5	-12.5 ± 1.6	-5.4 ± 2.4

training, in PostMatch, the SID returned towards resting values more rapidly, being higher at 10 ($P<0.05$) and 20 min ($P<0.001$) post-exercise (although still remaining well below the resting value; Figure 3.11A).

PreExh vs PostExh

Main effects for time ($P<0.001$) and training ($P<0.01$) were evident for the SID in the tests conducted to exhaustion. In PostExh the plasma SID was lower by a mean difference of 2.9 ± 0.5 mmol·l^{-1} (Figure 3.11B). The nadir of the SID was strongly positively correlated ($r=0.81$; $P<0.001$) with the amount of work produced before exhaustion, such that the more work produced, the lower the SID.

(ii) Plasma total weak acids - A_{tot}

PreExh vs PostMatch

The concentration of plasma total weak acids ([A_{tot}]) increased with exercise, continued to rise until 2 min of recovery ($P<0.001$), then fell towards resting values. After training [A_{tot}] was lower by a mean difference of 0.82 ± 0.11 mmol·l^{-1} ($P<0.01$; Figure 3.12A).

PreExh vs PostExh

Main effects of time ($P<0.001$) and training ($P<0.01$) for [A_{tot}] were also found for the tests to exhaustion. [A_{tot}] was 0.52 ± 0.09 mmol·l^{-1} lower (mean difference) in PostExh than PreExh (Figure 3.12B).

(iii) Carbon dioxide tension - PCO_2

PreExh vs PostMatch

Arterialised carbon dioxide tension (PCO_2) peaked immediately after exercise, then fell for the remainder of recovery ($P<0.001$; Figure 3.13A). In the PostMatch test, the PCO_2 increase following exercise was lower than in PreExh ($P<0.05$).

PreExh vs PostExh

PCO_2 rose sharply with exhaustive exercise, then decreased throughout the recovery period ($P<0.001$), and was not significantly different after training (Figure 3.13B).

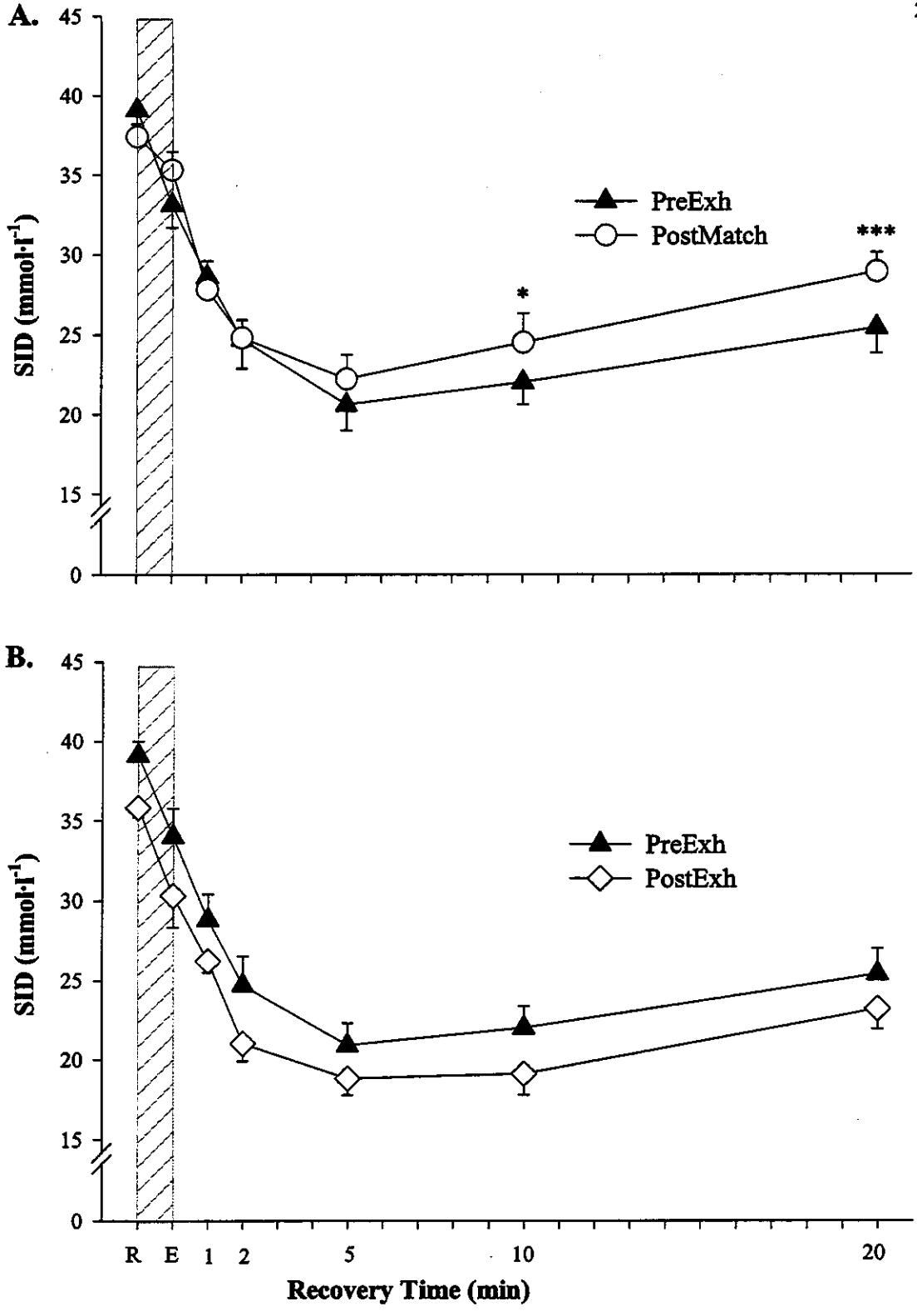


Figure 3.11 A,B Plasma strong ion difference (SID) (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E ($n = 5$), and 1 and 5 ($n = 6$). A main effect for time ($P<0.001$) was found. The interaction between time and training status was significant ($P<0.05$). *$P<0.05$, *** $P<0.001$, PostMatch > PreExh. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). Main effects for time ($P<0.001$) and training status ($P<0.01$) were found.

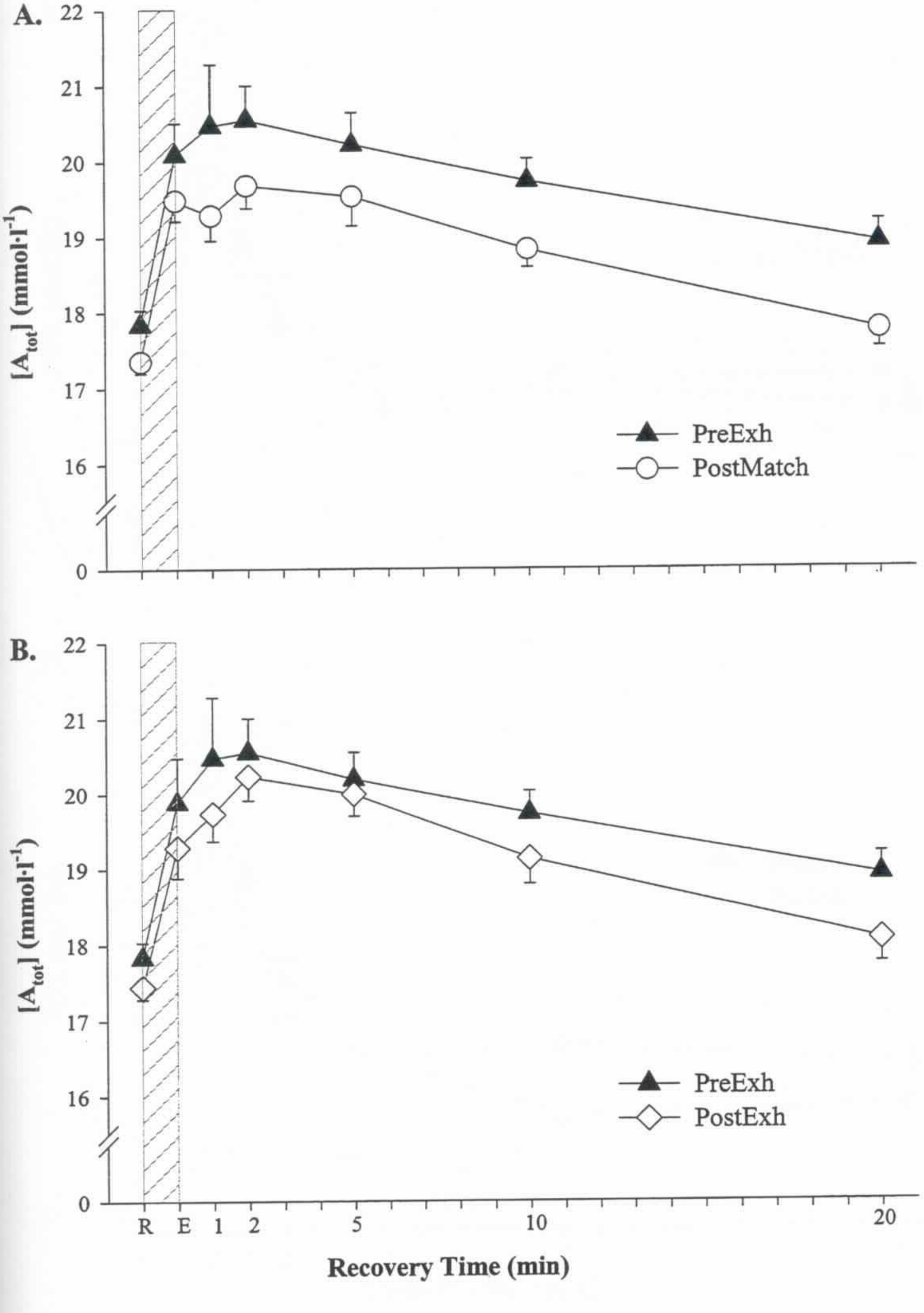


Figure 3.12 A,B Plasma total weak acids ($[A_{tot}]$) (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E ($n = 5$), and 1 and 5 ($n = 6$). Main effects for time ($P<0.001$) and training ($P<0.01$) were found. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). Main effects for time ($P<0.001$) and training status ($P<0.01$) were found.

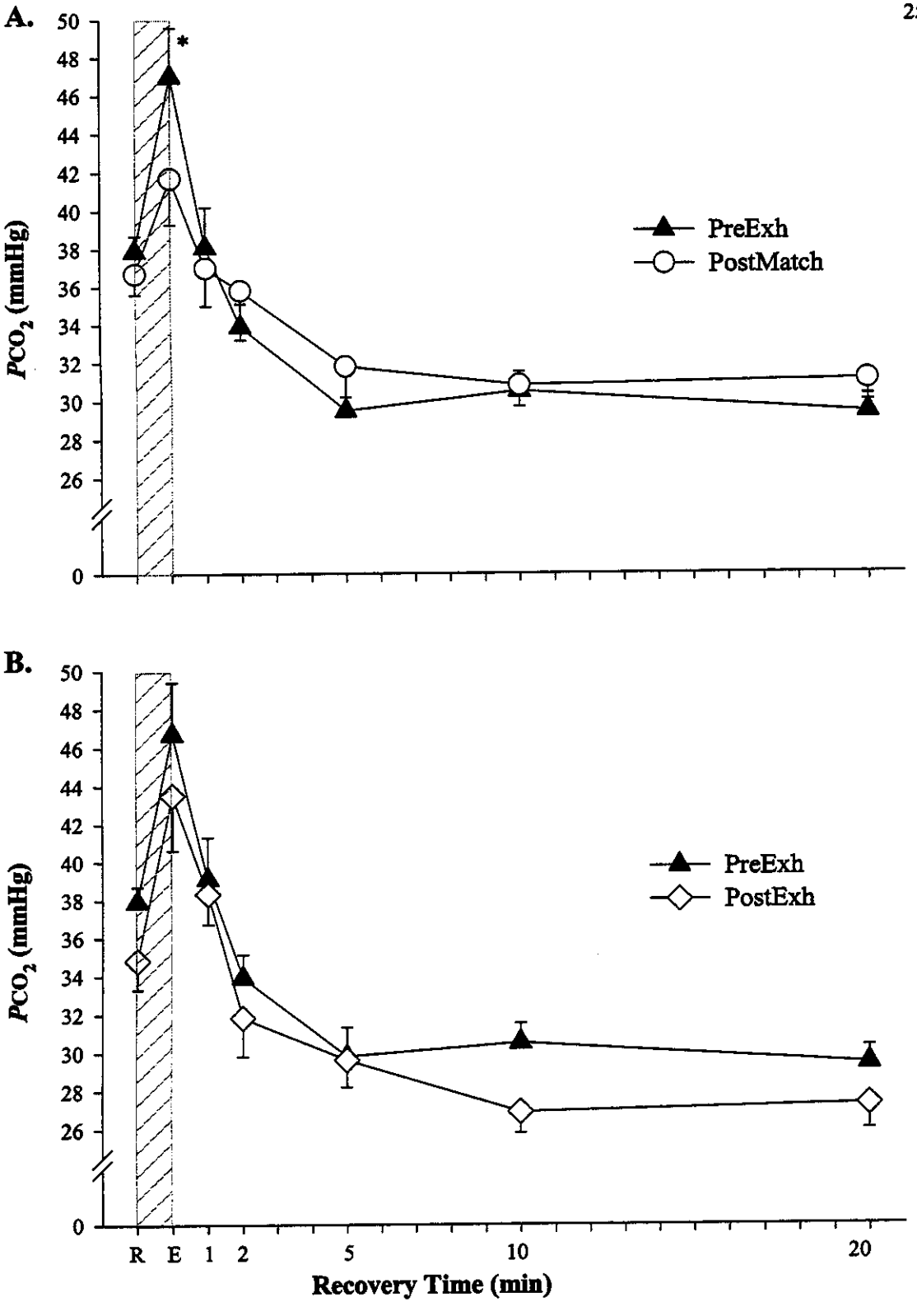


Figure 3.13 A,B Arterialised venous carbon dioxide tension ($P\mathrm{CO}_2$) (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}\mathrm{O}_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E ($n = 5$), and 1 and 5 ($n = 6$). A main effect for time ($P<0.001$) and an interaction effect (*$P<0.05$; PreExh>PostMatch) occurred. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). A main effect for time ($P<0.001$) was evident.

(iv) Plasma hydrogen ion - [H^+]

PreExh vs PostMatch

In PreExh, plasma hydrogen ion concentration ([H^+]) rose 46% after exercise, peaked at 5 min of recovery, and remained 37% above resting concentrations at the final measurement time ($P<0.001$; Figure 3.14A). Plasma [H^+] was 6.7 ± 0.7 nmol·l^{-1} lower (mean difference; $P<0.001$) in PostMatch than in PreExh. A smaller rise in [H^+] with exercise, a lower peak, and more rapid recovery was also evident in PostMatch ($P<0.001$).

PreExh vs PostExh

Plasma [H^+] rose markedly with exercise ($P<0.001$) both before and after training when cycling to fatigue, however reached a higher peak at 5 min post-exercise in PostExh ($P<0.05$; Figure 3.14B).

(v) Bicarbonate concentration - [HCO_3^-]

PreExh vs PostMatch

The calculated bicarbonate concentration ([HCO_3^-]) fell from a resting value of 22.8 ± 0.4 mmol·l^{-1} to a nadir of 10.4 ± 0.8 mmol·l^{-1} 5 min after the cessation of exercise, and remained 42% lower than rest after 20 min of recovery ($P<0.01$, Figure 3.15A). A similar pattern occurred after training, however [HCO_3^-] was significantly higher by a mean difference of 1.6 ± 0.3 mmol·l^{-1} ($P<0.05$).

PreExh vs PostExh

In contrast, [HCO_3^-] was significantly lower in PostExh than PreExh by a mean difference of 0.9 ± 0.2 mmol·l^{-1} ($P<0.001$), and the nadir (9.6 ± 0.8 mmol·l^{-1}) occurred later, after 10 min of recovery. Plasma [HCO_3^-] remained 42% and 45% below resting levels after 20 min recovery in PreExh and PostExh, respectively ($P<0.001$; Figure 3.15B).

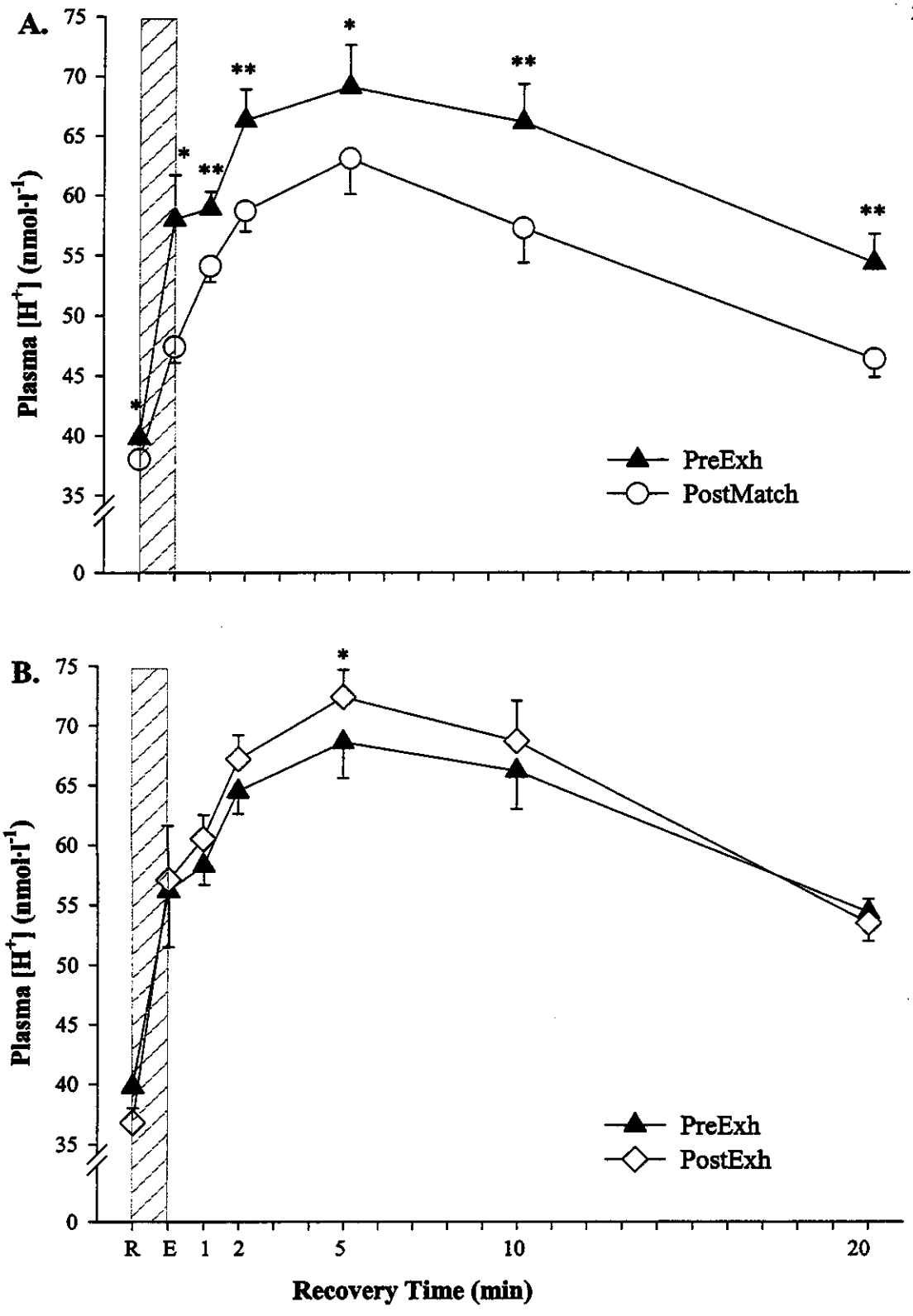


Figure 3.14 A,B Plasma hydrogen ion concentration ($[H^+]$) (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E ($n = 5$), and 1 and 5 ($n = 6$). Main effects for time ($P<0.001$) and training ($P<0.001$), and an interaction effect ($P<0.001$) occurred, $^*P<0.05$, $^{**}P<0.01$, PreExh>PostMatch. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). A main effect for time ($P<0.001$), and an interaction effect ($^*P<0.05$) was evident.

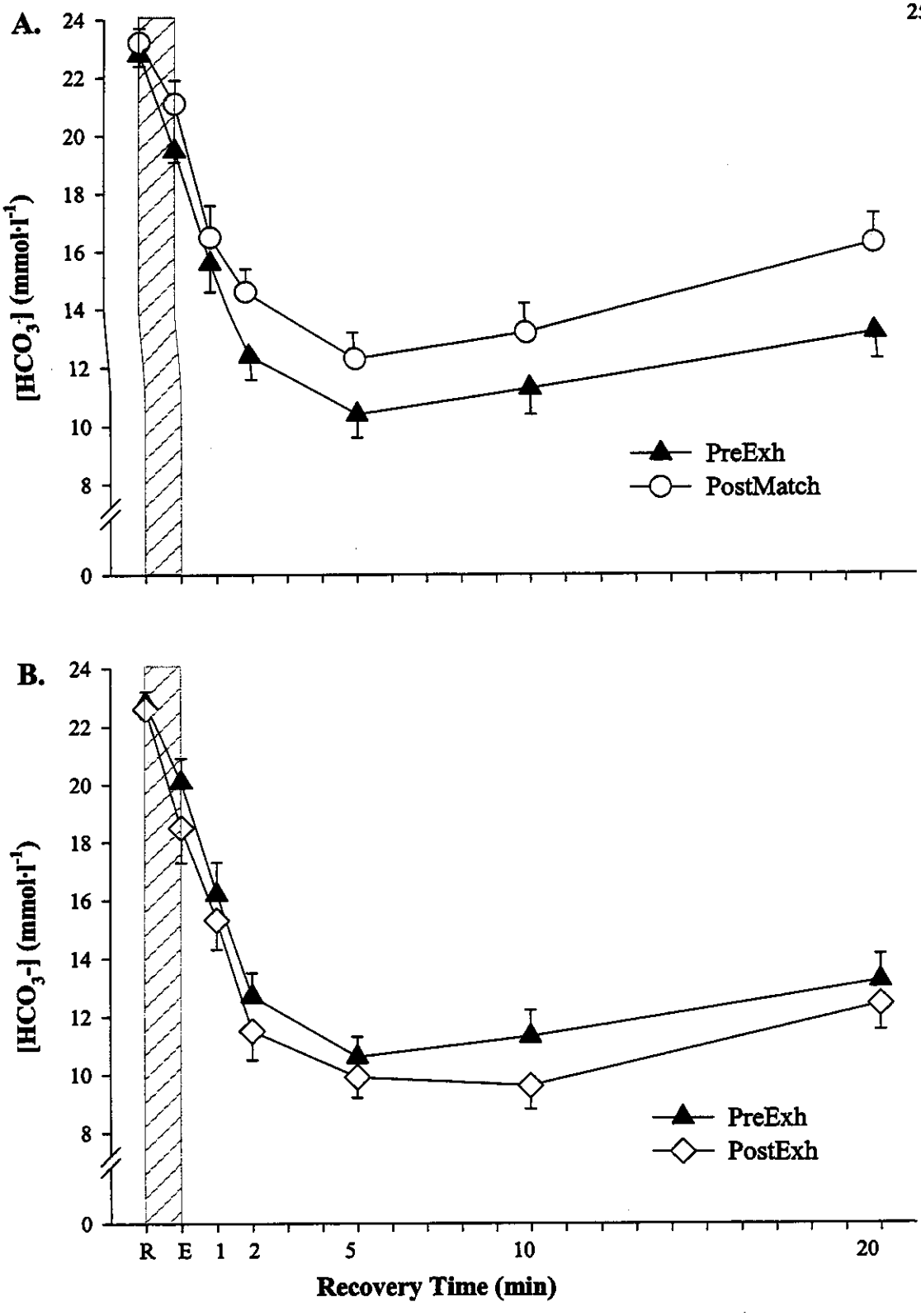


Figure 3.15 A,B Plasma bicarbonate concentration ($[HCO_3^-]$) (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E ($n = 5$), and 1 and 5 ($n = 6$). Main effects for time ($P<0.001$) and training ($P<0.05$) occurred. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). Main effects for time and training ($P<0.001$) were evident.

3.4.4.5 Extent of venous arterialisation

The extent of venous arterialisation is assessed by examining the P_{O_2}, P_{CO_2}, and pH of the sample. Values for arterialised P_{CO_2} are detailed above in section 3.4.4.4(iii). pH was converted to $[H^+]$ and is reported above in 3.4.4.4(iv), however is also reported in this section as itself to add clarity and aid interpretation.

(i) Oxygen tension - P_{O_2}

PreExh vs PostMatch

Arterialised venous blood had a mean oxygen tension (P_{O_2}) of 73 ± 3 mmHg prior to training, and was lower after training by a mean difference of 5 ± 2 mmHg ($P<0.05$; Figure 3.16A). P_{O_2} fell during exercise, and recovered rapidly thereafter in both tests ($P<0.001$).

PreExh vs PostExh

In both the pre- and post-training exhaustion tests, the P_{O_2} measured immediately after exercise was lower than resting values ($P<0.01$; Figure 3.16B). There was no difference in the P_{O_2} in PreExh and PostExh (means of 73 ± 3 and 74 ± 3 mmHg, respectively).

(ii) pH

PreExh vs PostMatch

Prior to training, pH fell from a resting value of 7.40 ± 0.00 to 7.24 ± 0.03 with exercise, and continued to fall reaching a nadir of 7.16 ± 0.02 after 5 min of recovery (Figure 3.17A). Substantially less acidosis was evident in PostMatch ($P<0.001$).

PreExh vs PostExh

The pH fell significantly with exercise and remained below resting values at every sampling time in recovery ($P<0.001$) in both PreExh and PostExh. However, after training, the pH reached a lower nadir 5 min post-exercise ($P<0.001$), then recovered more rapidly to reach the same final pH as in PreExh at 20 min (Figure 3.17B).

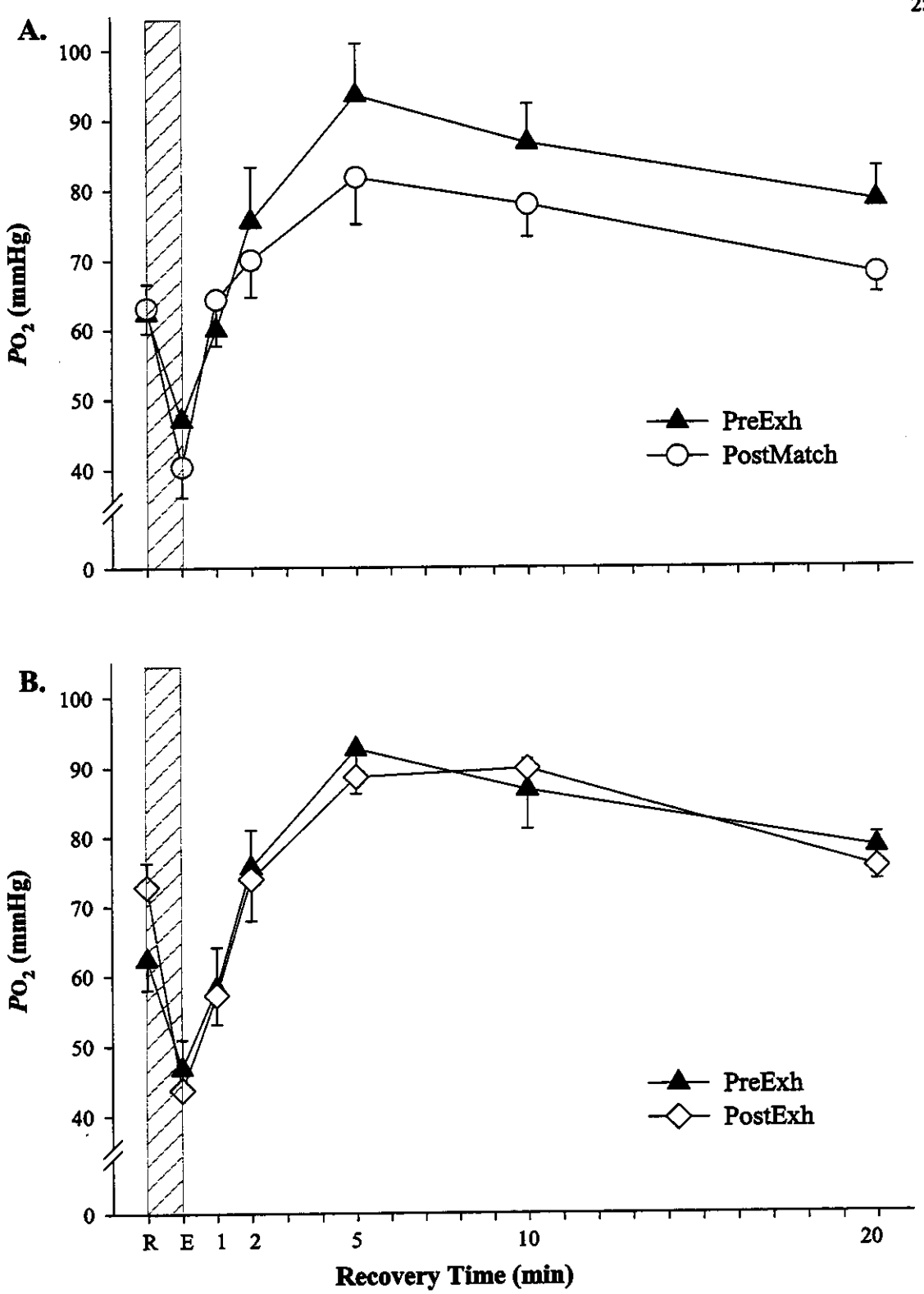


Figure 3.16 A,B Arterialised venous oxygen tension (P_{O_2}) (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E ($n = 5$), and 1 and 5 ($n = 6$). Main effects for time and training ($P<0.001$) occurred. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). A main effect for time ($P<0.001$) was evident.

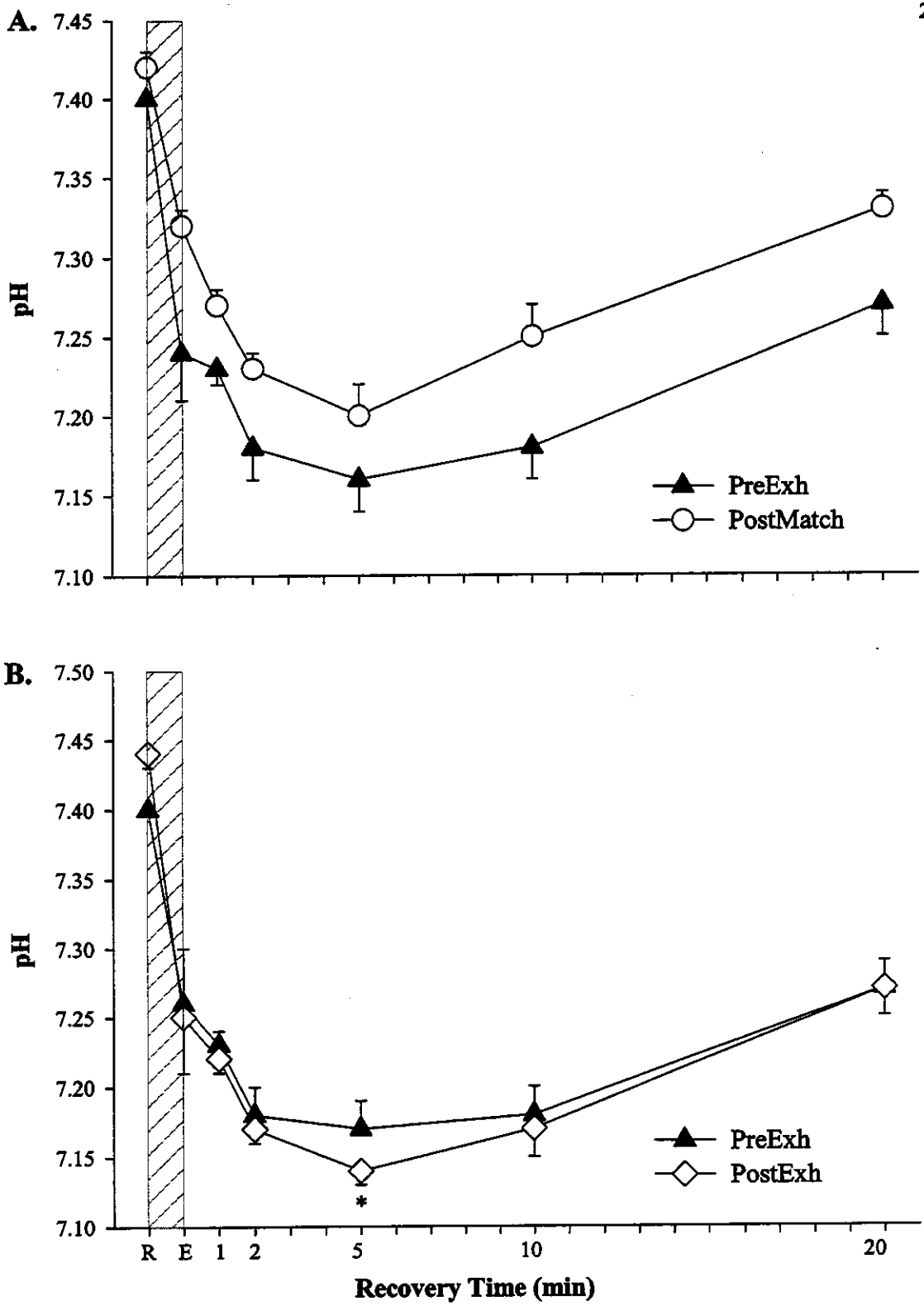


Figure 3.17 A,B pH (mean ± S.E.M.) at rest (R), immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$, and in recovery (numbered *x* axis labels). Hatched bar represents the period of exercise. A. PreExh vs PostMatch. $n = 7$, except E ($n = 5$), and 1 and 5 ($n = 6$). Main effects for time and training ($P<0.001$), and an interaction effect ($P<0.001$) occurred. B. PreExh vs PostExh. $n = 7$, except E and 1 ($n = 5$). A main effect for time ($P<0.001$) and an interaction effect ($P<0.001$) occurred. *$P<0.05$, PostExh<PreExh.

3.4.5 Summary of blood results in Study 1

Table 3.11 *Summary of blood results in Study 1.*

Variable		Change with training	
		PreExh versus:	
Blood Results		PostMatch same work	PostExh greater work
Catecholamines	NAdr	↓	↑
	Adr	no change	↑
Electrolytes	Lac^-	↓	↑
	K^+	↓	no change
	Na^+	no change	no change
	Cl^-	no change	no change
Haematology	Hct	↓	no change
	Hb	↓	no change
	PPr^-	↓	↓
	Δ PV	↓	no change
Acid-base	SID	↑	↓
	A_{tot}	↓	↓
	PCO_2	↓	no change
	H^+	↓	↑
	HCO_3^-	↑	↓

3.4.6 Muscle Metabolism

Individual data for all muscle variables appear in Appendix C (C14-20).

3.4.6.1 Percentage water content of muscle

The percentage water content of the biopsied muscle did not differ between test day (P=0.93), however was higher after exercise than at rest (75.7 ± 0.3% *vs* 75.3 ± 0.3%, respectively; P<0.05).

3.4.6.2 Total creatine concentration (TCr: PCr + creatine)

TCr did not differ with test day (P=0.74) or with time (rest *vs* exercise, P=0.29; Table 3.12), with the mean across all biopsies being 115.6 ± 4.5 mmol·kg^{-1} d.m. However, peak TCr was 4.4 ± 1.4% greater after training (119.5 ± 4.3 *vs* 124.8 ± 5.0 mmol·kg^{-1} d.m., pre- *vs* post-training, respectively; P<0.05).

Table 3.12 *Total creatine content in the pre- (PreExh) and post-training (PostExh) tests to exhaustion, and in the post-training matched-work test (PostMatch).*

PreExh		PostExh		PostMatch	
Rest	Exercise	Rest	Exercise	Rest	Exercise
109.9 ± 4.9	119.3 ± 4.4	115.1 ± 3.3	115.6 ± 5.0	116.8 ± 4.9	116.9 ± 7.9

Values are mean ± S.E.M. n = 7. All values are expressed as mmol·kg^{-1} d.m.

3.4.6.3 Muscle metabolite contents at rest

The two post-training invasive sprint tests (PostExh, PostMatch) were conducted in random order for each subject, resulting in three subjects performing the exhaustion test first, and four subjects performing the matched-work test first after training. Resting values for each metabolite were compared between the three tests (Table 3.13) to determine whether or not resting values were influenced by training.

Table 3.13 *Muscle metabolite contents at rest in the invasive sprint test prior to training (PreExh) and the two invasive sprint tests conducted in random order after training (PostExh, PostMatch).*

Variable	PreExh	PostExh	PostMatch	Post-test 1	Post-test 2
ATP (E)	21.6 ± 1.0	20.6 ± 0.6	19.4 ± 0.5	19.9 ± 0.3*	19.5 ± 1.0*
ATP (HPLC)	23.8 ± 1.4	19.9 ± 1.2	19.2 ± 0.9	20.0 ± 1.2**	19.2 ± 0.8**
ADP	2.41 ± 0.20	2.15 ± 0.24	2.07 ± 0.15	2.21 ± 0.22	2.02 ± 0.17
AMP	0.10 ± 0.01	0.11 ± 0.03	0.08 ± 0.01	0.11 ± 0.03	0.09 ± 0.01
TAN	26.3 ± 1.6	22.1 ± 1.4	21.4 ± 1.0	22.3 ± 1.4*	21.3 ± 0.9*
IMP	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01
PCr	80.9 ± 3.4	84.4± 3.9	84.4 ± 3.9	83.9 ± 4.3	84.9 ± 3.4
Creatine	38.7 ± 2.9	40.5 ± 2.8	40.4 ± 2.5	41.0 ± 2.2	39.9 ± 3.1
Lactate	5.7 ± 1.0	4.7 ± 0.8	5.0 ± 1.0	5.3 ± 0.9	4.3 ± 0.8
Glycogen	348 ± 30	376 ± 30	320 ± 25	348 ± 47	349 ± 22
$[H^+]$	62.2 ± 2.6	61.2 ± 1.7	60.0 ± 2.3	60.2 ± 1.25	60.9 ± 2.62

Values are means ± S.E.M.; n = 7 subjects. All units are mmol·kg^{-1} d.m., except $[H^+]$ which is nM. Muscle ATP was assayed using both enzymatic, fluorometric (E), and HPLC techniques. *P<0.05, **P<0.01, PreExh>Post-training 1 and 2.

An effect of training was found for ATP and the derived total adenine nucleotide (TAN) content (Table 3.13). In the PostMatch test, resting ATP and TAN tended to be lower than pre-training (PreExh) values (P=0.052, P=0.053, respectively), whilst values for PostMatch and PostExh were similar(P=0.64, P=0.62, respectively). Resting glycogen content was lower in PostMatch than PostExh (P<0.01), however PostMatch was not different to PreExh (P=0.23). When the two post-training tests were examined according to the order in which they were conducted (i.e. first or

second post-training tests), ATP and TAN were significantly lower than in PreExh ($P<0.05$) (Table 3.13), however there was no difference between post-training tests.

3.4.6.4 High-energy phosphates and degradation products

(i) Creatine phosphate and creatine

PreExh vs PostMatch

Resting creatine phosphate (PCr) content was reduced 64% as a consequence of the brief intense exercise to exhaustion in PreExh (Figure 3.18A). The contribution of creatine phosphate to ATP buffering during exercise was not altered in PostMatch, with a similar exercise-induced reduction in PCr content evident. The rise in creatine (Cr) content with exercise ($P<0.001$) was stoichiometric with the fall in PCr, and was not different after training in PostMatch.

PreExh vs PostExh

Exhaustive exercise resulted in a marked fall in PCr content ($P<0.001$) and a stoichiometric rise in Cr content that was not altered by training (Figure 3.18B).

(ii) ATP

Adenosine 5'-triphosphate (ATP) content was measured using both an enzymatic fluorimetric, and an HPLC technique. An excellent correlation was found between the two techniques ($r = 0.92$, $P<0.001$; Figure 3.19). HPLC mean data is reported in the body of this text, and both HPLC and enzymatic ATP reported as individual data in Appendix C (C16-17).

PreExh vs PostMatch

Prior to training, ATP content was significantly reduced from rest to be 16.0 ± 0.9 $mmol \cdot kg^{-1}$ d.m. following exhausting exercise ($P<0.01$). A significant exercise-induced reduction in ATP also occurred in PostMatch, however, ATP content only fell by 10% (*vs* 33% in PreExh; $P<0.01$; Figure 3.20A).

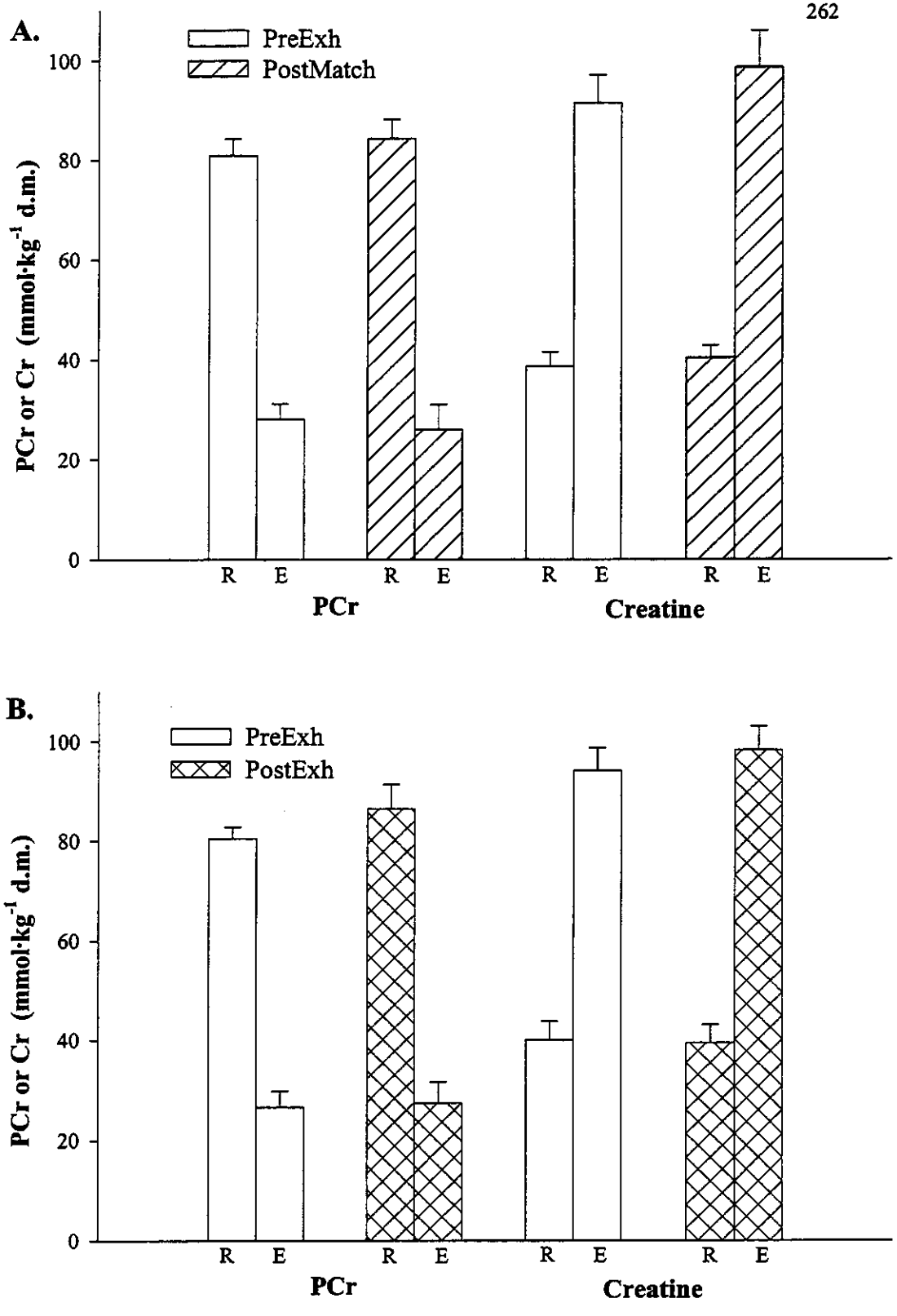


Figure 3.18 A,B Creatine phosphate (PCr) and creatine contents (Cr) (mean ± S.E.M.) at rest (R) and immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$. A. PreExh vs PostMatch, $n = 7$. B. PreExh vs PostExh, $n = 5$.

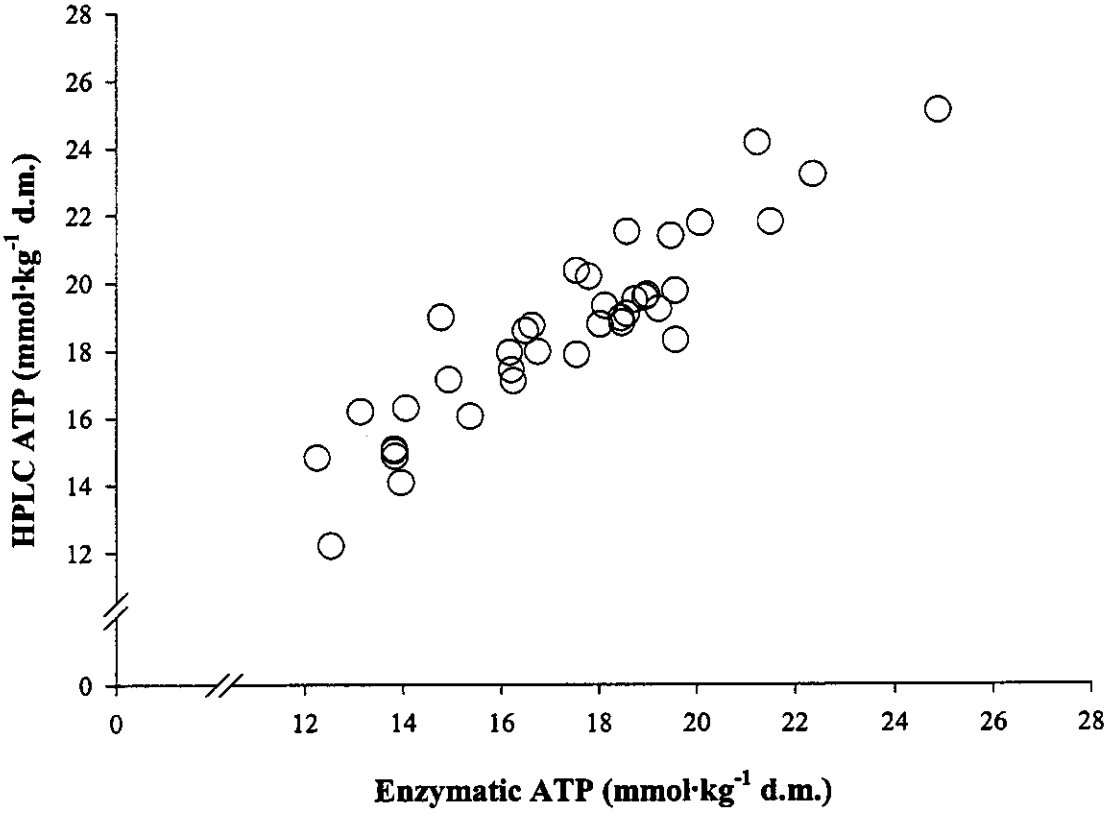


Figure 3.19 Relationship between the measurement of muscle ATP by enzymatic fluorimetric, and HPLC techniques. r = 0.92, $P<0.001$.

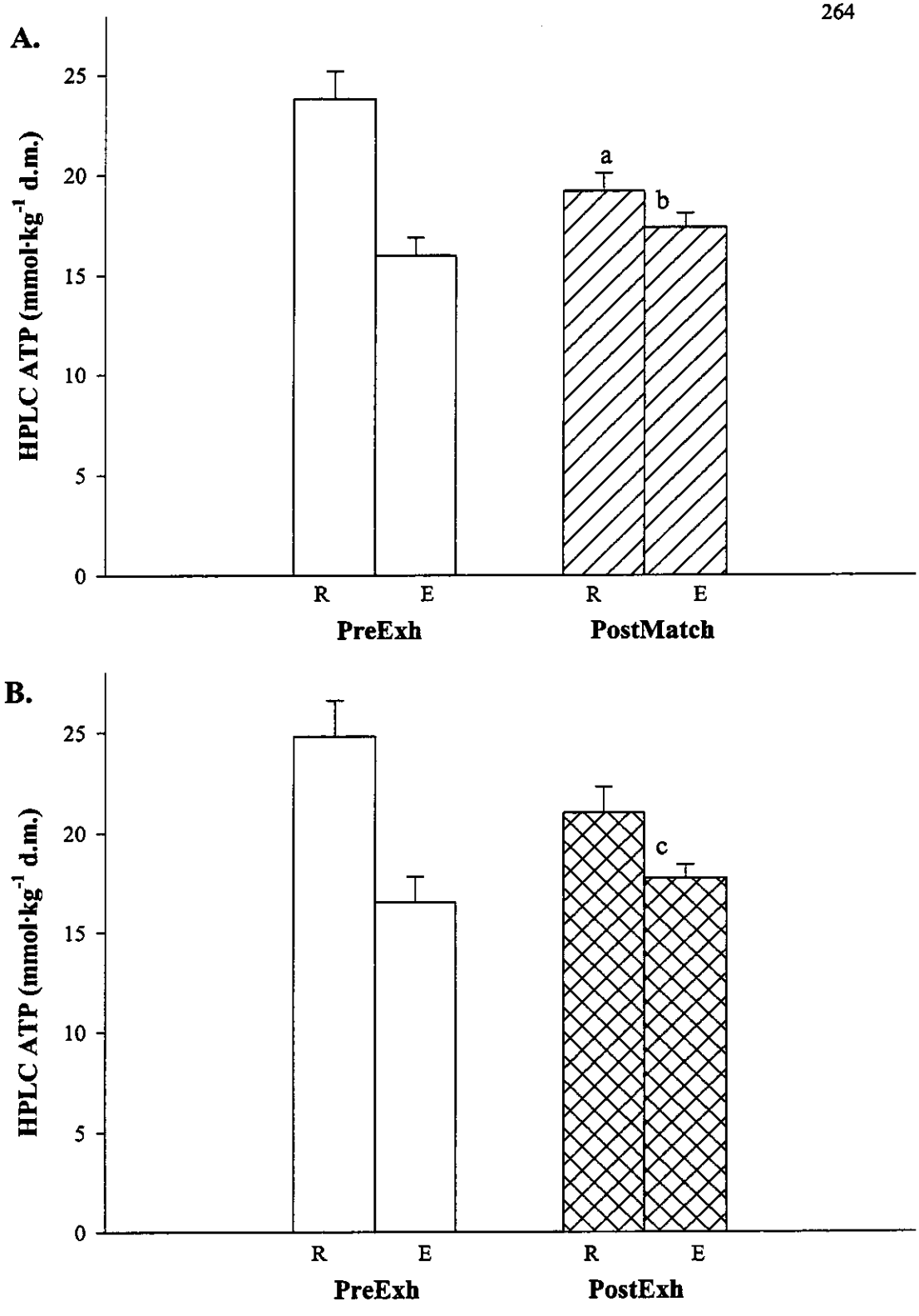


Figure 3.20 A,B HPLC Adenosine 5'-triphosphate (ATP) content (mean ± S.E.M.) at rest (R) and immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$. A. PreExh vs PostMatch, $n = 7$. ATP at rest, a, PreExh>PostMatch, P=0.052. A main effect for time (P<0.01) and an interaction effect (b, P<0.01) occurred. B. PreExh vs PostExh, $n = 5$. A main effect for time (P=0.01) and an interaction effect (c, P<0.05) occurred.

PreExh vs PostExh

Similar effects to those demonstrated in PostMatch were found when exercising to exhaustion in PostExh. ATP degradation in PostExh was halved with respect to the 34% fall evident in PreExh ($P<0.05$), despite cycling for a longer period before exhaustion (Figure 3.20B).

(iii) Total ADP – ADP_t

Total adenosine 5'-diphosphate (ADP_t) content was not affected by acute exercise (Table 3.14).

(iv) Total AMP – AMP_t

Total adenosine 5'-monophosphate (AMP_t) was not affected by acute exercise (Table 3.14).

(v) TAN

PreExh vs PostMatch

Reflecting changes in ATP, the TAN fell with exercise both pre- and post-training ($P<0.05$). Resting TAN content was lower after training, and the exercise-induced fall was diminished, being only 9% in PostMatch, compared with 31% in PreExh ($P<0.01$; Figure 3.21A).

PreExh vs PostExh

Greater protection of the adenine nucleotide pool (largely ATP) after training may have occurred during exercise to exhaustion in PostExh, demonstrated by the tendency for the fall in TAN to be reduced ($P=0.057$) when compared to PreExh (Figure 3.21B).

(vi) IMP

PreExh vs PostMatch

Supporting the reduced degradation of ATP in PostMatch was a significant 74% lower rise in inosine 5'-monophosphate (IMP) content ($P=0.001$; Figure 3.22A) in the PostMatch test, compared to PreExh.

Table 3.14 *Total ADP (ADP_t) and AMP (AMP_t) content measured in the invasive sprint tests to exhaustion conducted pre- (PreExh) and post-training (PostExh), and in the post-training matched work test (PostMatch).*

	PreExh *vs* PostMatch				PreExh *vs* PostExh			
	Rest	Ex	Rest	Ex	Rest	Ex	Rest	Ex
ADP_t	2.41 ± 0.20	2.05 ± 0.17	2.07 ± 0.15	2.02 ± 0.13	2.49 ± 0.28	2.13 ± 0.20	2.35 ± 0.27	2.19 ± 0.20
AMP_t	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.12 ± 0.04	0.07 ± 0.01

Contents are means ± S.E.M., and expressed as $mmol \cdot kg^{-1}$ d.m. $n = 7$ for PreExh *vs* PostMatch; $n = 5$ for PreExh *vs* PostExh. Ex, exercise.

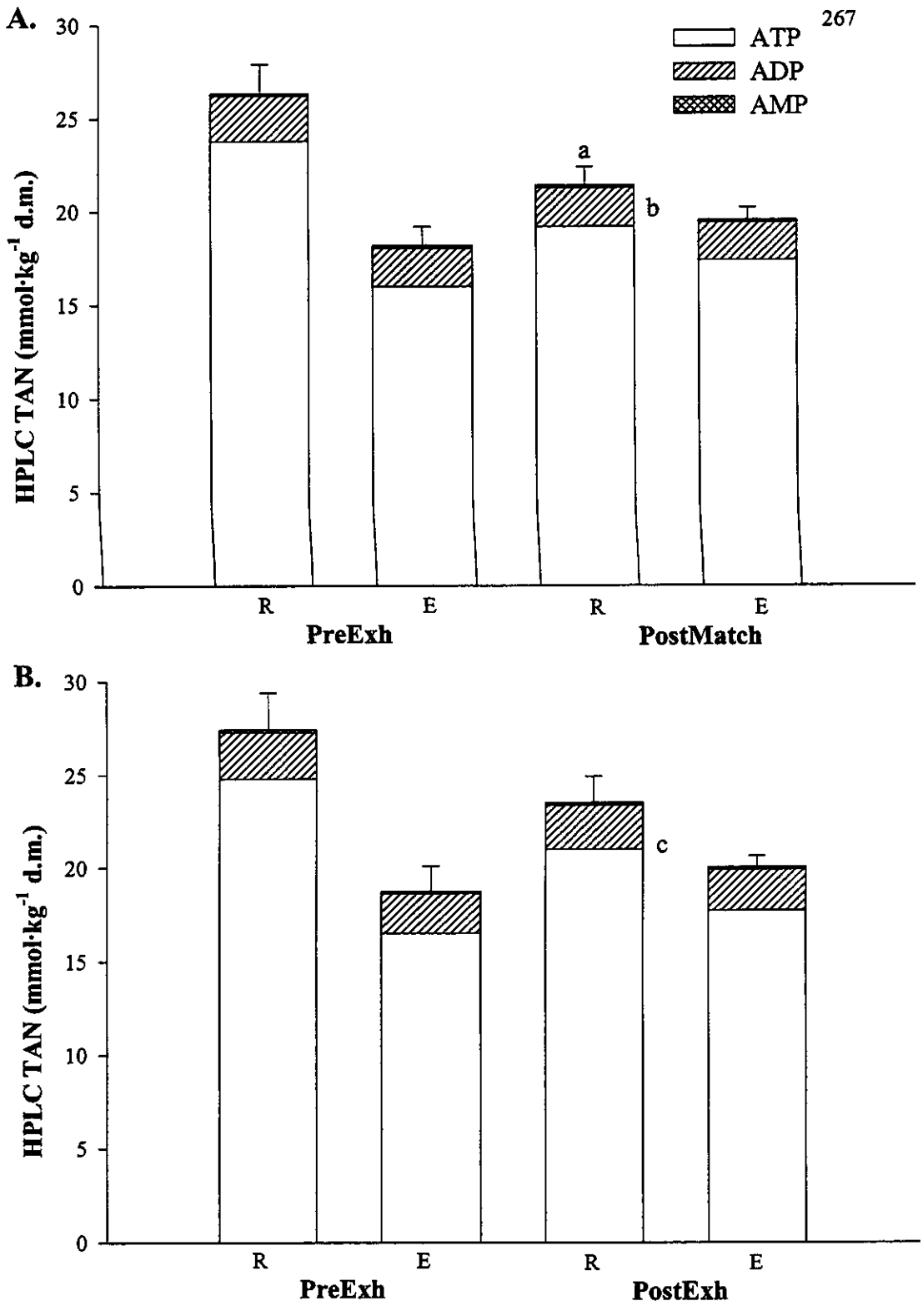


Figure 3.21 A,B Total adenine nucleotide content (TAN = ATP+ADP+AMP) (mean ± S.E.M.) at rest (R) and immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$. A. PreExh vs PostMatch, $n = 7$. TAN at rest, a, PreExh>PostMatch, P=0.053. A main effect for time (P<0.01) and an interaction effect (b, P<0.02) occurred. B. PreExh vs PostExh, $n = 5$. A main effect for time (P<0.05) and an interaction effect (c, P=0.057) occurred.

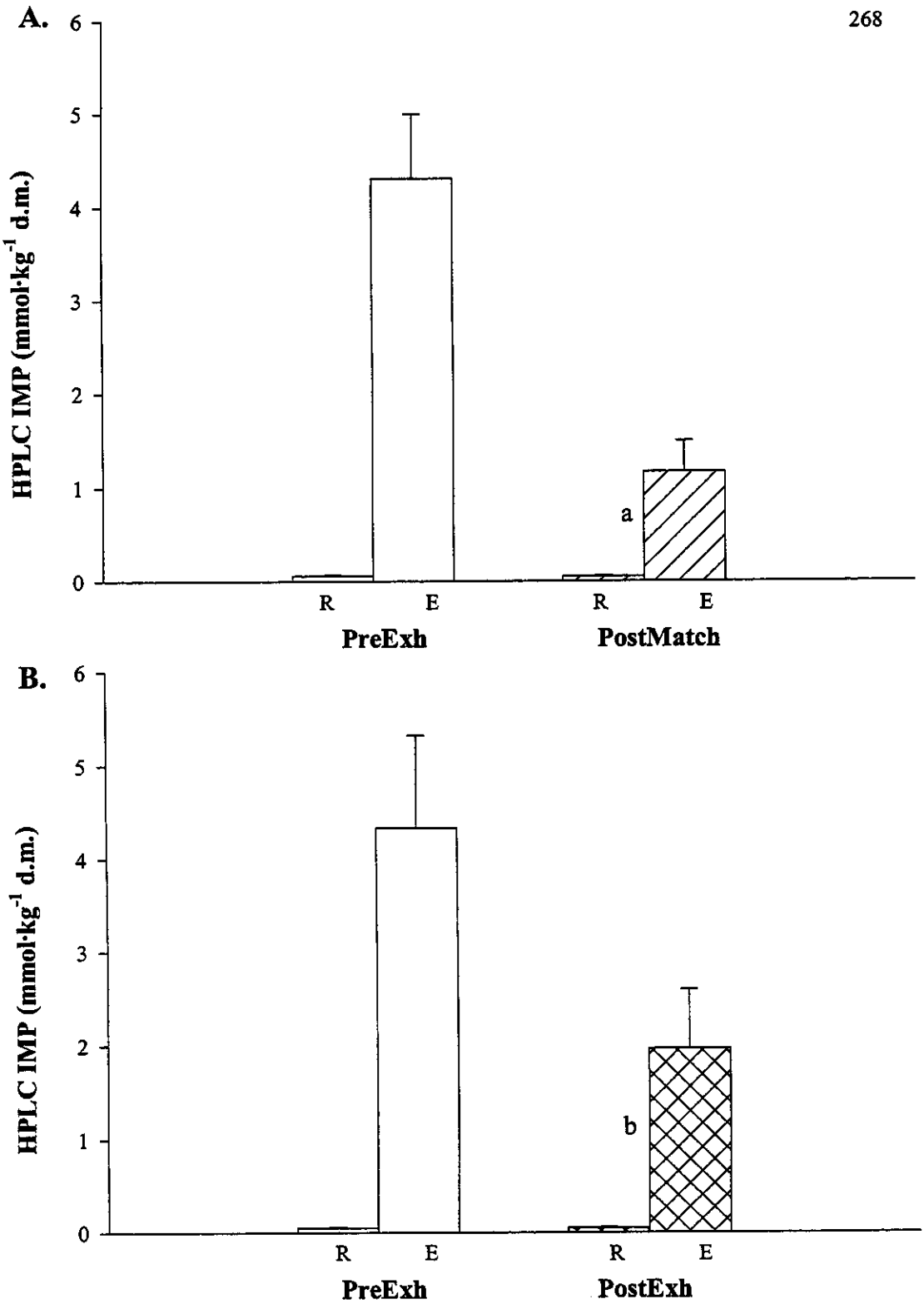


Figure 3.22 A,B HPLC inosine 5'-monophosphate content (IMP) (mean ± S.E.M.) at rest (R) and immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$. A. PreExh vs PostMatch, $n = 7$. Main effects for time (P=0.001) and training status (P=0.001), and an interaction effect (a, P=0.001) occurred. B. PreExh vs PostExh, $n = 5$. Main effects for time (P<0.02) and training status (P<0.05), and an interaction effect (b, P<0.05) occurred.

PreExh vs PostExh

The rise in IMP with exhaustive exercise was 55% lower in PostExh than PreExh, despite performing more work after training ($P<0.05$; Figure 3.22B).

3.4.6.5 Acid-base status

(i) *In vitro* buffering capacity

The mean difference for duplicate measures of *in vitro* buffering capacity ($\beta_{in\ vitro}$) was 0.07 ± 1.1 μmol $HCl \cdot g^{-1} d.m. \cdot pH^{-1}$. $\beta_{in\ vitro}$, measured on muscle sampled at rest, was not altered by high intensity training ($P=0.88$), being 156.6 ± 3.0, 157.0 ± 2.1, and 155.7 ± 3.4 μmol $HCl \cdot g^{-1} d.m. \cdot pH^{-1}$, in PreExh, PostMatch and PostExh, respectively (Figure 3.23A). A comparison of the $\beta_{in\ vitro}$ performed on the muscle samples taken at rest, versus those taken after exercise, revealed a mean difference of 2.6 ± 2.6 μmol $HCl \cdot g^{-1} d.m. \cdot pH^{-1}$ that was not significant ($P>0.05$; $n=18$, pooled pre- and post-training data).

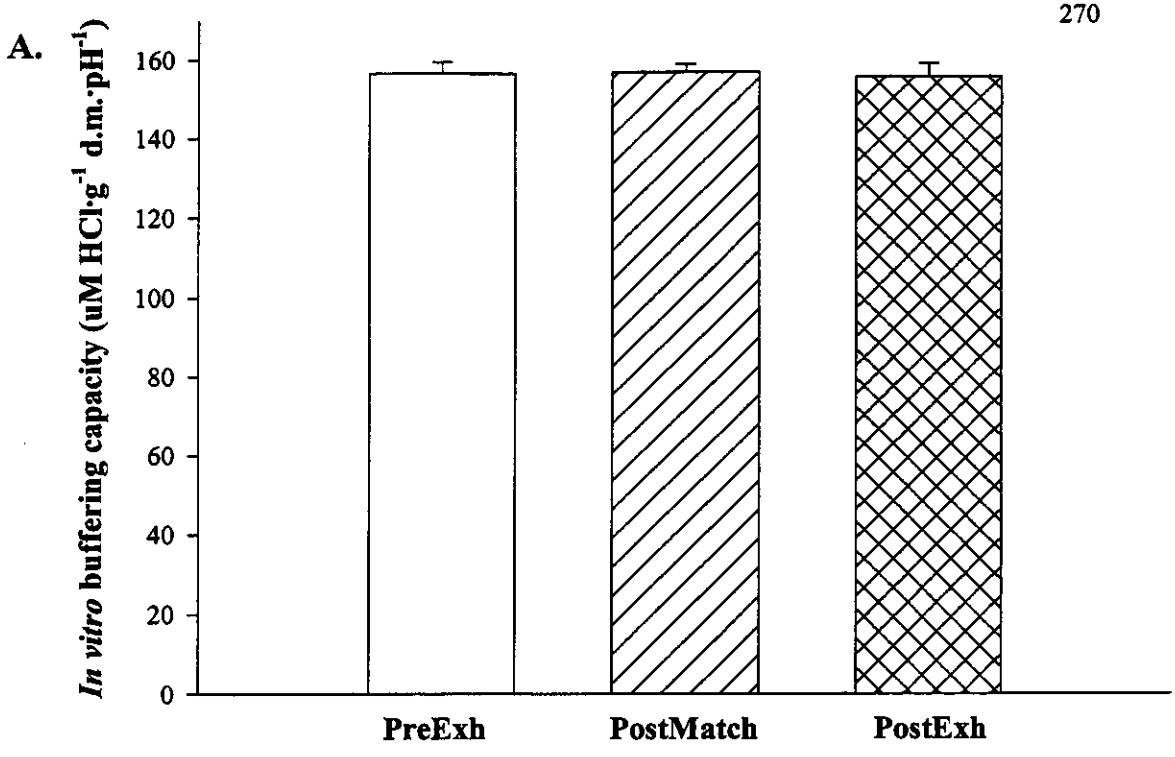
(ii) *In vivo* buffering capacity

In vivo buffering capacity ($\beta_{in\ vivo}$) was not significantly altered by sprint training, being 178.9 ± 4.7 and 187.4 ± 22.6 μmol $Lac^- \cdot g^{-1} d.m. \cdot pH^{-1}$ in PreExh and PostMatch, respectively ($P=0.71$; $n=6$); and 181.8 ± 6.0 and 186.3 ± 9.2 μmol $Lac^- \cdot g^{-1} d.m. \cdot pH^{-1}$ in PreExh and PostExh, respectively ($P=0.47$; $n=4$; Figure 3.23B). Correction of lactate to either mean or peak TCr had no affect upon results for $\beta_{in\ vivo}$.

(iii) $[H^+]$ and $\Delta[H^+] \cdot work^{-1}$

PreExh vs PostMatch

In PreExh muscle hydrogen ion concentration ($[H^+]$) rose dramatically ($P<0.01$) with exercise from a resting value of 62.5 ± 3.0 nmol (pH 7.21 ± 0.02) to reach 269.8 ± 31.3 nmol (pH 6.58 ± 0.05). The high resting value for pH is probably due to an alkaline shift induced by the process of freeze-drying (Harris *et al.*, 1989). After training in PostMatch, muscle $[H^+]$ rose 40% less ($P<0.05$) with exercise to reach 182.6 ± 30.2 nmol (pH 6.76 ± 0.07; Figure 3.24A). Despite the lack of training effect for $\beta_{in\ vitro}$ and $\beta_{in\ vivo}$, $\Delta[H^+] \cdot work^{-1}$ was 41% less in PostMatch ($P<0.05$; $n=6$).



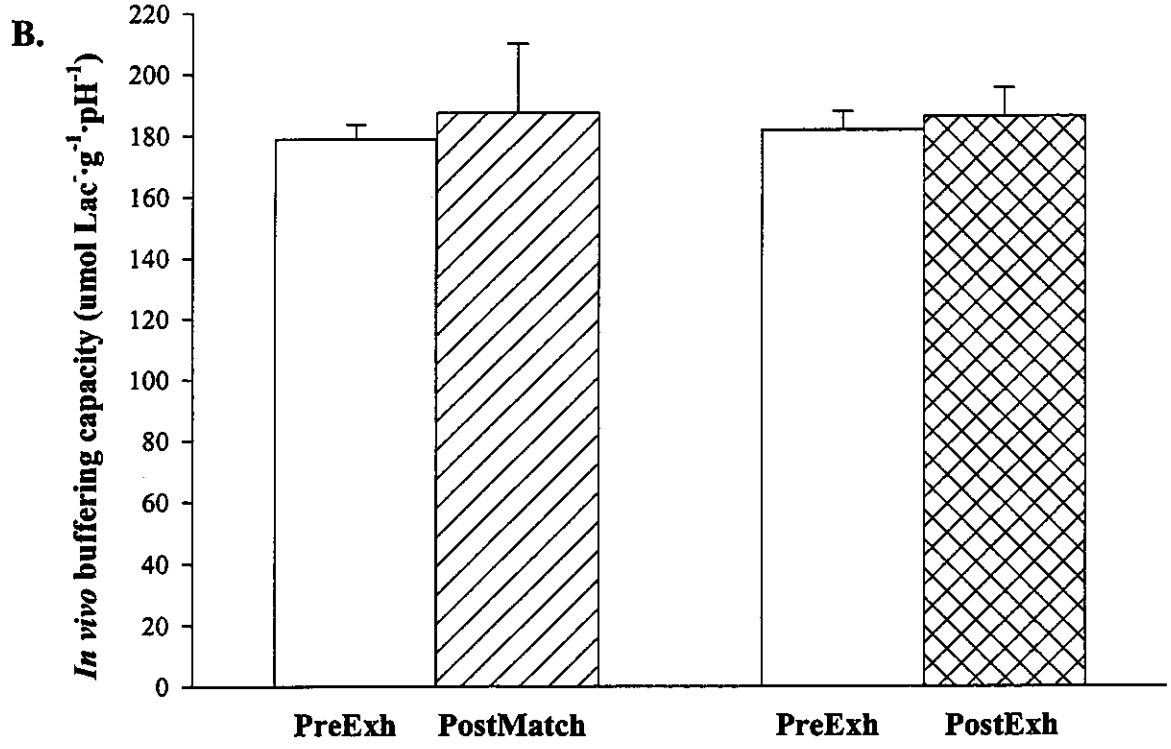


Figure 3.23 A,B Muscle buffering capacity (β; mean ± S.E.M.). A. *In vitro* β. *n* = 7. B. *In vivo* β. PreExh vs PostMatch, *n* = 6. PreExh vs PostExh, n = 4.

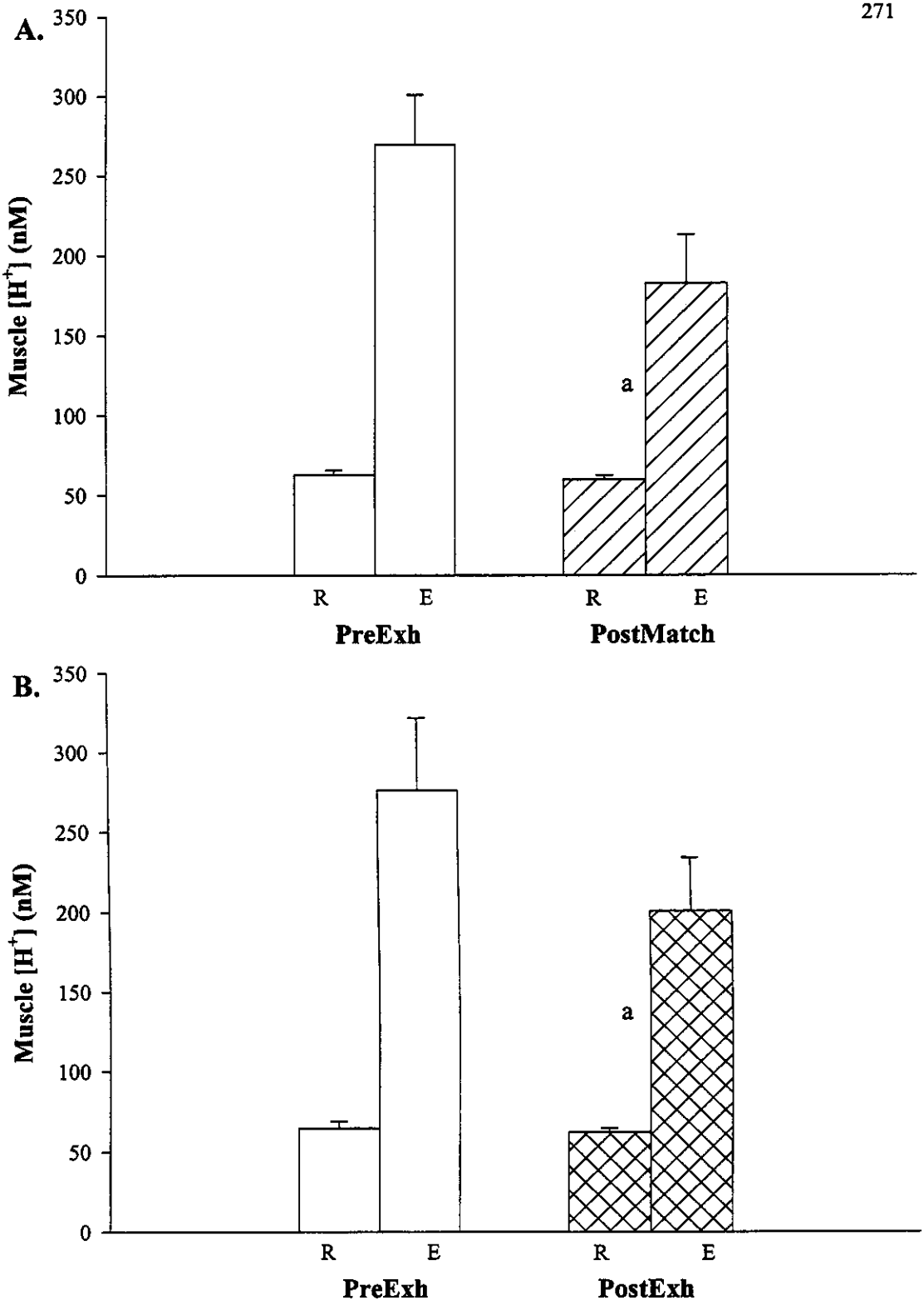


Figure 3.24 A,B Muscle [H^+] concentration (mean ± S.E.M.) at rest (R) and immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$. A. PreExh vs PostMatch, n = 6. A main effect for time (P<0.01) and an interaction effect (a, P<0.05) occurred. B. PreExh vs PostExh, n = 4. A main effect for time (P<0.01) and an interaction effect (a, P<0.05) occurred.

PreExh vs PostExh

In PostExh, greater work was performed, yet muscle [H^+] was lower (P<0.05; Figure 3.24B). $\Delta[H^+]\cdot work^{-1}$ was 45% less in PostExh (P<0.05; n = 4).

3.4.6.6 Metabolism

(i) Glycogen

PreExh vs PostMatch

The exercise-induced reduction in muscle glycogen content from rest was substantially attenuated in PostMatch, compared to PreExh (P<0.01;Figure 3.25A).

PreExh vs PostExh

Muscle glycogen content after exercise was reduced to a similar extent before and after training when cycling to fatigue (Figure 3.25B).

(ii) Lactate

PreExh vs PostMatch

The exercise-induced rise (P<0.001) in muscle lactate ([Lac^-]) was reduced 20% in PostMatch, compared to PreExh (P<0.05; Figure 3.26A).

PreExh vs PostExh

Muscle [Lac^-] rose dramatically with exercise to exhaustion (P<0.001), and was not different after training (Figure 3.26B).

(iii) Estimated muscle anaerobic ATP production/ production rate

PreExh vs PostMatch

Muscle anaerobic ATP production tended to be lower (17%) in the PostMatch test compared with PreExh (191 ± 24 and 229 ± 15 mmol $ATP\cdot kg^{-1}$ dm, respectively), although failed to attain statistical significance (P=0.09). Similarly, whilst the rate of anaerobic ATP production tended to be lower (21%) after training (PreExh, 3.02 ± 0.39; PostExh, 2.37 ± 0.27 mmol $ATP\cdot kg^{-1}$ $dm\cdot s^{-1}$), it did not attain statistical significance (P=0.10).

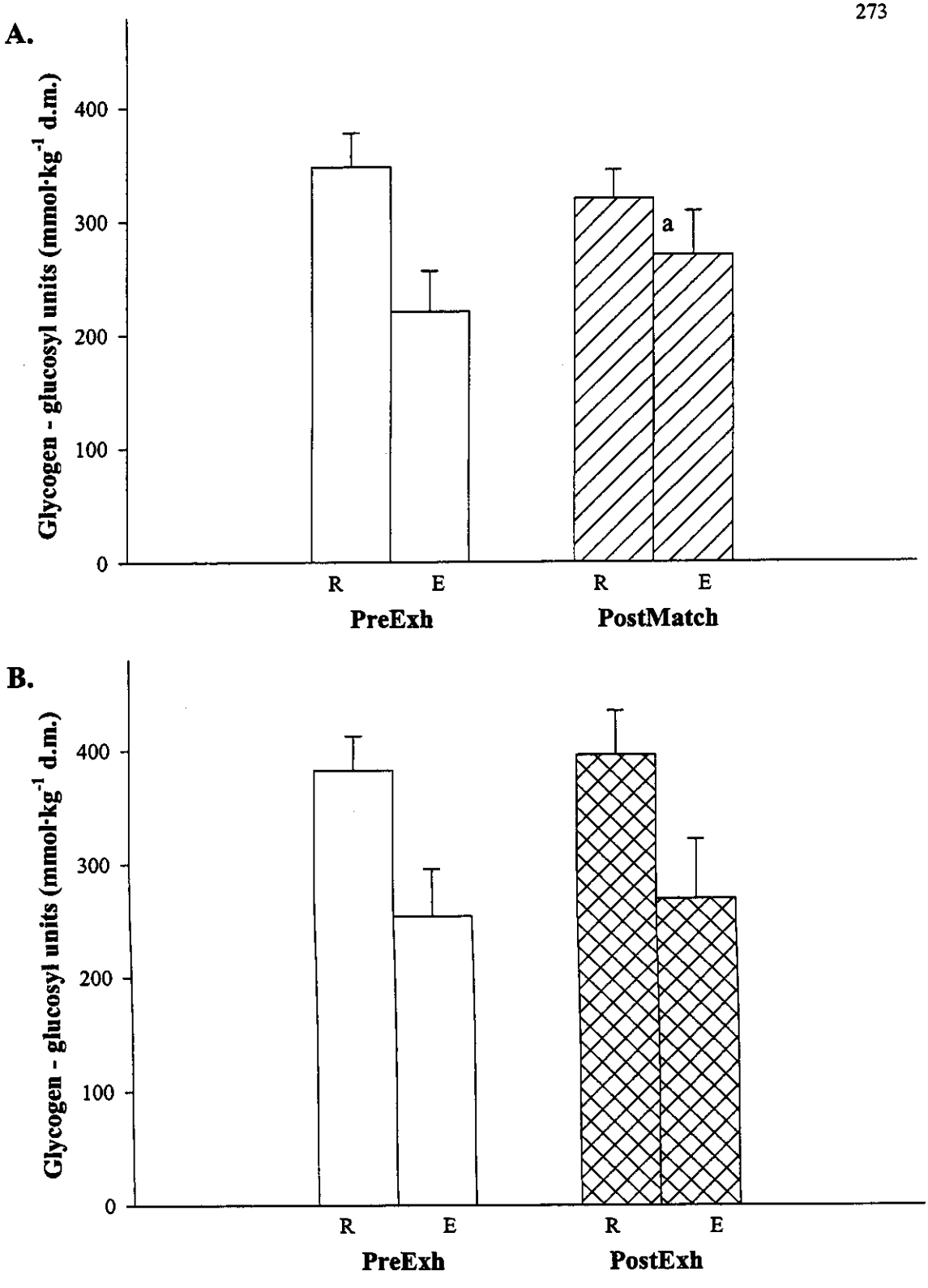


Figure 3.25 A,B Muscle glycogen content (mean ± S.E.M.) at rest (R) and immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$. A. PreExh vs PostMatch, n = 7. A main effect for time (P<0.01) and an interaction effect (a, P<0.01) occurred. B. PreExh vs PostExh, n = 5. A main effect for time (P<0.01) occurred.

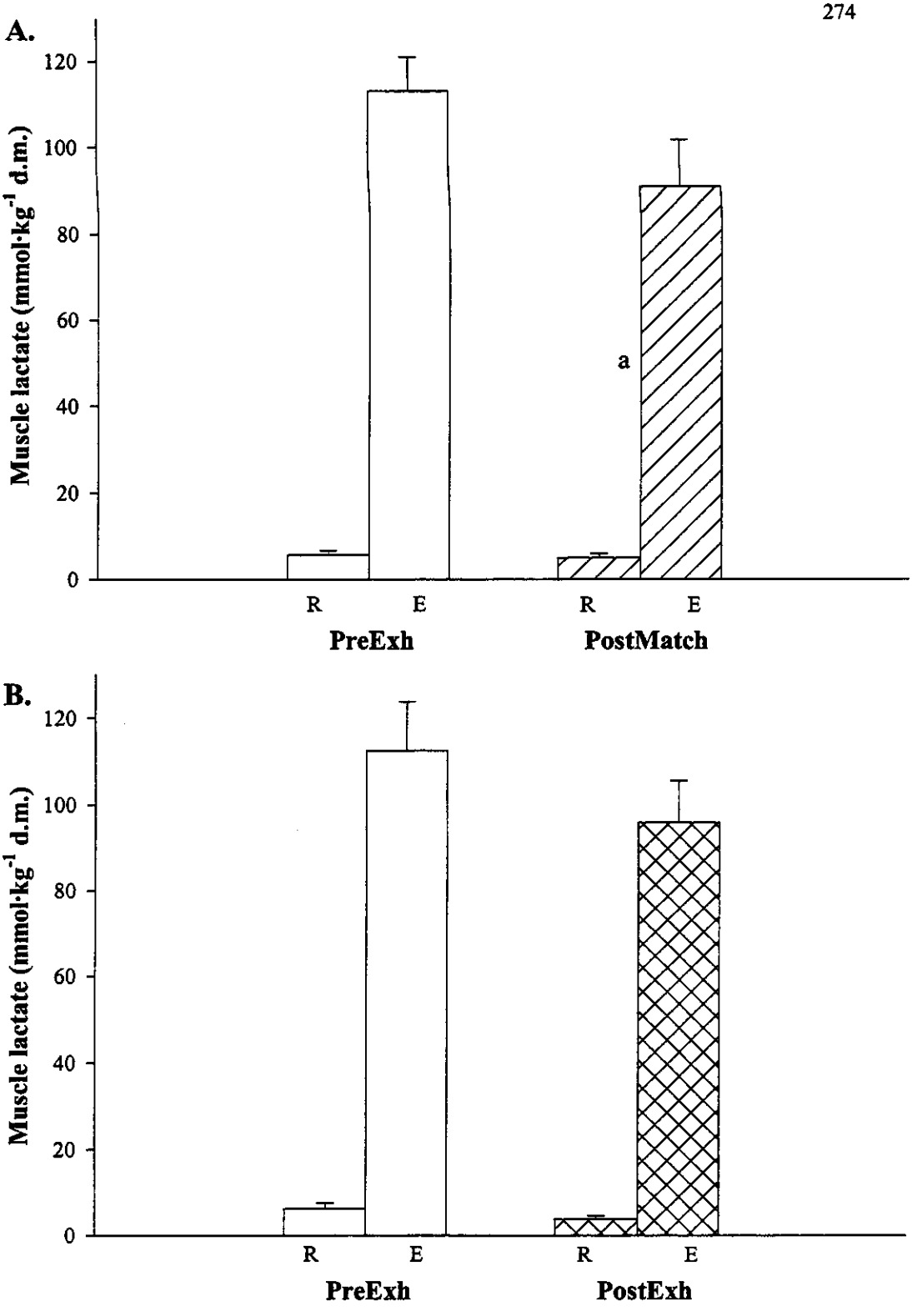


Figure 3.26 A,B Muscle lactate content (mean ± S.E.M.) at rest (R) and immediately after exercise (E) at 130% pre-training $\dot{V}O_{2peak}$. A. PreExh vs PostMatch, n = 7. Main effects for time (P<0.001) and training status (P<0.05), and an interaction effect (a, P<0.05) occurred. B. PreExh vs PostExh, n = 5. A main effect for time (P<0.001) occurred.

PreExh vs PostExh

Muscle anaerobic ATP production did not differ between PreExh (230 ± 19 mmol ATP·kg^{-1} dm) and PostExh (204 ± 20 mmol ATP·kg^{-1} dm) although the work accomplished after training in PostExh was significantly greater than that in PreExh. Hence, the rate of anaerobic ATP production tended to be lower after training (PreExh, 3.12 ± 0.54; PostExh, 2.29 ± 0.40 mmol ATP·kg^{-1} dm·s^{-1}; P=0.06).

3.4.6.7 Summary of Muscle Results in Study 1

Table 3.15 *Summary of muscle results for the invasive sprint tests pre- (PreExh) vs post-training (PostMatch, PostExh).*

		PreExh versus:	
		PostMatch	PostExh
Variable		same work	greater work
	Total creatine	no change	
High energy phosphates & degradation products	PCr	no change	no change
	creatine	no change	no change
	ATP	less degradation	less degradation
	ADP_t	no change	no change
	AMP_t	no change	no change
	IMP	less increase	less increase
Acid-base status	$\beta_{in\ vitro}$	no change	no change
	$\beta_{in\ vivo}$	no change	no change
	H^+	reduced	reduced
Metabolism	Glycogen	less degradation	no change
	Lac^-	less increase	no change
	Est. muscle ATP productn	tended to be lower	same
	Est. muscle ATP prod. rate	same	tended to be lower

3.5 Discussion

3.5.1 Cardiorespiratory responses and performance during maximal exercise after sprint training

3.5.1.1 Incremental exercise

The peak values for $\dot{V}_E$ and $\dot{V}CO_2$ were unchanged, whilst a tendency (P=0.07) for a small rise in peak $\dot{V}O_2$ (7%) was found in the incremental test to exhaustion after sprint training in the current study. It is probable that the lack of significance is due to a type II error. This is supported both by the significance of the increase in peak values for $\dot{V}O_2$ (P<0.01), $\dot{V}_E$ and $\dot{V}CO_2$ (P<0.05) when the results for non-diabetic subjects from studies 1 and 2 were pooled and analysed, and by the significant result for study 2 (see 4.5.1.2). The pooled results support the only previous study to comprehensively report the effects of sprint training upon ventilation and gas exchange during incremental exercise (McKenna *et al.*, 1997b), which demonstrated small increases in peak values for each of $\dot{V}_E$, $\dot{V}CO_2$, and $\dot{V}O_2$, using the sensitive breath-by-breath analysis technique.

3.5.1.2 Constant load exercise

The time to exhaustion in the pre-training respiratory test (PreResp) tended (P=0.051) to be briefer than the time to exhaustion in the pre-training test in which blood and muscle were sampled (PreExh). A familiarisation session was undertaken prior to the performance of the PreResp test, however, since the PreResp test necessarily preceded the PreExh test, it is possible that further 'learning' occurred, which may have resulted in an extension of the time to exhaustion in the latter. It is also possible that the level of verbal encouragement and motivation was greater in the PreExh test than in the PreResp test. Two or three experimenters were present during the PreResp test, whilst a number of people were required to assist with subject handling, and blood and muscle sampling and handling in the PreExh test. A combined effect of learning and added encouragement may also have occurred.

Following sprint training in the current study, time to exhaustion in the non-invasive, respiratory test (PostResp) was increased 29%, and was associated with a 10% higher

peak $\dot{V}_E$, but no change in peak exercise $\dot{V}O_2$, $\dot{V}CO_2$, or HR when compared to the PreResp test. Other than the increase in peak $\dot{V}_E$ following training, these findings support the hypothesis (section 3.2.2.3.1) that more work would be accomplished after training for a similar cardiorespiratory response, i.e. that the cardiorespiratory response to heavy exercise may be attenuated after sprint training. When accumulated values were normalised for the exercise duration, the resulting mean $\dot{V}_E$ was also 10% higher after training, whilst mean $\dot{V}O_2$ (6%; P=0.12) and $\dot{V}CO_2$ (4%) were not significantly increased. Although mean $\dot{V}O_2$ during maximal constant load exercise was not significantly altered after training in the present study, a higher $\dot{V}O_2$ was found in very early recovery following maximal constant load exercise in another sprint training study (McKenna *et al.*, 1997b), in which more sensitive measuring techniques were employed.

An increased absolute mean and peak $\dot{V}_E$ during exhausting exercise after training would increase the work of breathing and hence the respiratory muscle $\dot{V}O_2$ requirement (Harms & Dempsey, 1999). Elevation of the work of breathing by ~50% (by increasing inspiratory resistance), albeit with no change in maximal $\dot{V}_E$, increased leg vascular resistance and reduced leg blood flow and $\dot{V}O_2$ in trained cyclists when exercising at $\dot{V}O_{2max}$, however had no affect upon whole body $\dot{V}O_2$, cardiac output ($\dot{Q}$), oxygen extraction, MAP or HR (Harms *et al.*, 1997; Harms *et al.*, 1998). The relatively small increase in $\dot{V}_E$ in the current study may have facilitated a small increase in whole body $\dot{V}O_2$ (which may have been too small to detect by respiratory gas analysis) at the expense of a small increase in the work of breathing, however the effects of sprint training upon relative partitioning of whole body versus leg $\dot{Q}$ and $\dot{V}O_2$ during exercise to exhaustion are unknown.

3.5.1.3 Performance

Performance was improved in each of the three high-intensity exercise tests, i.e. incremental, constant-load (130% $\dot{V}O_{2peak}$), and the 'all out' 30-s air-braked cycle. The role of metabolic and ionic adaptations induced by sprint training in contributing

to the improved performance is discussed below. Whilst failing to attain significance, calculated muscle volume tended to be higher (P=0.08) after sprint training. This may suggest that muscle cross-sectional area tended to increase, however it was beyond the scope of the current study to include this measurement. Therefore it cannot be excluded that some contribution to improved performance was made via such a mechanism. Similarly, an enhanced recruitment of synergistic muscles, which would effectively increase the active muscle cross-sectional area, may contribute to performance. It is also possible that a psychological component, i.e. learning to tolerate the effects of intense exercise, contributed to improved performance. However, it is likely that adaptations within the muscle play a significant role in allowing an increased tolerance to intense exercise.

In summary, $\dot{V}O_{2\,peak}$ tended to be higher after sprint training, however just failed to attain statistical significance (P=0.07), likely due to a type II error. During constant load exhausting exercise, a small increase in $\dot{V}_E$ was found, which may be expected to require a small increase in $\dot{V}O_2$, however no significant change was found after sprint training. It is possible that any changes in $\dot{V}O_2$ and $\dot{V}CO_2$ may have been too small to detect by the method of gas analysis employed in the current study. It is also possible that the ventilatory response to heavy exercise may be attenuated following sprint training, given that time to exhaustion increased 29% with no change in $\dot{V}O_2$ or $\dot{V}CO_2$. Performance was improved in each of the exercise tests. Metabolic and ionic mechanisms for such an improvement are discussed below.

3.5.2 Effects of sprint training on ion regulation during maximal exercise

3.5.2.1 K^+ regulation

The major finding with regard to potassium regulation in the present study was a reduction in exercise-induced hyperkalaemia during matched-work exercise after 7 weeks of sprint training. This finding confirms the hypothesis (section 3.2.2.2.2) that sprint training would result in a reduction in exercise-induced hyperkalaemia when

pre- and post-training work was matched. Complementing this finding was a similar peak plasma [K^+] immediately after an exhausting cycle sprint, in which more work was accomplished after sprint training. The previous inability to demonstrate reduced hyperkalaemia, despite improved K^+ regulation (McKenna *et al.*, 1993; McKenna *et al.*, 1997a), is now explained and clarified on the basis of greater work being performed after training.

The reduced rise in plasma [K^+] immediately following exercise after training in the current study thus extends the findings of McKenna *et al.* (1993; 1997a), who examined K^+ regulation during intense exercise after sprint training. In those studies, despite the completion of greater work in the maximal 30-s exercise bout/s after training, the rise in arterial and femoral venous plasma [K^+] was unchanged (McKenna *et al.*, 1997a), and when corrected for fluid shifts, the rise in arterialized venous plasma [K^+] was lower (McKenna *et al.*, 1993). In addition, net muscle K^+ uptake was greater in the final seconds of exercise after training for each subject (McKenna *et al.*, 1997a). The authors concluded that sprint training enhanced K^+ regulation, however, the anticipated reduction in exercise-induced hyperkalaemia was not demonstrated (McKenna *et al.*, 1997a). The use of the matched-work test after sprint training in the present study resulted in reduced hyperkalaemia, similar to findings when submaximal work was matched after endurance training (Green *et al.*, 1993).

The present study also appears to support a previous cross-sectional study, in which trained sprinters had similar peak femoral venous plasma [K^+] to endurance-trained subjects, despite the performance of greater work, and exercising at a higher relative intensity during exercise that lead to exhaustion in ~ 60 s (Medbø & Sejersted, 1994). However, since no untrained subjects were included for comparison, it is difficult to draw definitive conclusions. However, it is likely that both the sprint-trained, and those who undertake a programme of sprint training, exhibit enhanced K^+ regulation.

All muscle fibres in the vastus lateralis are likely to be recruited at $\dot{V}O_{2peak}$ (Vøllestad & Blom, 1985). However total electrical activity continues to increase linearly with power above $\dot{V}O_{2peak}$, and hence the rate of K^+ efflux also increases linearly

(Vøllestad *et al.*, 1994). In the present study, the relative intensity during the invasive exercise test was reduced ~5% after training, and thus slightly lower exercise-induced hyperkalaemia may be anticipated (Vøllestad *et al.*, 1994). However, whilst the rate of rise in K^+ efflux during exercise was not measured in the current study, peak plasma [K^+] was reduced 11% and the absolute rise in [K^+] with exercise was reduced 31% following training, so the slight lowering of intensity cannot fully explain the training effect.

Mechanisms that have been suggested to contribute to sprint training-induced improvements in K^+ regulation during and after intense exercise include a greater Na^+,K^+-ATPase content (McKenna *et al.*, 1993), and greater Na^+,K^+-ATPase activation of active or inactive skeletal muscle or other tissues (e.g. liver), or reduced release of K^+ from the active muscle (McKenna, 1995; McKenna *et al.*, 1997a).

A mechanism that may contribute to the reduced hyperkalaemia during matched-work exercise is a change in blood flow to the trained muscle, which could alter net K^+ release; however no human sprint training study has examined this possibility. The tendency for lower noradrenaline concentration during matched-work exercise after training in the current study may contribute to an increased blood flow to active muscle, via a reduction in vasoconstrictor outflow (Rowell, 1993). Such a change would tend to increase net K^+ release, but may also increase K^+ redistribution to inactive tissues and hence enhance removal. Thus, it is not clear if alterations in blood flow contribute to the improved K^+ regulation observed during and following intense matched-work exercise after sprint training in the current study.

Reduced exercise-induced hyperkalaemia may reflect reduced interstitial K^+, and therefore be consistent with augmented muscular performance (Overgaard *et al.*, 1999). Thus, the prolongation of time to fatigue during maximal exercise after sprint training in the current study may have been permitted by tighter K^+ regulation. The catecholamines are important in reducing the lag in activation of the Na^+,K^+-ATPase at the onset of exercise (Hallén *et al.*, 1994; Gullestad *et al.*, 1995). In the present study, the higher catecholamine concentrations found when exercising to exhaustion after sprint training may have further reduced the lag of Na^+,K^+-ATPase pump activation at the onset of exercise and perhaps resulted in a reduced net K^+ release.

This would be consistent with the increased net uptake of K^+ in the contracting muscle in the final seconds of an exhausting 30-s cycle sprint after 7 weeks of sprint training (McKenna *et al.*, 1997a), and with the notion that both catecholamines and elevated arterial [K^+] are important stimuli for re-uptake of K^+ by the contracting muscle during exercise (Juel *et al.*, 1999).

In summary, sprint training enhanced K^+ regulation during maximal exercise. A novel finding was reduced exercise-induced hyperkalaemia during maximal matched-work exercise. That finding, coupled with a finding of similar peak plasma [K^+] in exhausting maximal exercise, during which more work was performed after sprint training, explained findings in other studies of enhanced K^+ regulation, but no change in exercise-induced hyperkalaemia.

3.5.2.2 [H^+] regulation

This study is the first to demonstrate reduced muscle H^+ accumulation during maximal exercise after sprint training. Reduced H^+ accumulation ($P<0.05$) was evident both when exercising to exhaustion, and when performing identical work after sprint training.

The latter confirms the hypothesis (section 3.2.2.1.4) that sprint training would enhance H^+ regulation when examined under identical exercise conditions. However, the finding of lower muscle [H^+] after exercise to exhaustion, in which more work was performed after sprint training, was somewhat unexpected. This contrasts with the hypothesis that muscle [H^+] accumulation would be similar pre- and post-training when exercising to exhaustion (3.2.2.1.1). Lower muscle [H^+] under both post-training exercise conditions signals a marked improvement in the muscle's ability to regulate [H^+] after sprint training.

Reduced [H^+] accumulation may be due to reduced H^+ production, and/or enhanced H^+ removal. Plasma [H^+] was markedly lower ($P<0.001$) after matched-work exercise and during recovery (PostMatch) after sprint training. Lower H^+ accumulation in muscle and in blood in PostMatch provides strong evidence for a reduction in H^+ production after training, and is consistent with the findings of lower muscle and plasma Lac^-, and the tendency for a lower rate of anaerobic ATP production (see

section 3.4.6.6.3). However, muscle [H^+] was also lower than in PreExh when exercising to exhaustion after training (PostExh), despite a similar Lac^- accumulation and performance of more work, whereas plasma [H^+] and [Lac^-] were both higher. These findings suggest that sprint training may have also enhanced H^+ clearance.

Muscle $\beta_{in\ vitro}$ was unchanged after sprint training in the present study, consistent with other studies (Nevill *et al.*, 1989; Mannion *et al.*, 1994), and thus cannot explain the lower muscle [H^+]. Muscle $\beta_{in\ vivo}$ was also unchanged after training, similar to Nevill *et al.* (1989), but in contrast to another sprint training study (Sharp *et al.*, 1986). Despite the lack of change in muscle $\beta_{in\ vitro}$ and $\beta_{in\ vivo}$, the calculated ratio of $\Delta[H^+]\cdot work^{-1}$ was reduced in both PostMatch and PostExh, even though the *n* was only four for the latter.

Muscle intracellular [H^+] depends upon the concentrations of the intracellular strong ions, principally Na^+, K^+, Lac^-, and Cl^-, as well as PCO_2 and the concentration of weak acids (Kowalchuk *et al.*, 1988b). Of these variables the present study only examined muscle Lac^-. However, lactate accounted for much of the change in the strong ion difference (SID) in skeletal muscle after exhausting exercise, and increased [H^+] accumulation was primarily effected by the fall in SID (Kowalchuk *et al.*, 1988b). Thus, in the present study, the lower accumulation of muscle Lac^- in PostMatch would have lessened the fall in muscle SID and hence reduced the rise in muscle H^+. However, in PostExh, muscle Lac^- did not differ from that in PreExh, and thus improved muscle [H^+] regulation in PostExh cannot be attributed to an altered SID.

Muscle H^+ clearance may be enhanced by increased membrane transport proteins. Following 8 weeks of one-legged high intensity interval training in habitually active men, the rate of Lac^-/H^+ transport in sarcolemmal giant vesicles was 12% higher in the trained leg than in the untrained leg (Pilegaard *et al.*, 1999), consistent with a 33-70% greater content of monocarboxylate transporters in the trained leg (Pilegaard *et al.*, 1999). Additionally, after high-intensity training in rats (Juel, 1998), the Na^+/H^+ exchange capacity was increased and may contribute to enhanced muscle [H^+] regulation in human muscle after sprint training, although this has not been investigated.

Although not the first study to examine the effect of sprint training upon muscle H^+, this study is the first to directly demonstrate improved H^+ regulation during intense exercise. The use of the matched-work exercise test, as well as the test to exhaustion, provided an improved testing model in the current study.

Only one other study (Nevill *et al.*, 1989) used a matched-work exercise test after sprint training. However, as reviewed (section 2.15.5), the metabolic perturbation was not extensive and may have been insufficient to allow discrimination of an effect of training on muscle H^+. One cross-sectional study that compared highly trained anaerobic athletes to sedentary men, found similar work, but a higher muscle pH at exhaustion in the former group (Sahlin & Henriksson, 1984), supporting the results of the matched-work test in the current study.

The few other sprint training studies which have examined (or provided sufficient data to examine) muscle H^+, used an exercise test to exhaustion in which greater work was usually performed after training. Thus, to account for differences in work, the effect of training on $[H^+]$ regulation was assessed by calculating the ratio of the change in H^+ relative to the amount of work performed during exercise ($\Delta[H^+]\cdot work^{-1}$). Supporting the current study, calculation of $\Delta[H^+]\cdot work^{-1}$ from other studies consistently demonstrated that sprint training of 7 to 8 weeks duration improved muscle H^+ regulation during intense exercise, as reviewed (section 2.15.5) and tabulated (Table 3.16).

As Table 3.16 shows, muscle H^+ accumulation at exhaustion was not significantly different pre- and post-training in three studies. In contrast, in the current study, muscle H^+ accumulation was reduced when exercising to exhaustion. Supporting the current study, Bell & Wenger (1988) found a higher muscle pH (lower H^+ accumulation) after one-legged sprint training, in a muscle sample taken 1 min after the completion of an exhausting 60-s cycle. The difference between the current study (and that of Bell & Wenger, 1988), and the three previous studies may reside in the nature of the protocols used to train and test subjects, or other study differences.

Table 3.16 *The difference (relative to pre-training) in hydrogen ion concentration ($[H^+]$) accumulation, work, and the calculated ratio of the change in $[H^+]$ relative to the amount of work performed during exercise ($\Delta[H^+]\cdot work^{-1}$), after sprint training.*

Reference	Test mode; intensity; endpoint	Pre- vs post-training, % change		
		$\Delta[H^+]$	Work	$\Delta[H^+]\cdot work^{-1}$
Present study	Cycle; fixed; 83s	↓40%	fixed	↓41%
	Cycle; fixed; exhn	↓34%	↑21%	↓45%
(Sharp *et al.*, 1986)	Cycle; incr.; exhn	ns	↑*	↓*
(Bell & Wenger, 1988)	Cycle (1 leg: Tr); 'all out'; 60s	↓53%	↑18%	↓60%
	Cycle (1 leg: UTr); 'all out'; 60s	↓45%	↑8%	↓49%
(Nevill *et al.*, 1989)	Tm; 'all out'; 30s	↓11% ns	↑6% ns	↓15%
	Tm; fixed; 120s	↓8% ns	fixed*	↓~8%†
(Pilegaard *et al.*, 1999)	Kick (1 leg); 'all out'; exhn	ns*‡	↑132%‡	↓~132%†‡

Exhn, exhasution; incr., incremental; ns, not significant; *insufficient data to calculate % change; Tr, trained leg; UTr, untrained leg; Tm, treadmill; †, insufficient data reported, so calculated assuming constant denominator or numerator; ‡, differences between the trained and untrained legs after training.

Sharp *et al.* (1986) utilised an incremental exercise test, rather than a constant-load or constant-time test, which presents a markedly different challenge to acid-base regulation. Nevill *et al.* (1989) found no significant change in mean power, and hence work (but increased peak power), in an exhausting 30-s treadmill run following training. Pilegaard *et al.* (1999), who utilised one-legged training, did not obtain pre-training resting, or pre-exercise, muscle pH samples, rather, they sampled muscle from the trained and the untrained leg at exhaustion after training. Additionally, data appears to have combined from two different testing protocols (incremental to exhaustion; and a constant-load test to exhaustion), with an *n* of 2 and 3 subjects, respectively. As Table 3.16 shows, one-legged sprint training may also induce a training effect in the untrained leg (Bell & Wenger, 1988), and therefore a finding of no difference in muscle pH between trained and untrained legs with a single post-exercise/ post-training sample may not accurately reflect the effect of sprint training on the trained leg. In the current study, muscle samples were obtained at rest and

after exercise in both the untrained and trained states, thus providing a stronger comparison.

In summary, muscle H^+ regulation was improved during maximal exercise following sprint training. Reduced H^+ accumulation was demonstrated in both maximal exhausting exercise and in matched-work exercise after sprint training. Reduced H^+ accumulation may be accounted for by lower production, suggested by a lower rate of anaerobic glycolysis, lower [Lac^-] accumulation and therefore less of a fall in SID in PostMatch. In PostExh, anaerobic ATP production did not differ from that in PreExh, and hence H^+ production was probably similar when exercising to exhaustion. However, H^+ clearance was probably also improved after sprint training.

3.5.3 Effects of sprint training on muscle metabolism during maximal exercise

3.5.3.1 Muscle high-energy phosphates

Reduced resting muscle ATP content after sprint training

Muscle ATP content at rest was reduced 16-20% after 7 weeks of sprint training in the present study; with a similar reduction evident in TAN. Two days to 7 weeks of sprint training reduced resting ATP content similarly in three previous studies (Green *et al.*, 1987b; Hellsten-Westing *et al.*, 1993; Stathis *et al.*, 1994); however several other studies reported no effect (Thorstensson *et al.*, 1975; Boobis *et al.*, 1983; Boobis *et al.*, 1987; Nevill *et al.*, 1989; Linossier *et al.*, 1993). The loss of adenine nucleotides from muscle, consequent to repeated severe exercise during the sprint training programme, has been suggested to account for the reduction in resting ATP content which persisted for at least 72 hours after the final training session (Green *et al.*, 1987b; Hellsten-Westing *et al.*, 1993; Stathis *et al.*, 1994). The present study supports and extends these results, with reduced resting ATP content still evident 5 days after the cessation of training. (Although, since several post-training exercise tests were conducted within this period, it cannot be excluded that they may have affected adenine nucleotide recovery. However, since the tests did not involve

repeated sprints, a major effect is unlikely.) Whilst the loss of adenine nucleotides is thought to be a result of the diffusion of the small molecules inosine and hypoxanthine from muscle to blood, it is possible that sarcolemmal damage may also allow leakage, however the latter has never been demonstrated. It has been suggested (Hellsten-Westing *et al.*, 1993; Stathis *et al.*, 1994) that the lack of training effect on resting ATP content in other studies was due to the use of very brief, or fewer sprints, with more extended recovery intervals between bouts, each of which would be anticipated to reduce the net loss of adenine nucleotides from the exercising muscle.

An interesting issue is whether or not the improvement in performance in the exhausting test in the present study may have been even greater if resting ATP content had not been reduced. Acute reduction in resting ATP content did not reduce performance in an 'all out' 30-s cycle bout in humans, suggesting that lower resting ATP did not impair high intensity dynamic exercise (Hargreaves *et al.*, 1998). Studies in which ATP content at rest (and the decrement during exercise) was not altered by sprint training demonstrated similar improvements in performance during intense exercise to the present study and to other studies demonstrating reduced resting ATP (Green *et al.*, 1987b; Stathis *et al.*, 1994) and thus by inference, a small reduction in resting ATP content after sprint training is probably not detrimental to performance.

Improved balance between ATP hydrolysis and ATP resynthesis during intense exhausting exercise after sprint training

Confirming the hypothesis (section 3.2.2.1.2), the net degradation of ATP was markedly reduced after 7 weeks of sprint training when exercising to exhaustion, despite the lower resting content, similar to the findings of two previous sprint training studies (Green *et al.*, 1987b; Stathis *et al.*, 1994), and one cross-sectional study that compared sprinters with long-distance runners (Rehunen *et al.*, 1982), but in contrast to several others (Boobis *et al.*, 1983; Boobis *et al.*, 1987; Nevill *et al.*, 1989; Linossier *et al.*, 1993). Based upon the results of Green *et al.* (1987b) and Stathis *et al.* (1994), it was hypothesized (section 3.2.2.1.4) that an even more pronounced attenuation of ATP degradation would occur when the work accomplished during exhausting exercise prior to training was matched after sprint

training. This hypothesis was strikingly confirmed, with a marked attenuation of net ATP degradation (from 33 to 10%) during PostMatch.

Reduced ATP degradation during exercise provides evidence that the rate of ATP utilization was reduced during exercise and/or that the rate of ATP resynthesis was more rapid after sprint training. This finding is further strengthened by the marked reduction in muscle IMP accumulation during both PostExh and PostMatch in the present study.

A reduced rate of ATP utilization is somewhat unlikely, although it is possible that changes in overall muscle efficiency, i.e. ATP cost per muscle contraction (Westra *et al.*, 1985), and muscle recruitment could have occurred after sprint training, as has been previously discussed (Stathis *et al.*, 1994).

Greater ATP resynthesis may be achieved by a more rapid/ greater contribution from PCr hydrolysis, a higher glycolytic rate, and/or greater oxidative metabolism. The resting content and degradation of PCr during exercise was unchanged after sprint training in the current study, supporting a number of other sprint training studies (see Table 2.13). Although endurance training has been demonstrated to attenuate the degradation of PCr during exercise at the same absolute submaximal workload (Green *et al.*, 1992; Cadefau *et al.*, 1994; Green *et al.*, 1995), maximal exercise necessitates an extremely rapid rate of ATP resynthesis, with effective depletion of PCr evident between ~10 to 30 s of exercise (Hirvonen *et al.*, 1987; Withers *et al.*, 1991; Bogdanis *et al.*, 1996; Parolin *et al.*, 1999). It has been suggested that even in the absence of a measured change in PCr content, sprint training may increase the rate of PCr degradation, and hence ATP resynthesis, within the first seconds of maximal exercise; an effect that would be undetectable if muscle sampling occurred after PCr depletion (Nevill *et al.*, 1989). Hence, in the present study in which either the end of exercise or exhaustion was reached in ~80 to 100 s, a change in PCr degradation after training would be unlikely to have been detected, and indeed was not. Thus a faster rate of PCr hydrolysis may have contributed to maintaining an improved balance between ATP degradation and resynthesis early in exercise after training, however cannot be determined from the present study.

It has been suggested that an enhanced glycolytic capacity may contribute to an improved ATP balance after sprint training (Stathis *et al.*, 1994). However, in the present study the anaerobic ATP production rate was unchanged (tended to be lower) during both matched-work and exhausting exercise after sprint training; nevertheless, the ATP balance was improved. Studies in which the anaerobic ATP production rate was enhanced (Boobis *et al.*, 1987; Nevill *et al.*, 1989; Linossier *et al.*, 1993) did not report an attenuated net degradation of ATP, however, greater work was accomplished for the same ATP degradation, and hence net degradation with respect to work must have been reduced. Thus it seems clear that sprint training reduces net ATP degradation during intense exercise, whether it be relative or absolute. However, the source of additional ATP resynthesis is probably dependent upon the nature of the sprint training programme. Programmes which included briefer sprints and relatively longer recovery periods, (e.g. Nevill *et al.*, 1989; Linossier *et al.*, 1993) may have more selectively trained the glycolytic system. The current study and two previous studies that found reduced net ATP degradation during intense exercise (Green *et al.*, 1987b; Stathis *et al.*, 1994) used longer sprint intervals and absolutely or relatively briefer recovery intervals. Repeated 30-s sprint bouts necessitate progressively greater contribution from oxidative metabolism (Putman *et al.*, 1995a; Parolin *et al.*, 1999), and thus constitute a greater oxidative stimulus than very brief sprints.

Therefore, an alternative, strongly suggested by the results of the present study, is that the oxidative generation of ATP may have been greater during both matched-work and exhausting exercise after sprint training (see below).

3.5.3.2 Muscle metabolism

The lower glycogen degradation, coupled with lower muscle Lac^- accumulation and lower plasma [Lac^-], indicate attenuated glycogenolysis (and possibly also glycolysis) during intense matched-work exercise after sprint training. Reduced glycogenolysis may be caused by the attenuated net ATP degradation, resulting in lower free AMP, reduced phosphorylase activation and less release of PFK inhibition. Additionally, there was a tendency for a reduction in muscle anaerobic ATP production during matched-work exercise (P=0.09). The lack of statistical significance is likely due to a type II error, since when data from Study 1 was combined with the ND group's data from Study 2, the reduction in both the anaerobic ATP production (P<0.01), and the

rate of production ($P<0.05$) during matched-work exercise, were both significant, with no group differences evident. It is possible that lower plasma [Lac^-] may reflect greater uptake by other tissues after sprint training, however this has not been investigated. However, the finding that glycogen degradation was lower during matched-work exercise tends to support the notion of a change occurring within the muscle.

When exercising to exhaustion, glycogen degradation, lactate accumulation, and the muscle anaerobic ATP production were all unchanged after training, despite a 21% longer exercise duration. Consequently, the anaerobic ATP production rate tended to be lower (25%) than in PreExh, although just failed to attain significance ($P=0.06$). Reduced anaerobic metabolism during intense exercise following sprint training in the current study is consistent with findings of unchanged (Stathis *et al.*, 1994) or lower muscle Lac^- accumulation (Pilegaard *et al.*, 1999), attenuated ATP degradation (Stathis *et al.*, 1994), and an unchanged arterio-venous Lac^- difference (McKenna *et al.*, 1997a; Pilegaard *et al.*, 1999) when greater work was performed during an exhaustive exercise bout after sprint training. Unchanged muscle lactate accumulation and anaerobic ATP production, but higher plasma [Lac^-] with exhausting exercise suggests that sprint training may enhance blood flow and/or lactate transport; with the latter shown recently (Pilegaard *et al.*, 1999). If so, this suggests that the muscle anaerobic ATP production may have been underestimated after training. Even so, the potential increase in muscle lactate transport after sprint training (Pilegaard *et al.*, 1999) is less than half the calculated reduction in the rate of muscle anaerobic ATP production, so the conclusions may not be significantly affected by this consideration.

These findings, considered with the improved balance between ATP degradation and resynthesis evident (section 3.5.3.1), provide strong metabolic evidence that both exhausting and matched-work maximal exercise were accomplished more oxidatively after sprint training.

A small increase in oxidative ATP generation during maximal exercise would provide a considerable energetic advantage, and may allow exercise to continue for a longer period before fatigue, such as was evident in PostExh. Such an adaptation would also

result in less metabolic perturbation during identical post-training maximal exercise conditions, as evidenced in PostMatch, or as has been previously suggested, may allow a higher power output in a set time period (McKenna *et al.*, 1997b; Howlett *et al.*, 1999).

The mechanism/s whereby greater oxidative muscle metabolism may occur during maximal exercise after sprint training may include a greater increase in the active fraction of pyruvate dehydrogenase (PDH*a*) activity during exercise, a greater ATP contribution from intramuscular triglycerides, and/or faster onset kinetics for different variables. To date, no sprint training studies have examined these possibilities.

Sprint training has been reported to increase several mitochondrial enzymes, e.g. citrate synthase (CS), β-hydroxyacyl-CoA dehydrogenase (HAD), and succinate dehydrogenase (SDH) (Jacobs *et al.*, 1987; Cadefau *et al.*, 1990; MacDougall *et al.*, 1998; Pilegaard *et al.*, 1999), and may thus increase mitochondrial density and hence total PDH (PDH*t*). Since, brief, intense exercise results in a rapid and complete conversion of PDH*t* to PDH*a* (Putman *et al.*, 1995), increased PDH*t* may therefore result in greater PDH*a* during exercise. However, the few studies that have examined the effect of other forms of exercise training on PDH*t* have demonstrated no change (see Table 2.18). Even if PDH*a* was unchanged, a slower rate of pyruvate presentation would probably permit a greater proportion to be oxidized, thus constituting a considerable energetic advantage after training. Intramuscular triglyceride use may be significant during brief exercise bouts (Essén *et al.*, 1977; Essén, 1978; McCartney *et al.*, 1986), however the effect of sprint training is unknown.

In summary, strong metabolic evidence is presented to support the hypothesis that sprint training induces an adaptation whereby oxidative metabolism is enhanced during maximal exercise. The cellular mechanisms leading to such an adaptation remain to be investigated.

3.5.4 Study 1 Conclusions

Seven weeks of sprint training resulted in improvements in performance, and in muscle metabolic and ionic regulation during maximal exercise in young untrained men. Major findings in the PostMatch test (matched-work) were improved cellular energy balance, indicated by reduced net ATP degradation and IMP accumulation, less muscle H^+ accumulation, lower glycogen degradation, lower muscle and plasma lactate accumulation, and a tendency for reduced muscle anaerobic ATP production, during maximal exercise. Plasma K^+ regulation was also enhanced after sprint training. In the PostExh test, in which the exercise time to exhaustion was extended 21% (i.e. performance was improved), major findings included reduced net ATP degradation and IMP accumulation, less muscle H^+ accumulation, similar glycogen degradation and muscle lactate accumulation, but higher plasma [Lac^-], and a tendency for reduced muscle anaerobic ATP production, compared with the pre-training test to exhaustion (PreExh).

Each of these factors is likely to have been of importance in allowing a longer time before exhaustion in the PostExh test, and in resulting in less metabolic and ionic perturbation in PostMatch. Thus, the metabolic evidence presented in Study 1 strongly suggests that after sprint training a higher proportion of ATP generation was derived from oxidative metabolism during maximal exercise, both when exercising to exhaustion and when performing identical pre- and post-training work. However, the current study cannot determine the source of potentially higher oxidative ATP generation. The respiratory responses during maximal exercise after sprint training may also support these findings. In addition, the examination of both matched and greater work conditions during maximal exercise after sprint training allowed clarification of some discordant findings in previous studies.

4. STUDY 2

4.1 Introduction

Study 2 investigated the effects of a 7-week sprint training programme on integrated metabolic, ionic, and cardiorespiratory responses during acute maximal exercise under identical work conditions in young, untrained subjects with and without type 1 DM. Further, the study evaluated the effect of a 7-week sprint training programme on metabolic control in the subjects with type 1 DM.

4.2 Study 2 Aims and Hypotheses

4.2.1 Aims:

1. To characterise the effect of sprint training on metabolic control in subjects with type 1 DM.
2. To characterise the metabolic, ionic, and respiratory responses during maximal exercise to exhaustion and in recovery in a group with type 1 DM, and to compare the responses with those of a matched non-diabetic group.
3. To characterise the metabolic, ionic and respiratory adaptations to a 7-week programme of sprint training during exercise conditions of identical pre- and post-training work in a group with type 1 DM, and to compare the responses with those of a matched non-diabetic group.

4.2.2 Experimental hypotheses:

4.2.2.1 Glucoregulation

1. HbA_{1c} will be unchanged or reduced after 7 weeks of sprint training in the group with type 1 DM.
2. Daily insulin dosage will be reduced after 7 weeks of sprint training in the group with type 1 DM.
3. The rise in plasma glucose concentration will be attenuated during and after matched-work maximal exercise in both groups.

4.2.2.2 Metabolism

1. PDH*a* will be lower at rest in the T1D group than in the ND group.
2. After acute exhausting maximal exercise the muscle Lac^- content will be higher in the T1D group, associated with lower PDH*a* than in the ND group.
3. After training, during matched-work maximal exercise (identical exercise conditions), the degradation of glycogen and ATP, and the accumulation of glycolytic intermediates and Lac^- will be attenuated in both groups.
4. After training, during matched-work maximal exercise (identical exercise conditions), the rates of glycogenolysis, glycolysis and anaerobic ATP production will be attenuated and ATP generation will be accomplished more oxidatively in both T1D and ND groups

4.2.2.3 Ion regulation

1. Plasma $[K^+]$ and $[Na^+]$ will be similar at rest in the T1D and ND groups providing BGL is <10.5 mM, otherwise, plasma $[K^+]$ and $[Na^+]$ at rest will be respectively higher and lower in the T1D group.
2. Plasma $[K^+]$ will be higher in the T1D group in recovery from maximal exercise after the catecholamines have returned to resting levels.
3. Muscle [^{3}H]ouabain binding site content will be increased after sprint training in both groups.
4. After training, during matched-work maximal exercise (identical exercise conditions), peak plasma $[K^+]$ will be lower, i.e. a reduction in exercise-induced hyperkalaemia will be evident, and $[H^+]$ and $[Lac^-]$ will be reduced in both groups.

4.2.2.4 Cardiorespiratory variables

1. Greater work will be performed in the respiratory test during maximal exercise to exhaustion after sprint training (PostFResp), however peak cardiorespiratory responses will be similar to those evident during exhausting exercise prior to training (PreFResp), with no differences between groups.
2. During the matched-work respiratory test (PostMResp) $\dot{V}O_2$ will be higher and $\dot{V}_E$, $\dot{V}CO_2$ and HR lower than in the pre-training exhausting respiratory test (PreFResp) with no difference between groups.

4.3 Methods

4.3.1 Subjects

Seventeen recreationally active subjects volunteered to participate in the study. Each subject was informed in writing of the purpose of the study and was verbally apprised of all experimental procedures. Subjects completed a medical screening questionnaire and gave signed informed consent prior to inclusion in the study. The subjects comprised two groups: eight subjects with type 1 (insulin-dependent) diabetes mellitus, and nine non-diabetic subjects. Subjects were required to be between the ages of 18 and 35 years, and not currently undertaking any high-intensity exercise training.

4.3.1.1 Group with type 1 diabetes mellitus (T1D)

Eight healthy subjects (5 M, 3 F) with type 1 diabetes comprised the T1D group. No subject smoked or took any prescription medications other than insulin. Potential subjects with diabetes were screened by their endocrinologists for the presence of any of the following possible complications of diabetes:

(i) Proteinuria > 200 mg / 24 hours

(ii) Microalbuminuria

(iii) Retinopathy - > 10 microaneurysms in the last year

(iv) Neuropathy - absence of ankle jerks, evidence of sensory loss

(v) Autonomic neuropathy - evidence of postural hypotension

If any of the above complications was present the potential subject was excluded from participation in the study. The above criteria excluded three potential subjects. In addition, subjects were required to have had diabetes for at least 2 years duration and a glycosylated haemoglobin, A_{1c} fraction (HbA_{1c}) of less than 10 %.

4.3.1.2 Non-diabetic Group (ND)

Nine healthy subjects (6 M, 3 F) without evidence (Fasting plasma glucose concentration < 5.4 $mmol \cdot l^{-1}$, and HbA_{1c} < 6%) or family history of diabetes, or of any other metabolic disorder were recruited to the non-diabetic group (ND). One male subject (SG), who completed all pre-training tests, dropped out after the first training session. Another male subject (BH) completed the training programme, however then played a full week of rugby at the University Games in the week of his scheduled

post-testing. Data from both subjects appear in Appendix E with other individual results, however have been excluded from statistics. Data for the ND group is thus presented for four males and three females. No subject smoked or took any prescription medication. Non-diabetic subjects were matched with the T1D subjects for age, BMI, and $\dot{V}O_{2peak}$.

4.3.2 Research Design and Experimental Overview

Figure 4.1 illustrates the longitudinal research design and experimental overview of Study 2. All experimental procedures and protocols were approved by the University of Sydney Human Ethics Committee and the South Western Area Health Service Research Ethics Committee.

In the 2 weeks prior to training subjects completed two incremental tests to assess $\dot{V}O_{2peak}$, and two constant load maximal sprints to fatigue at a power output calculated to elicit 130% $\dot{V}O_{2peak}$. Gas exchange and ventilation were assessed in the first of the constant load tests (termed 'PreFResp'; non-invasive), whilst muscle and arterialised venous blood were sampled in the second of the 130% $\dot{V}O_{2peak}$ tests (termed 'PreFB'; invasive).

Subjects then participated in 7 weeks of supervised cycle sprint training. In the 8 days following the final sprint session, three constant load maximal tests were conducted at the power output calculated to elicit 130% pre-training $\dot{V}O_{2peak}$, and one incremental test to re-assess $\dot{V}O_{2peak}$ was performed. One constant load test (termed 'PostFResp'; non-invasive) was continued until fatigue, during which respiratory gas exchange and ventilation were assessed. The two other constant load tests were time-matched with the pre-training exhaustion time, rather than extending until fatigue. Analysis of expired gases and ventilation was performed in one of the time-matched tests (termed 'PostMResp'; non-invasive), whereas blood and muscle samples were collected in the other (termed 'PostMB'; invasive).

Figure 4.1 Study 2 Research Design and Experimental Overview (cont. over page)

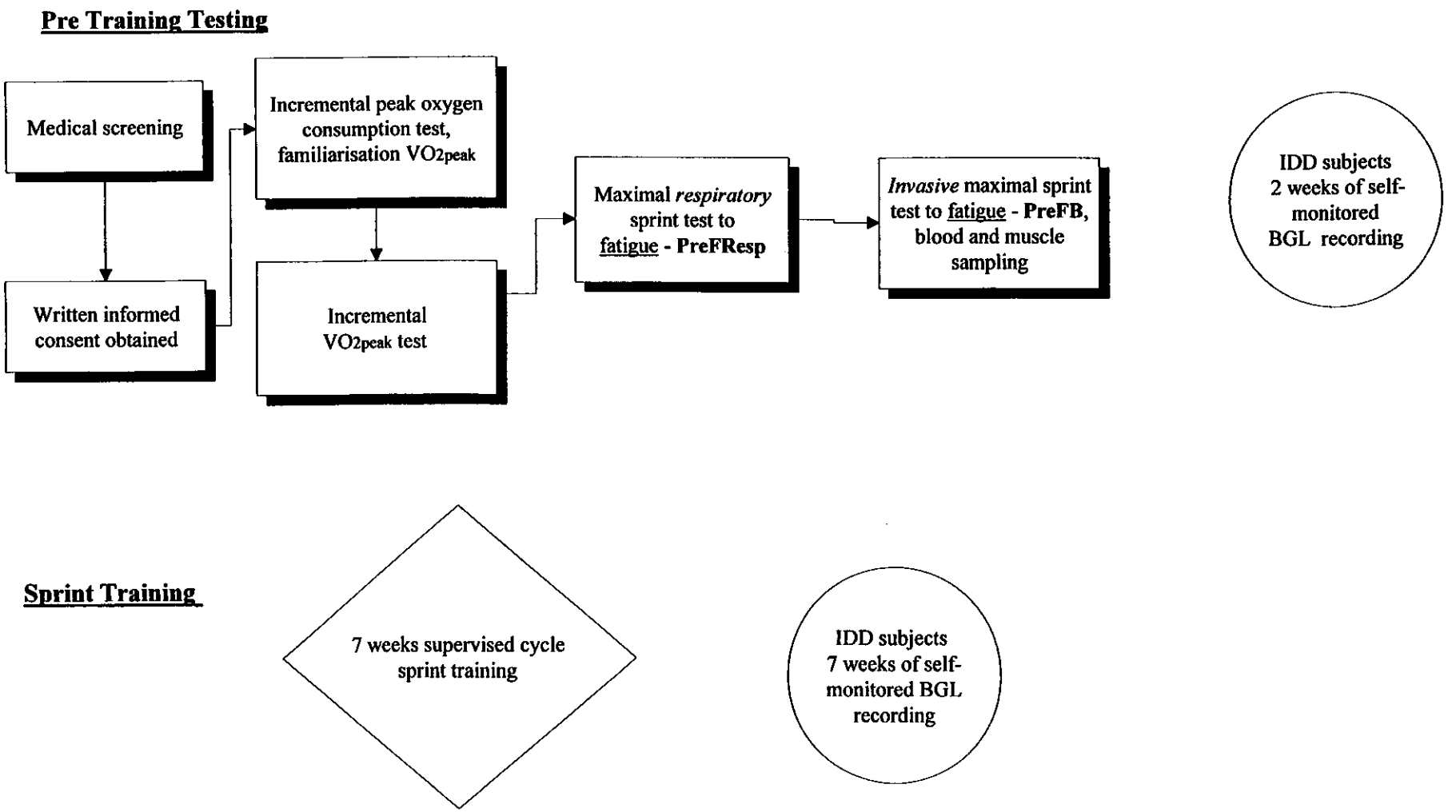
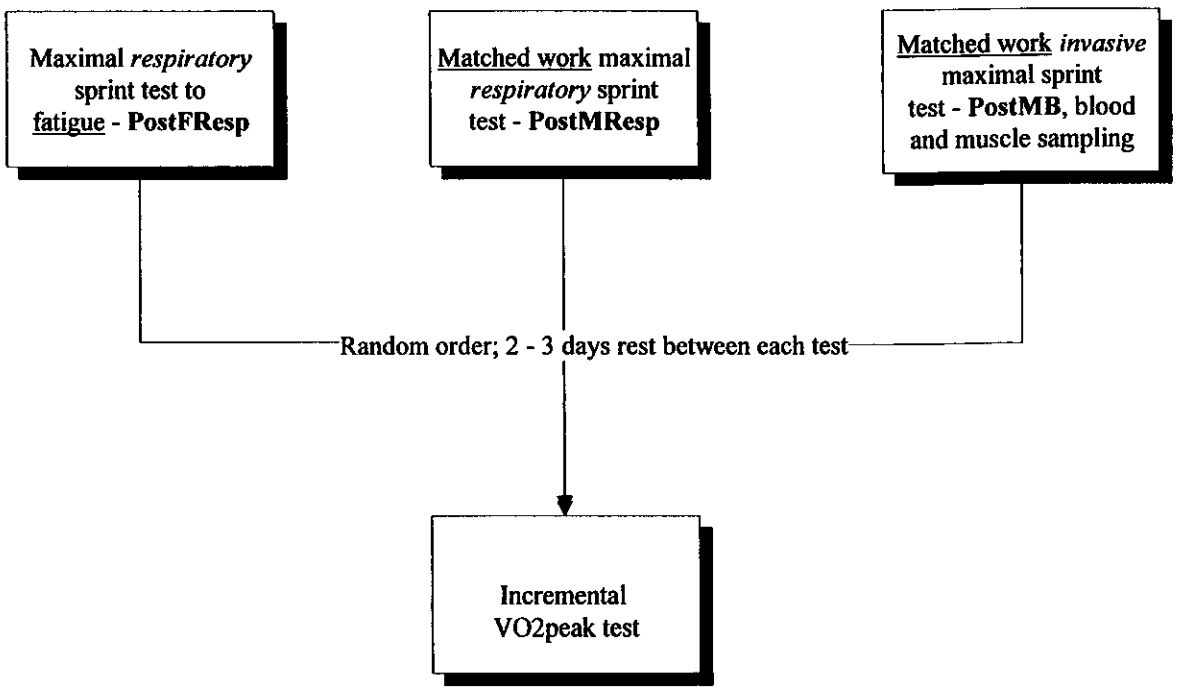


Figure 4.1 (cont.)

Post Training Testing



4.3.3 Training

The training programme was identical to that employed in the first study. Each subject completed 21 sessions of supervised sprint training over a 7-week period. Refer to the study 1 methods section 3.3.3 for details.

4.3.4 Test Procedures

4.3.4.1 Incremental tests to determine $\dot{V}O_{2peak}$

Pre-Training. Subjects completed two incremental tests to determine peak oxygen consumption ($\dot{V}O_{2peak}$) on an electronically braked cycle ergometer (Siemens 380B, Siemens Elema, Sweden) prior to training. The first test served to familiarise each subject with the cycling protocol and the expired gas collection equipment and procedures. The equipment used to collect and analyse expired gases, and to assess ventilation, was identical to that described in the methods section of Study 1 (3.3.4.2). The incremental test was repeated two days later and submaximal steady state and maximal values for oxygen uptake ($\dot{V}O_2$) were obtained.

Subjects were weighed, wearing a pair of shorts (and exercise bra top for females), to the nearest 0.01 kg (Wedderburn Scales, Australia) and had ECG electrodes attached to the chest in the CM5 position. Electrocardiograms were displayed (Phillips, Australia) and monitored throughout the test. The subject was seated at a predetermined saddle height on the electro-magnetically braked cycle ergometer (Siemens 380B, Siemens Elema, Sweden). For each cycle test, feet were secured in toe-clips, and seat and handlebar height kept constant for each subject.

Subjects cycled for 4 min at each of four power outputs, i.e. 60, 90, 120, and 150 W, to allow determination of steady state oxygen uptake. Subjects were rested after the submaximal test until heart rate had returned to within 10 beats·min^{-1} of resting values. At such time a 3 min warm up at 20W was conducted, then the cycle power was incremented to 60W for the commencement of an incremental (10 W·30 s^{-1}) cycle test to volitional fatigue, from which $\dot{V}O_{2peak}$ was obtained. $\dot{V}O_{2peak}$ was defined as the mean of the three highest consecutive 10-s values for $\dot{V}O_2$; as for Study 1.

Post-Training. An incremental test to reassess $\dot{V}O_{2peak}$ was conducted 8.2 ± 1.1 days after training had finished. Procedures were identical to those during the second of the pre-training incremental tests.

4.3.4.2 Calculation of the power output required to elicit 130 % $\dot{V}O_{2peak}$

The procedure for the calculation of the power output required to elicit 130 % $\dot{V}O_{2peak}$ was identical to that described in the methods section of study 1 (section 3.3.4.4). Three female subjects (two in T1D group, one in ND group) were unable to complete 4 min at 150 W. Regression equations were formulated based on three submaximal power outputs (60, 90, 120 W) for two subjects, and four submaximal power outputs for the other female subject (60, 90, 110, 120 W). The Siemens 380B cycle ergometer had power increments of 10 W, consequently the power to elicit 130 % $\dot{V}O_{2peak}$ was rounded up or down to the nearest 10 W.

4.3.4.3 Constant power tests conducted at 130 % $\dot{V}O_{2peak}$

Pre-Training

Prior to training, and after a familiarisation trial, two maximal sprint tests conducted at the power output calculated to elicit 130% $\dot{V}O_{2peak}$, were performed on different days to fatigue. The first was a non-invasive respiratory test, whilst the second was invasive and involved collection of blood and muscle samples. The respiratory test was conducted separately to the invasive sprint, both for practical reasons and to minimise the stressful effects of complex testing procedures on subjects.

1. Non-invasive test: gas exchange and ventilation (PreFResp). The procedure and calculations pertaining to this test were identical to that described in the methods section of Study 1 (section 3.3.4.5). Time to fatigue was recorded.

2. Invasive test: blood and muscle sampling (PreFB). Blood and muscle samples were taken in the second test to fatigue. Time to fatigue was also recorded for this test. Testing procedures were very similar to those for the PreExh test, described in Study 1 (sections 3.3.4.5, 3.3.5.2, 3.3.6.2), except that two muscle biopsies, rather than one, were obtained at each sampling time, and extra blood samples were obtained. See Figures 4.3 and 4.4 for the schemes of handling and analysis procedures for blood and muscle samples respectively, and refer to sections 4.3.6.3 and 4.3.7 for attendant descriptions.

Post-training

Three constant load maximal tests, with 48 - 72 hours rest between each test, were conducted after training at the power output calculated to elicit 130 % of pre-training $\dot{V}O_{2peak}$:

1. Two non-invasive respiratory tests:

(a) A respiratory test to fatigue (PostFResp) was repeated at the same power output, and using identical procedures as in the pre-training respiratory test (PreFResp).

(b) Another respiratory test was performed in which both the power and the time to fatigue in PreFResp were matched exactly (PostMResp).

The post-training respiratory tests were conducted in random order for each subject, and preceded the invasive test.

2. Invasive test with muscle and blood sampling:

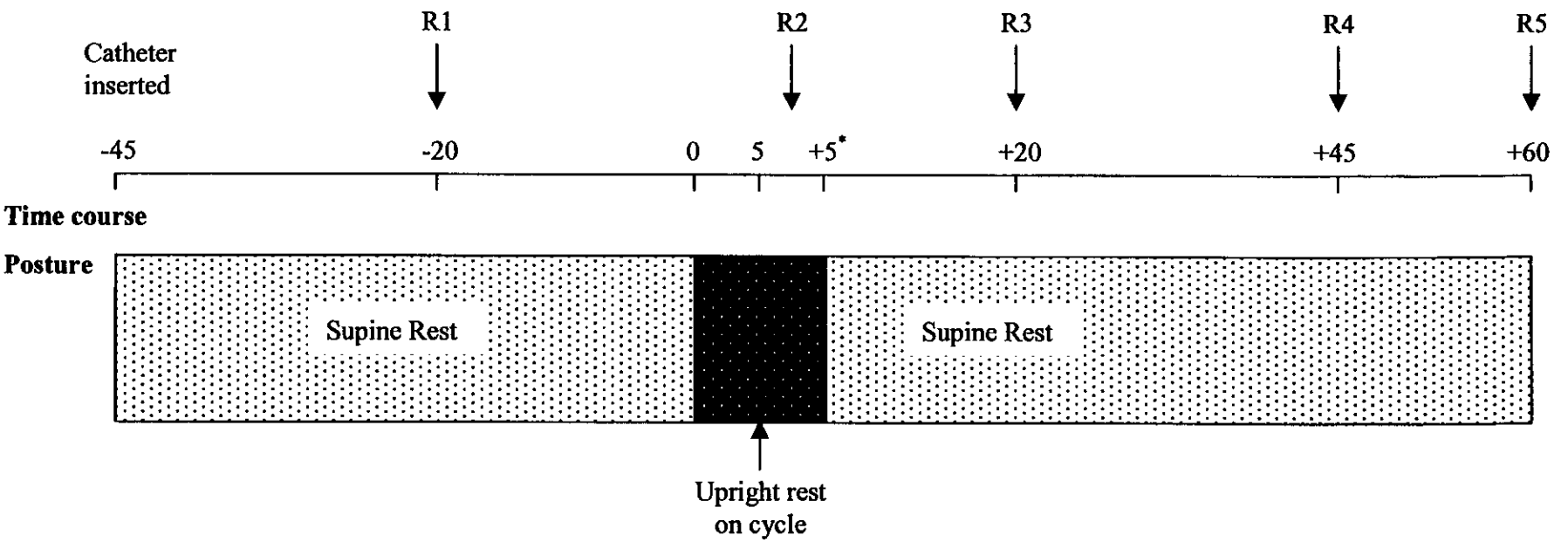
The invasive supramaximal sprint test (PostMB) in which both the exercise time and power output were identical to the PreFB test was conducted 5.9 ± 0.4 days after the final training session. Muscle and blood sampling times were matched as closely as possible to those of PreFB. The total time spent in the laboratory for each of PreFB and PostMB was approximately 2.5 hours.

4.3.5 Resting Study in the T1D group

On a separate day, each of the eight subjects with type 1 diabetes attended the laboratory for collection of resting blood data. This test was primarily designed to examine the effect of withholding insulin administration upon metabolic control in the subjects in the T1D group, thus providing a non-exercise comparison for the invasive exercise tests. The subject preparation for this test was identical to that for the other blood tests, i.e. 10 – 12 hour overnight fast and delayed administration of the morning insulin dose for the duration of the test. The resting study mimicked the time of day (commencing at 6–7 am), the total test time, the preparation and procedures for blood sampling, and the postures adopted in the 130% $\dot{V}O_{2peak}$ tests in which blood and muscle were sampled (PreFB, PostMB). The exercise bout, muscle sampling, and some of the blood sampling performed in PreFB and PostMB was omitted.

In the resting study, the subject lay supine on a plinth for 45 min, during which time the dorsal hand circulation was arterialised and the blood sampling catheter inserted; blood samples were then taken after 25 min supine rest (see Figure 4.2 for schema of procedures, times and postures). After another 20 min supine rest (during which resting muscle samples were taken in the PreFB, PostMB tests), the subject moved to sit passively on the cycle ergometer, on which they remained seated for 10 min (during which time the warm-up, exercise bout, post-exercise muscle biopsies, and 1 and 2 min post-exercise blood samples were obtained in the PreFB, PostMB tests).

Figure 4.2 Schema of the procedures, time course and postures adopted during the 'Resting Study' in the subjects with type 1 diabetes mellitus.



† Dorsal hand circulation arterialised throughout test

* Numerals preceded by addition signs indicate the period of 'recovery' which commenced after 5 min passive upright sitting on the cycle ergometer. The subject remained seated on the cycle ergometer for the first 5 min of recovery, then lay supine on a plinth for the remainder of recovery.

The subject then resumed the supine posture on the plinth and remained there for a further 60 min (corresponding to the exercise recovery period, during which blood samples were obtained in PreFB and PostMB). Arterialised venous blood was sampled at times corresponding to rest (R1), and 2 (R2), 20 (R3), 45 (R4), and 60 min (R5) of recovery in the invasive exercise tests (PreFB, PostMB), and was assayed for concentrations of free insulin, free fatty acids (FFA), glucose, lactate (Lac^-), potassium(K^+) and sodium (Na^+). The total time the subject was present in the laboratory during the resting study was approximately 2.5 hours.

4.3.6 Blood sampling, handling and analyses

4.3.6.1 Subject preparation

Each subject abstained from caffeine and alcohol consumption, and refrained from strenuous exercise for the 48 hours prior to each exercise test. Subjects documented their dietary intake for the 2 days prior to the test in which blood and muscle were sampled. The individual dietary intake records were photocopied and given back to each subject. Pre-training diets were then replicated for the 2 days preceding the post-training test in which blood and muscle were sampled.

All subjects presented at the laboratory after an overnight fast of 10–12 hours duration. T1D subjects reduced their usual evening dose of insulin by 1-2 U to prevent a hypoglycaemic episode on the morning of the tests, and delayed their usual morning dose of insulin until after testing had been completed.

Female subjects underwent blood and muscle testing in the luteal phase of the menstrual cycle (determined by subject feedback and plasma progesterone concentration). However, two female subjects, one in each of the T1D and ND groups, had very irregular menstrual cycles, and therefore testing could not be accurately timed with the luteal phase.

Subjects lay supine with a hand and forearm immersed in warm water (43°C) for 10 min. A 22G flexible catheter (Optiva 225, Johnson & Johnson, Australia) was inserted using aseptic technique into a dorsal hand vein and secured with a clear

waterproof dressing and micropore tape. Minimum volume extension tubing (25 cm; Tuta Laboratories, Australia), with a deadspace of ~ 0.5 ml, was connected to the catheter. A one-way valve (Safsite, B. Braun,U.S.A.) was attached to the extension tubing to enable rapid sampling. The hand was placed inside a plastic bag and re-immersed in warm water for the duration of each test (except during the collection of the 1 and 2 min recovery blood samples during the PreFB and PostMB tests when the post-exercise muscle biopsy procedures necessitated rapid alteration of posture on the cycle ergometer). A heating fan was applied over the dorsal aspect of the hand to maintain venous arterialisation during these periods. The catheter was kept patent by administration of sterile isotonic saline after the final blood sample at each collection time, and by further periodic flushing with saline in the 10–60 min recovery period.

4.3.6.2 Blood sampling times

After training, in PostMB, blood sampling times were matched as closely as possible to those recorded for the invasive pre-training test (PreFB).

Rest

Several blood samples were taken prior to the muscle biopsies, after 20 – 25 min supine rest. Another sample was obtained with the subject seated on the cycle ergometer, prior to the warm-up. (In addition, prior to training, a 4 ml blood sample was obtained after an overnight fast, and analysed for plasma glucose concentration as described below. This sample was taken on a different day to the invasive test to permit screening of fasting plasma glucose without the added stress of impending biopsy procedures.)

Exercise

Blood collection was commenced in the final seconds of the sprint at volitional fatigue (PreFB), or in the final seconds of exercise in the case of the matched-time test (PostMB).

Recovery

Blood was sampled at 1, 2, 5, 10, 20, 45 and 60 min of recovery from these exercise tests. The 1 and 2 minute samples were collected while in a semi-reclined position on the cycle ergometer. The remainder of the recovery samples were taken with the subject resting on a plinth in a supine position.

4.3.6.3 Blood handling and analyses

Figure 4.3 depicts the scheme for apportioning and handling of blood.

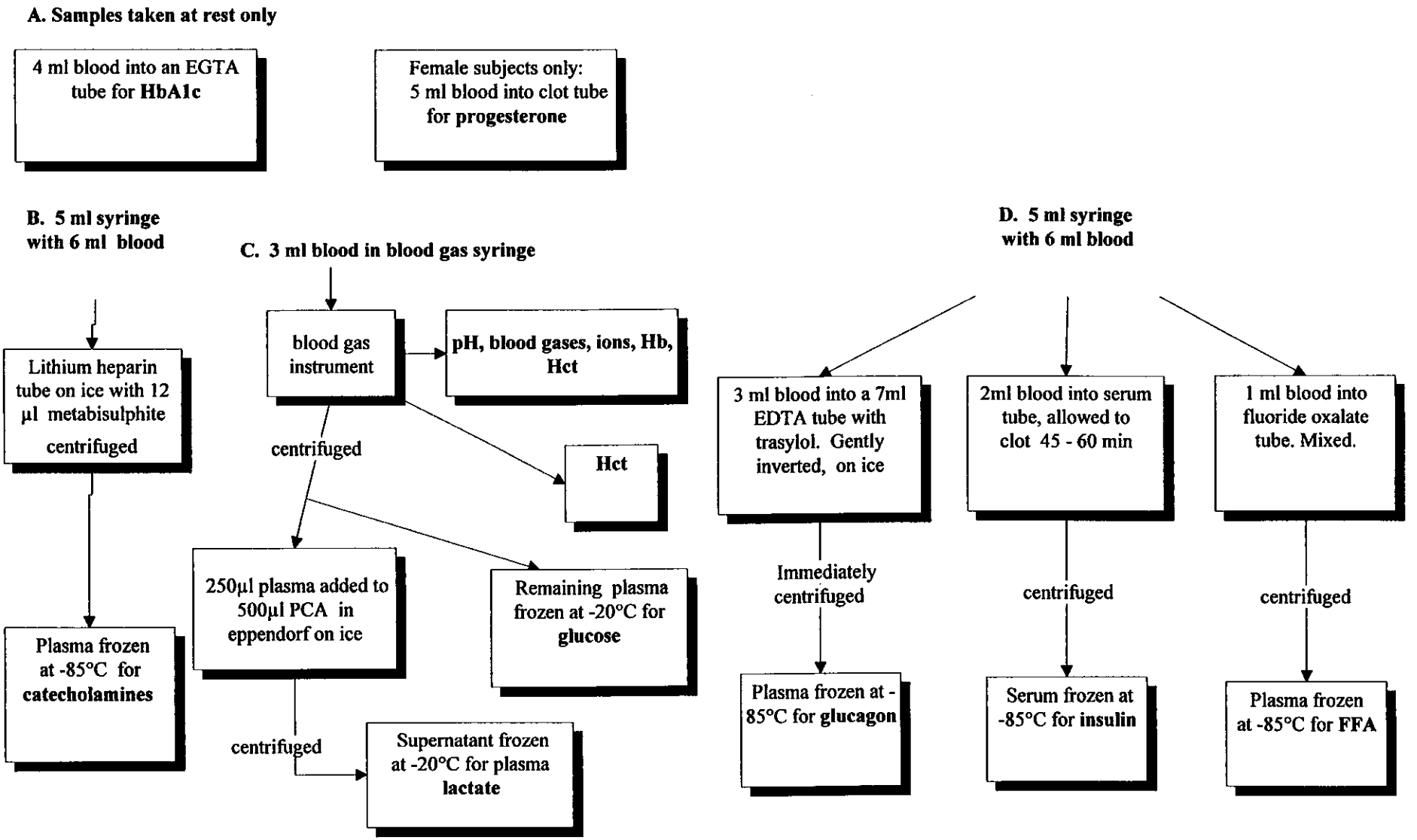
To account for dead space, 1 - 1.5 ml of blood were withdrawn and discarded prior to the first sample being collected at each time point. One 4 ml whole blood sample was taken at rest only from all subjects before and after training in the invasive tests. The 4 ml sample was withdrawn and ejected into an EGTA tube, gently mixed, then briefly stored at 4°C until being delivered on ice the same day to Bankstown Hospital Pathology Department; and was subsequently analysed for percent HbA_{1c} using an HPLC technique by the Liverpool Hospital Biochemistry Department. A further 5 ml blood sample was obtained from each female subject at rest in the pre- and post-training invasive tests. The sample was centrifuged and the plasma was stored at –80°C until analysed for progesterone concentration by the the Liverpool Hospital Biochemistry Department.

For rest, exercise and recovery in the invasive tests, two to three samples were taken at each time point (refer to Figure 4.3), with the catecholamine sample being taken first, then the blood gas sample, then the sample for insulin, glucagon and FFA.

Sample 1: A 6 ml blood sample was taken at each sampling point (except at 45 min of recovery), ejected into an ice-cold tube containing 12 µl sodium metabisulphite ($5g \cdot dl^{-1}$), and used to determine catecholamine concentrations as previously described (section 3.3.5.3ii).

Sample 2: At each sampling time 3 ml of blood were withdrawn into a 'RapidLyte' blood gas syringe (Chiron Diagnostics Corp., U.S.A.) containing lithium heparin. Air bubbles were expelled from the syringe, the syringe re-capped and the blood gently mixed. The syringe was immediately connected to the blood gas analyser (Corning 865, Chiron Diagnostics Corp., U.S.A.), or briefly kept in an ice slurry if immediate analysis wasn't possible, for auto-sampling and analysis. Immediately after auto-

Figure 4.3 Study 2 Blood handling and aliquoting schema



sampling, the syringe was removed and placed in an ice slurry. All samples were analysed in duplicate (or triplicate if required).

The following variables were obtained from the blood gas analysis: PO_2 and PCO_2, pH, [Hb], plasma [Na^+], [K^+], [Cl^-], and [HCO_3^-]. The remaining blood was transferred into a 4 ml tube on ice. Haematocrit samples were then drawn into microcapillary tubes (duplicate), before the 4 ml tube was centrifuged at 0°C and 12,000 rpm for 10 min. The change in plasma volume, relative to rest, (ΔPV), the [H^+] and strong ion difference (SID) was calculated as for Study 1 (section 3.3.5.3ii). The plasma was removed and placed into eppendorf tubes on ice. 250 µl of plasma was removed, ejected into an eppendorf tube containing 500 µl 0.6 M PCA, centrifuged, the supernatant removed and stored at –20°C until analysis for lactate concentration as previously described (Study 1, section 3.3.5.3ii). The remaining plasma was frozen at –20°C until analysed for glucose concentration. Plasma glucose was assayed enzymatically at 37°C using a commercial kit (Trace Scientific, Melbourne, Australia), and read at 340 nm on a spectrophotometer (UV-1601PC; Shimadzu, Tokyo, Japan).

Sample 3: A further 6 ml blood sample was taken at the following time points – rest, end of exercise, and 5, 20, 45, and 60 min of recovery, and apportioned for later analysis of glucagon, FFA, and insulin (and free insulin in subjects with type 1 DM) concentrations.

Insulin, free insulin, and glucagon assays were performed by Mr K. Li and Ms D. Wilks at the Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, Sydney, Australia. The blood sample was mixed gently and immediately aliquoted into three tubes. 3 ml were ejected into a 7 ml ice-cold tube containing EDTA, heparin ($15U \cdot ml^{-1}$) and aprotinin ($10,000\ IU \cdot ml^{-1}$), inverted, and stored briefly on ice until centrifugation at 4,000 rpm for 10 min. The plasma was removed, placed in a plastic tube and stored at –85°C until analysed for glucagon concentration by a kit method (Euro Diagnostica, Malmö, Sweden) double-antibody radioimmunoassay (RIA) using a polyclonal antiserum, ^{125}I-glucagon, and a synthetic human glucagon standard. A further 1 ml of whole blood was aliquoted into a

fluoride oxalate tube, inverted, and stored on ice until centrifugation at 4,000 rpm for 9 min. The plasma was removed, transferred to an iced eppendorf tube, and stored at –85°C until analysis for FFA concentration. Free fatty acids were assayed at 37°C using a commercial kit (NEFA C, Wako Pure Chemical Industries, Osaka, Japan), and an enzymatic, colorimetric method which utilised a spectrophotometer (UV-1601PC; Shimadzu, Tokyo, Japan) at 550 nm. The remaining whole blood was placed in a clot tube containing separation gel, allowed to sit at room temperature for 45 – 60 min, then centrifuged. The serum was removed and transferred into an eppendorf tube which was stored at –85°C until analysis for insulin concentration by double-antibody RIA using a polyclonal antiserum, ^{125}I-human insulin, and a highly purified human insulin standard (NOVO Industri, Bagsvaerd, Denmark). Due to the development of anti-insulin antibodies following treatment with bovine/porcine and biosynthetic (human) insulin, free (biologically active) insulin was measured in the subjects with type 1 diabetes by precipitating immunoglobulins from the sample with polyethylene glycol prior to radioimmunoassay of the supernatant (Kuzuya *et al.*, 1977). Haemolysis of the plasma sample, may significantly reduce plasma IRI concentration, depending on the assay (O'Rahilly *et al.*, 1987), so care was taken to ensure minimal haemolysis.

For the T1D Resting Study, plasma [K^+] and [Na^+] was analysed in triplicate using an auto-injection flame photometer (IL 943, Instrumentation Laboratory, Italy). Plasma glucose and Lac^-, FFA, and free insulin assays were performed as described above.

Excepting pH, blood gas analysis, and HbA_{1c}, all pre- and post-training blood samples for a particular assay were analysed together on the same day for each subject. When instrumentation was used to analyse samples, calibration was performed immediately prior to, and periodically throughout the analysis with both quality control assayed serum, and high and low precision standards.

4.3.7 Muscle Biopsies – sampling, handling and analyses

Four muscle samples were obtained prior to training in the PreFB test. The first two samples were taken at rest from the same incision with the subject supine, and the third and fourth samples were taken immediately at the cessation of exercise, whilst the subject was still seated, supported in a semi-reclined position on the cycle ergometer (illustrated in Figure 4.4). Due to the marked hyperaemia induced by intense exercise, each post-exercise muscle sample was taken from a separate incision on the same leg. After training, in the PostMB test, four muscle samples were taken from the contralateral leg, with post-exercise sampling times matched as closely as possible to those of the PreFB test.

4.3.7.1 Subject Preparation

Preparation of each subject for the muscle biopsies was identical to that described in section 3.3.6.1, except that three small incisions (~0.8 mm) were made in the anaesthetised skin overlying the vastus lateralis muscle. The first incision was made at the junction of the proximal two-thirds and distal third of the distance between the anterior superior iliac spine and the proximal pole of the patella. Each subsequent incision was located ~1-2 cm medially and ~2 cm distally to the preceding one. The first two biopsies were taken from the most distal incision, the third biopsy from the middle incision, and the fourth biopsy from the most proximal incision.

4.3.7.2 Muscle Handling and Analyses

All muscle samples or homogenates were stored in cryotubes immersed in liquid nitrogen until required for analysis. Figure 4.5 summarises muscle handling and analysis procedures.

(i) Biopsy apportioning

Biopsies 1 and 3 (i.e. the first sample taken at rest and immediately at the cessation of exercise). Fresh muscle samples were immmediately immersed, whilst still in the biopsy needle, in liquid nitrogen (N_2). Samples were carefully prised free of the needle and transferred to cryotubes for storage at –196°C until freeze-dried and assayed for metabolite concentration.

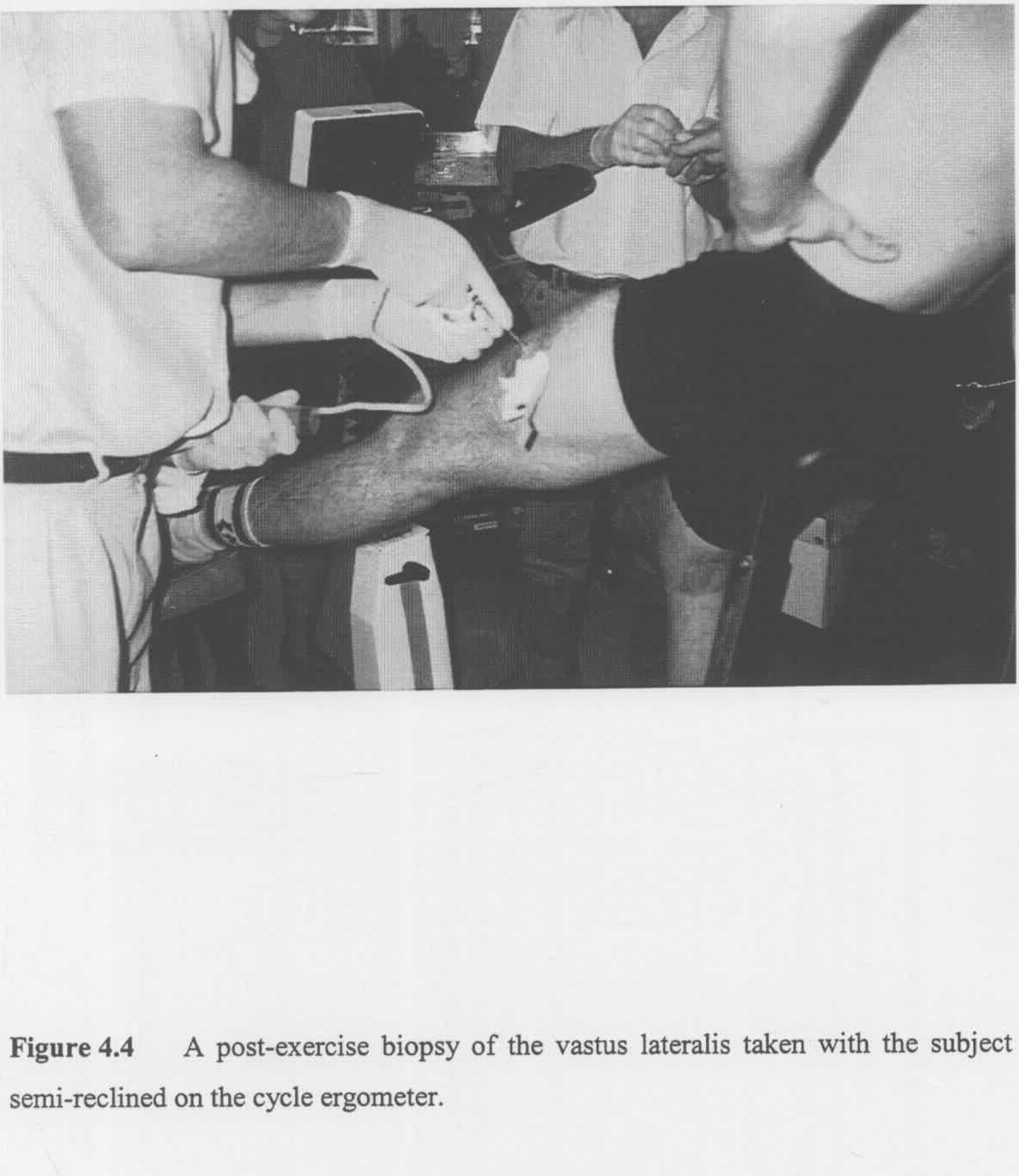
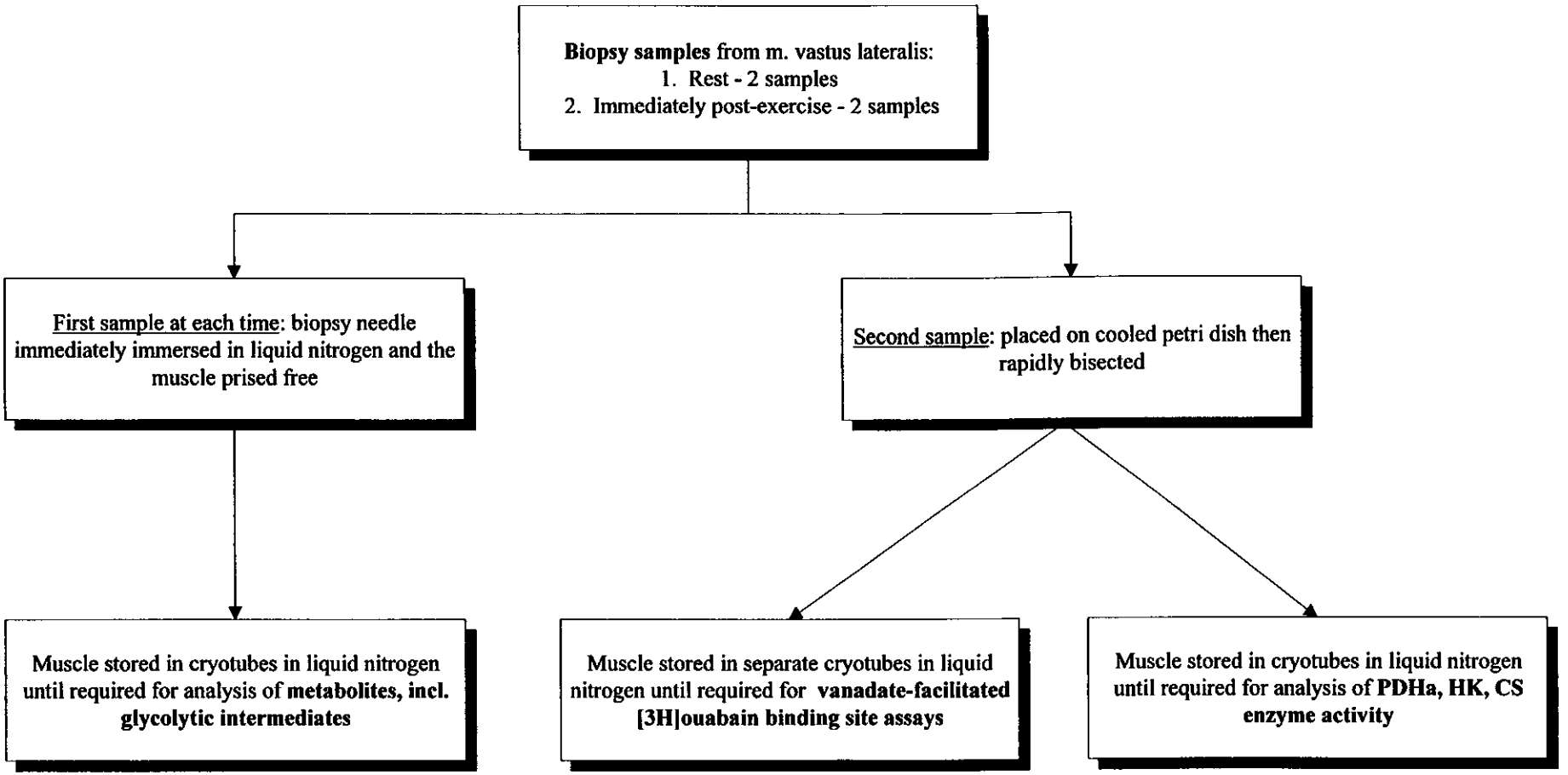


Figure 4.4 A post-exercise biopsy of the vastus lateralis taken with the subject semi-reclined on the cycle ergometer.

Figure 4.5 Study 2 Muscle handling and analysis schema



Biopsies 2 and 4 (i.e. the second sample obtained at rest and after exercise). Excised muscle was placed on a cooled petri dish, then quickly divided into two portions. Both portions were immediately frozen (in separate cryotubes) and stored under liquid N_2 for later analysis of muscle enzyme activity (PDH, HK, CS), and Na^+K^+-ATPase concentration.

(ii) Freeze-drying procedure prior to metabolite analyses

The muscle for metabolite analysis was freeze-dried, then dissected free of connective tissue and blood. The freeze-drying procedure is described in section 3.3.6.3(i).

(iii) Extraction procedures for metabolite assays

Extraction procedures have been previously described in section 3.3.6.3(ii). 6 mg of freeze-dried muscle was extracted per sample to enable determination of glycolytic intermediates, as well as the other muscle metabolites that were assayed in Study 1, and 1-2 mg of dried muscle was extracted for the glycogen assay. For each subject, muscle metabolites (except glycogen, glucose, Lac^-, and pyruvate) in pre- and post-training samples were corrected in relation to the peak total creatine concentration obtained before and after training, respectively, and expressed as $mmol \cdot kg^{-1}$ dry mass (dm).

(iv) Assay Procedures

Muscle metabolites

Assay procedures for muscle metabolites (exclusive of muscle glucose, G 6-P, G 1-P, F 6-P, and pyruvate) and protein concentration are described in section 3.3.6.3(iii). Metabolite assays were conducted on an Aminco Bowman Series 2 luminescence spectrometer (SLM Instruments, Urbana, IL), and protein assays on a spectrophotometer (UV-1601PC; Shimadzu, Tokyo, Japan).

Intracelluar glucose and selected glycolytic intermediates (G 6-P, G 1-P, F 6-P, and pyruvate) were assayed according to the methods of Lowry & Passoneau (1972). The reagent for the intracellular glucose, G 6-P, G 1-P, and F 6-P assay was comprised of 30 mM Tris (pH 8.1), 1 mM $MgCl_2$, 0.5 mM DTT, 0.3 mM ATP, 0.05 mM $NADP^+$, and 0.1 mM EDTA. Standards were assayed in triplicate and 100 µl extract samples

assayed in duplicate. After an initial reading at 365 nm absorption and 455 nm emission in the luminescence spectrometer, 10 µl of the diluted enzyme G-6-P dehydrogenase (10 µl enzyme diluted in 1.5 ml reagent) was added to each cuvette. The cuvettes were incubated for a 15 min period in a dark cupboard at room temperature, then read again. The same procedure was followed for the two diluted enzymes, phosphoglucomutase (20 µl enzyme diluted in 1.0 ml reagent) and phosphoglucoisomerase (10 µl enzyme diluted in 1.0 ml reagent), whilst 25 µl of diluted hexokinase (25 µl enzyme diluted in 1.0 ml reagent) was added for the final reaction.

The reagent for the pyruvate assay was composed of a 50 mM phosphate buffer (Na_2HPO_4, KH_2PO_4) and 0.75 µM NADH. Standards and 50 µl extract samples were assayed in triplicate. After an initial reading of the sample and reagent, 10 µl of diluted lactate dehydrogenase (10 µl diluted in 2 ml reagent) was added to each cuvette which was then incubated at room temperature in a dark cupboard for 20 min, then read again at 365 nm absorption and 455 nm emission in the luminescence spectrometer.

The rate of glycogenolysis in muscle was calculated as: ((delta G 1-P + delta G 6-P + delta F 6-P) + 0.5(delta Lac + delta Pyr))/time in s; and the rate of glycolysis as: 0.5(delta Lac + delta Pyr)/time in s (Spriet *et al.*, 1987).

Enzyme activity

The activities of the cytosolic enzyme hexokinase (HK) and the mitochondrial enzymes pyruvate dehydrogenase (active portion, PDH*a*) and citrate synthase (CS) were assayed by Mrs J. Bryson at the Human Nutrition Unit, Department of Biochemistry, The University of Sydney. All enzyme activities were corrected to the respective pre- or post-training peak total creatine.

Briefly, approximately 30 mg frozen wet muscle was weighed, then homogenised in ten volumes of a medium comprised of 50 mM Tris-HCl, 20 mM EGTA, 25 mM NaF, and 1 mM benzamidine; pH 7.4. This homogenate was used without further dilution

for each of the HK, PDH*a*, and CS activity assays, which were conducted spectrophotometrically (Cary3, Varian, Mulgrave, Victoria) at 30°C.

HK activity in the muscle homogenate (Gauthier *et al.*, 1992) was determined at 340 nm in an assay medium of 50 mM TRA, 7.5 mM $MgCl_2$, 1 mM EDTA, 1.5 mM KCl, 4 mM NADP, 25 mM ATP, 0.875 $U \cdot ml^{-1}$ G 6-P DH; pH 7.5. The reaction was initiated by the addition of 100 µl of 20 mM glucose to the cuvette.

PDH*a* activity was determined at 460 nm and assayed by coupling the production of acetyl-CoA to the acetylation of the dye p-(p-aminophenylazo)-benzenesulfonic acid (AABS) with arylamine transferase (Coore *et al.*, 1971). The dye was a gift from Professor Sir Philip Randle. The PDH*a* assay buffer was comprised of 0.1 mM Tris, 1 mM $MgCl_2$, 1 mM EDTA, 1% Triton X-100, 2 % AABS, and 0.35% mercaptoethanol; pH 7.4, to which was added arylamine acetyltransferase, 0.5 M NAD^+, 0.1 mM pyruvate, 0.1 mM coenzyme-A, cocarboxylase, and the muscle homogenate.

CS activity was determined at 412 nm and assayed in 700 µl of a buffer comprised of 0.1 M Tris, 1 mM $MgCl_2$, 1 mM EDTA, 4 µg 5, 5' dithiobis(2-dinitro)benzoic acid; pH 7.4, to which was added 25 µl acetyl-CoA (50 mg Co-A, 5 µl 1 M KH_2PO_4, 100 µl acetic anhydrite; pH 8.0) and 5 µl muscle homogenate (Coore *et al.*, 1971). The reaction was initiated by the addition of 25 µl of 0.1 M oxaloacetate.

Na^+-K^+ ATPase assay

Na^+K^+-ATPase pump density was assessed by vanadate-facilitated [^{3}H]ouabain binding, with minor modifications to the previously described standard method for small muscle samples (Nørgaard *et al.*, 1984). Both α_1 and α_2 isoforms of human skeletal muscle have a high affinity for ouabain allowing detection of both by the [^{3}H]ouabain binding site concentration assay (Clausen, 1998), which quantifies all functional Na^+,K^+ pumps (Clausen *et al.*, 1987).

[^{3}H]ouabain binding site concentration was assessed in muscle that had been stored under liquid nitrogen. Frozen muscle, kept cold on dry ice, was cut to yield pieces weighing 5-15 mg. Based on pilot results, slightly larger muscle pieces were used if the frozen muscle was surrounded by blood, and smaller pieces used when the muscle was relatively free from blood. Mean muscle wet weight prior to the first pre-wash was 9.72 ± 0.23 mg, however after incubation, washing, and blotting, the mean wet weight was 5.99 ± 0.19 mg. This apparent loss of 38 ± 1.5 % was consequent to removal of blood during the pre-washes, and occasionally from dissecting out obvious connective tissue before the final weighing. Pre- and post-training muscle samples were analysed together in the same assay run, and each sample was analysed in quadruplicate. The muscle samples were pre-washed 2 x 10 min at 0 °C in 1,000 µl of freshly made cold, unlabeled buffer (pH 7.3) comprised of 250 mM sucrose, 10 mM Tris(Hydroxymethyl)aminomethane chloride, 3 mM $MgSO_4$, and 1 mM $NaVO_3$, with the buffer being changed after the first 10 min wash. Muscle samples were loosely confined within an eppendorf tube with numerous drainage holes. The eppendorf tube and muscle were transferred together into fresh 2,000 µl tubes after each wash or incubation. This minor modification to the original method helped reduce the loss of muscle between transfers. Samples were then incubated, agitated by a stirred water bath, for 2 x 60 min at 37 °C in a medium comprised of the unlabeled buffer, 0.12 $\mu mol \cdot l^{-1}$ tritiated ouabain (1.8 $\mu Ci \cdot ml^{-1}$), and 0.88 $\mu mol \cdot l^{-1}$ unlabeled ouabain to achieve a final ouabain concentration of 1 $\mu mol \cdot l^{-1}$. The muscle was then washed 4 x 30 min at 0 °C with the cold, unlabeled buffer to reduce non-specific binding. Between each of the cold washes the muscle was blotted on filter paper to remove most of the previous wash buffer. After the final wash, the muscle was blotted on dry filter paper, weighed and left overnight in 1,000 µl 5 % trichloroacetic acid (TCA). The following day, the eppendorfs containing the muscle /TCA were centrifuged for 5 min, prior to adding 500 µl of the supernatant to scintillation vials containing 5,000 µl scintillation fluid (Optiphase, HiSafe 3, Wallac, Turku, Finland). A 500 µl TCA blank was included in each assay, in addition to three vials each containing 10 µl of the tritiated ouabain incubation media, 500 µl TCA and scintillation fluid. Tubes were counted in a WinSpectral 1414 Liquid Scintillation Counter (Wallac, Turku, Finland). The mean intra-assay coefficient of variation was 5.61 ± 0.73 %. [^{3}H]ouabain binding

site concentration was expressed as $pmol \cdot g^{-1}$ wet weight, and calculated according to the formula:

$$\frac{(2)\,(1.05)\,(10\ pmoles/\ 10\ \mu l\ media)\,(sample\ cpm\text{-}blank)}{(cpm\text{-}blank/\ 10\ \mu l\ media)\,(sample\ weight)}$$

where 2 corrects for the the use of 500 µl supernatant from the 1ml extraction; 1.05 is a correction factor for the loss of specifically bound [^{3}H]ouabain during the 4 x 30 min cold washes (Nørgaard *et al.*, 1984); the sample cpm was counted twice and the mean value used; and the media cpm counted three times, with the mean value used.

4.3.8 Statistics

Blood and muscle data were analysed using a repeated measures three-way ANOVA (sample time, training status, and group; SPSS 8.0 for Windows). Significant F ratios were further examined using an ANOVA contrast technique (SPSS). Repeated measures two-way ANOVA (training status/test day, group; SPSS) was used to compare the difference between the two groups for mean and peak cardiorespiratory variables and single pre- and post-training measures, e.g. $\dot{V}O_{2peak}$, body mass index, peak incremental power. SPSS ANOVA contrasts were used to define the effect when a significant F ratio was found for the mean and peak cardiorespiratory comparisons in PreFResp, PostMResp, and PostFResp. Statistical significance was accepted at $P<0.05$. Results are reported as the mean ± SEM, unless otherwise indicated.

4.4 Results

4.4.1 Subject characteristics

(i) Anthropometry

The physical characteristics of the eight subjects (5 males, 3 females) who were included in the group with type 1 diabetes mellitus (T1D), and the seven subjects (4 males, 3 females) who comprised the non-diabetic group (ND) are summarised in Table 4.1. The groups were well matched for physical characteristics, evidenced by no difference between the T1D and ND groups for any of the variables, either before or after training. Individual data appear in Appendix E1. The mean duration of type 1 diabetes mellitus in the T1D group, from the time of first diagnosis to the commencement of Study 2 was 7.1 ± 1.4 years (Appendix E2).

Table 4.1 *Subject characteristics of the T1D and ND groups.*

Characteristic	Pre/Post training	T1D	ND
Age (years)	*Pre*	25 ± 1	24 ± 2
Height (cm)	*Pre*	174.8 ± 4.4	174.4 ± 2.6
Mass (kg)	*Pre*	77.9 ± 4.9	73.2 ± 7.5
	Post	77.4 ± 5.1	73.2 ± 7.7
Body mass index ($kg \cdot m^{-2}$)	*Pre*	25.4 ± 1.1	23.8 ± 1.9
	Post	25.2 ± 1.1	23.8 ± 1.9

Values are means ± SEM. T1D, group with type 1 diabetes mellitus, $n = 8$; ND, non-diabetic group, $n = 7$.

4.4.2 Performance

Incremental tests to fatigue

The peak power attained during the incremental test was ~ 10 % greater after training ($P<0.01$; Table 4.2), with no difference between groups.

Table 4.2 *Peak power achieved by the non-diabetic (ND) group and group with type 1 diabetes (T1D) in the pre-and post-training incremental $\dot{V}O_{2\,peak}$ tests.*

	Peak power (W)	
Group	Pre	Post **
ND	270 ± 24	297 ± 26
T1D	269 ± 28	298 ± 33

Values are means ± SEM; n = 7, ND; 8, T1D. ** P<0.01, Post>Pre.

Constant power tests at 130 % pre-training $\dot{V}O_{2\,peak}$

The power output calculated to elicit 130% $\dot{V}O_{2\,peak}$ did not differ between ND and T1D groups (350 ± 39 and 340 ± 41, respectively; P=0.86). There was no difference between the times to fatigue in the non-invasive respiratory (PreFResp), and invasive (PreFB) tests prior to training for either group. Therefore, a pre-training mean time to fatigue has been calculated (average of PreFResp and PreFB; Table 4.3) and analysed in addition to the separate test data.

Time to fatigue in the 130 % $\dot{V}O_{2\,peak}$ test increased significantly (P<0.001) after training, with a 34 % increase for the ND group, and a 52 % increase for the T1D group, with no difference between groups. When examining each of the pre-training tests separately by comparing PreFResp and PostFResp, then PreFB and PostFResp, respectively, the T1D group demonstrated 64 and 42 % increases, whilst the ND group showed 33 and 35 % increases in time to fatigue (Table 4.3). The increase in time to fatigue in the post-training respiratory test (PostFResp *vs* PreFResp) tended to be greater (P=0.06) in the T1D group than in the ND group.

When exercising to fatigue after training (PostFResp), the work performed by both T1D (37 ± 4 kJ) and ND (29 ± 5 kJ) groups was significantly greater (P<0.001) than in either of the fatiguing pre-training tests, i.e. the non-invasive, PreFResp (22 ± 2, T1D; 22 ± 4

kJ, ND) or the invasive PreFB (25 ± 3, T1D; 21 ± 3 kJ, ND; Figure 4.6) tests, or the pre-training mean (24 ± 2, T1D; 21 ± 3 kJ, ND). The group by test interaction just failed to reach significance (P=0.08).

Table 4.3 *Time to fatigue in the 130 % $\dot{V}O_{2peak}$ tests, before and after training, in the non-diabetic (ND) group and the group with type 1 diabetes (T1D).*

	Time to fatigue (s)			
Group	PreFResp	PreFB	Mean Pre	PostFResp ***
ND	62 ± 8	62 ± 6	62 ± 7	83 ± 10
T1D	68 ± 4	78 ± 7	73 ± 5	111 ± 11

Values are means ± SEM; n = 7, ND; 8, T1D. PreFResp, pre-training respiratory test to fatigue; PreFB, pre-training invasive test to fatigue; Mean Pre, mean of the two pre-training tests, PreFResp and PreFB; PostFResp, post-training respiratory test to fatigue. *** P<0.001, PostFResp>PreFResp, PreFB, Mean Pre.

In the pre- (PreFResp) and post-training (PostMResp) matched work respiratory tests (Figure 4.7A), and in the pre- (PreFB) and post-training (PostMB) matched work invasive tests (Figure 4.7B), the exercise time, and hence work performed, was identical within each condition (respiratory; invasive) and each group (T1D; ND), and did not differ significantly between conditions or groups.

4.4.3 Cardiorespiratory Responses to Exercise and Training

(i) Incremental tests to fatigue

The peak cardiorespiratory responses in the incremental tests before and after training are summarised in Table 4.4. After training, in the incremental tests to fatigue, $\dot{V}O_{2peak}$ was significantly increased (P<0.05; Table 4.4), being 2.5% higher in the T1D group, and 7.2% higher in the ND group, with no statistical difference between the two groups.

Peak expired ventilation ($\dot{V}E_{peak}$) and carbon dioxide output ($\dot{V}CO_{2peak}$) were also significantly higher after training (Table 4.4). $\dot{V}E_{peak}$ was increased 10–12% (P<0.01),

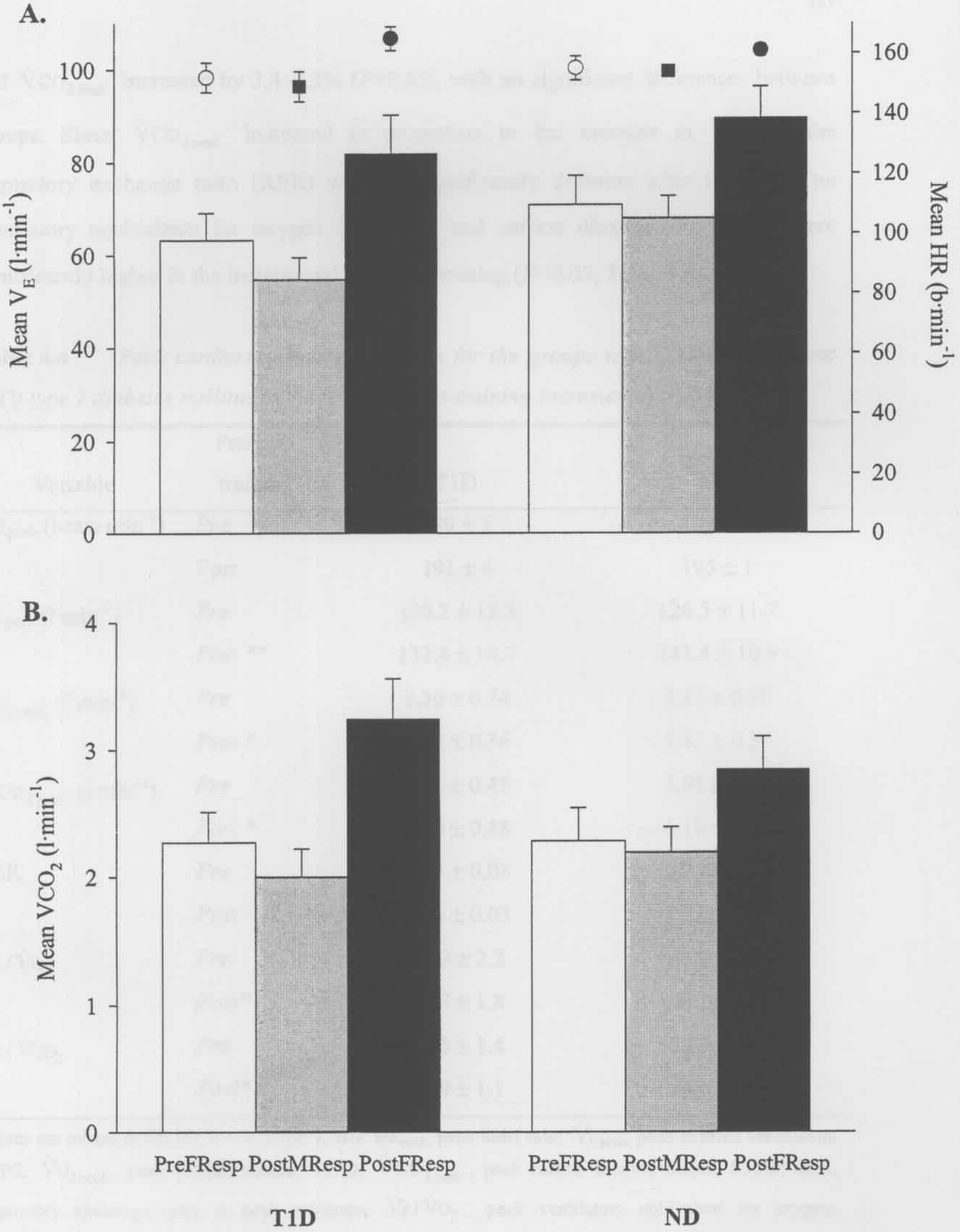


Figure 4.6 Mean values (± SEM) obtained during the constant power 130 % $\dot{V}O_{2peak}$ tests to fatigue before (PreFResp) and after training (PostFResp), and in the matched work test after training (PostMResp), for (A) Heart rate (right-hand *y* axis; O, PreFResp; ■, PostMResp; ●, PostFResp), and expired ventilation ($\dot{V}_E$; left-hand *y* axis); and (B) $\dot{V}CO_2$, in the groups with (T1D) and without (ND) type 1 diabetes. n = 8, T1D; n = 7, ND. Significant effects are reported in the text.

and $\dot{V}CO_{2\,peak}$ increased by 3.4–7.2% (P<0.05), with no significant differences between groups. Since $\dot{V}CO_{2\,peak}$ increased in proportion to the increase in $\dot{V}O_{2\,peak}$, the respiratory exchange ratio (RER) was not significantly different after training. The ventilatory equivalents for oxygen ($\dot{V}_E/\dot{V}O_2$) and carbon dioxide ($\dot{V}_E/\dot{V}CO_2$) were significantly higher in the incremental test after training (P<0.05; Table 4.4).

Table 4.4 *Peak cardiorespiratory responses for the groups with (T1D) and without (ND) type 1 diabetes mellitus in the pre- and post-training incremental tests to fatigue.*

Variable	Pre/Post training	T1D	ND
HR_{peak} (beats·min^{-1})	*Pre*	189 ± 3	193 ± 2
	Post	191 ± 4	195 ± 1
$\dot{V}_{E\,peak}$ (l·min^{-1})	*Pre*	120.2 ± 13.3	126.5 ± 11.7
	Post **	132.4 ± 14.7	141.4 ± 10.9
$\dot{V}O_{2\,peak}$ (l·min^{-1})	*Pre*	3.30 ± 0.34	3.17 ± 0.30
	Post *	3.38 ± 0.36	3.43 ± 0.37
$\dot{V}CO_{2\,peak}$ (l·min^{-1})	*Pre*	4.06 ± 0.45	3.91 ± 0.36
	Post *	4.20 ± 0.48	4.19 ± 0.39
RER	*Pre*	1.24 ± 0.03	1.25 ± 0.02
	Post	1.25 ± 0.03	1.27 ± 0.03
$\dot{V}_E/\dot{V}O_2$	*Pre*	37.9 ± 2.2	41.0 ± 2.4
	*Post**	40.5 ± 1.8	43.8 ± 1.9
$\dot{V}_E/\dot{V}CO_2$	*Pre*	30.5 ± 1.4	32.9 ± 1.8
	*Post**	32.9 ± 1.1	34.6 ± 1.0

Values are means ± S.E.M.; n = 8, T1D; 7, ND. HR_{peak}, peak heart rate; $\dot{V}_{E\,peak}$, peak expired ventilation, BTPS; $\dot{V}O_{2\,peak}$, peak oxygen uptake, STPD; $\dot{V}CO_{2\,peak}$, peak carbon dioxide output, STPD; RER, respiratory exchange ratio at peak workrate; $\dot{V}_E/\dot{V}O_2$, peak ventilatory equivalent for oxygen; $\dot{V}_E/\dot{V}CO_2$, ventilatory equivalent for carbon dioxide at peak workrate. * P<0.05, ** P<0.01, Post>Pre-training.

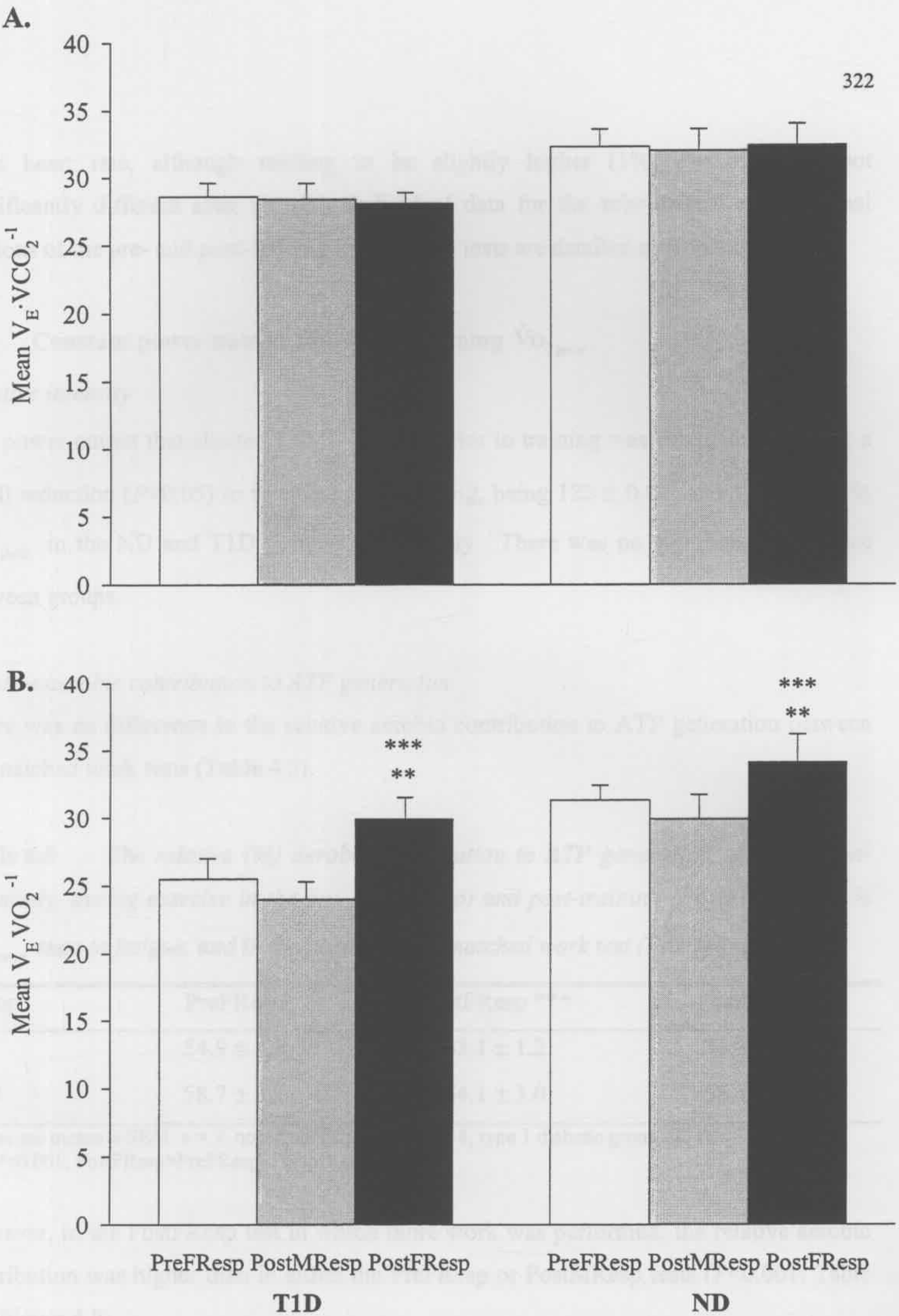


Figure 4.7 Mean (± SEM) values for (A) $\dot{V}_E \cdot \dot{V}CO_2^{-1}$, the ventilatory equivalent for carbon dioxide, and (B) $\dot{V}_E \cdot \dot{V}O_2^{-1}$, the ventilatory equivalent for oxygen, during constant power exercise at 130 % $\dot{V}O_{2peak}$, when exercising to fatigue before (PreFResp) and after training (PostFResp), and in the post-training matched work test (PostMResp), in the groups with (T1D) and without (ND) type 1 diabetes. n = 8, T1D; n = 7, ND. Mean $\dot{V}_E \cdot \dot{V}O_2^{-1}$ in PostFResp>PreFResp (**P<0.01) and PostMResp (***P<0.001). Both ventilatory equivalents were significantly lower across all three tests in the T1D group (P<0.05).

Peak heart rate, although tending to be slightly higher (1%; P=0.09), was not significantly different after training. Individual data for the submaximal and maximal sections of the pre- and post-training incremental tests are detailed in Appendix E(4-6).

(ii) Constant power tests at 130 % pre-training $\dot{V}O_{2peak}$

Relative intensity

The power output that elicited 130 % $\dot{V}O_{2peak}$ prior to training was calculated to evoke a small reduction (P<0.05) in intensity post-training, being 122 ± 0.04, and 127 ± 0.04 % $\dot{V}O_{2peak}$ in the ND and T1D groups, respectively. There was no significant difference between groups.

Relative aerobic contribution to ATP generation

There was no difference in the relative aerobic contribution to ATP generation between the matched work tests (Table 4.5).

Table 4.5 *The relative (%) aerobic contribution to ATP generation, assessed non-invasively, during exercise in the pre- (PreFResp) and post-training (PostFResp) 130 % $\dot{V}O_{2peak}$ tests to fatigue, and in the post-training matched work test (PostMResp).*

Group	PreFResp	PostFResp ***	PostMResp
ND	54.9 ± 1.8	63.1 ± 1.2	55.8 ± 0.8
T1D	58.7 ± 2.1	64.1 ± 3.0	55.1 ± 3.8

Values are means ± SEM. n = 7, non-diabetic group (ND); 8, type 1 diabetic group (T1D).
*** P<0.001, PostFResp>PreFResp, PostMResp.

However, in the PostFResp test in which more work was performed, the relative aerobic contribution was higher than in either the PreFResp or PostMResp tests (P<0.001; Table 4.5; Figure 4.8).

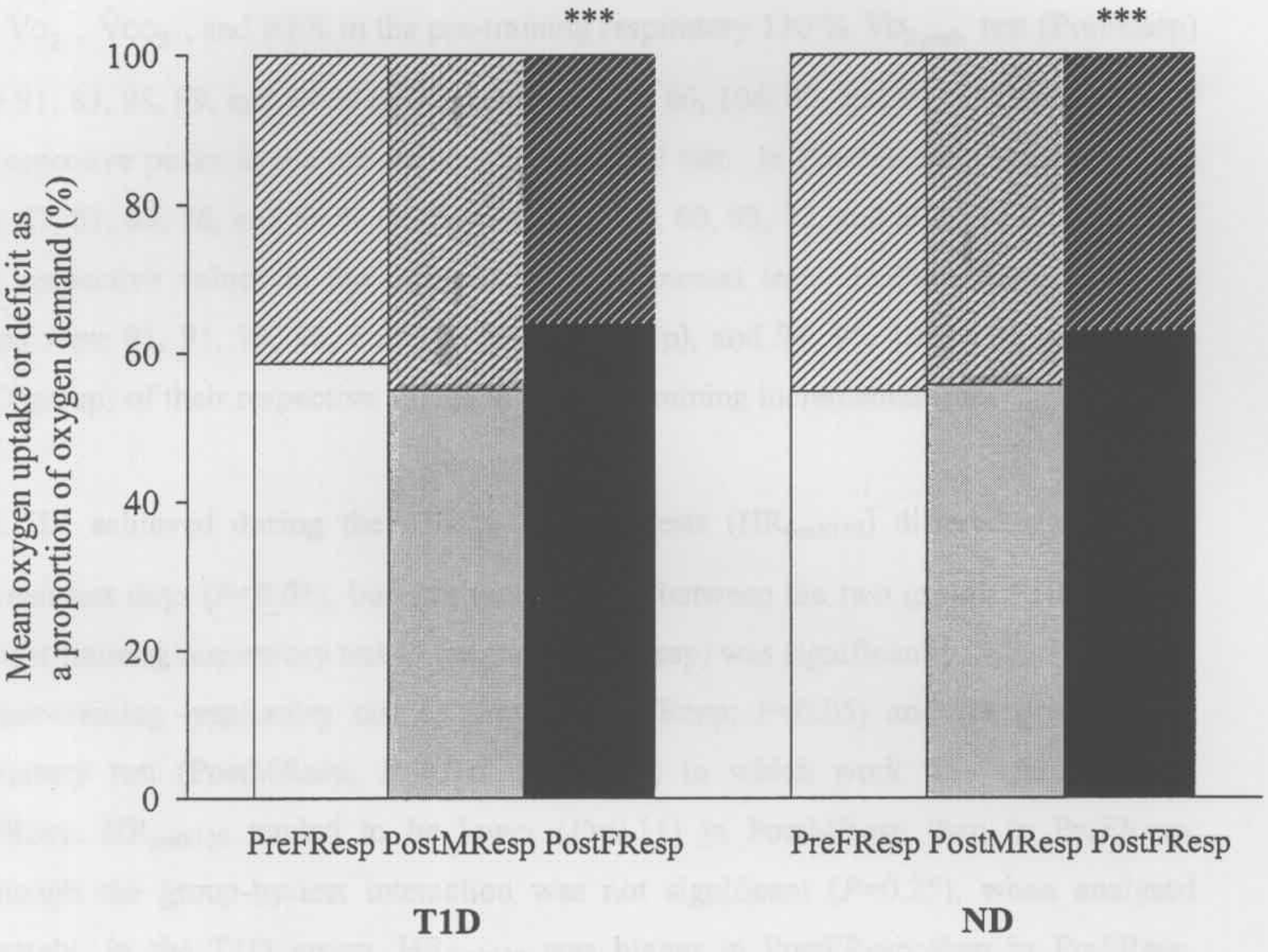


Figure 4.8 Mean oxygen uptake (solid, lower portion of bar) and oxygen deficit (hatched, upper portion of bar) expressed as a percentage of actual oxygen demand. Mean oxygen demand for the group with type 1 diabetes (T1D) was 4.29 ± 0.44 $l \cdot min^{-1}$, and for the non-diabetic group (ND) was 4.15 ± 0.36 $l \cdot min^{-1}$. Exercise was conducted to fatigue at constant power at 130 % $\dot{V}O_{2peak}$ before (PreFResp) and after training (PostFResp), or was work-matched to PreFResp after training (PostMResp). Mean oxygen uptake was significantly higher, and mean oxygen deficit significantly lower (*** $P<0.001$) in the PostFResp test than in PreFResp or PostMResp. PreFResp and PostMResp were not significantly different from each other. $n = 8$, T1D; $n = 7$, ND.

Peak values

Individual peak values for cardiorespiratory variables during the exhausting and matched-work respiratory tests appear in Appendix E4(ii). The respective peak values for HR, $\dot{V}_E$, $\dot{V}O_2$, $\dot{V}CO_2$, and RER in the pre-training respiratory 130 % $\dot{V}O_{2peak}$ test (PreFResp) were 91, 83, 98, 89, and 94 % (ND group), and 90, 80, 104, 87, and 90 % (T1D group) of the respective peaks in the pre-training incremental test. In PostMResp, the peak values were 87, 67, 88, 76, and 86 % (ND group), and 88, 60, 93, 72, and 80 % (T1D group) of their respective values in the post-training incremental test. In PostFResp, the peak values were 91, 91, 98, 98, and 108 % (ND group), and 94, 95, 103, 110, and 113 % (T1D group) of their respective values in the post-training incremental test.

Peak HR achieved during the 130 % $\dot{V}O_{2peak}$ tests ($HR_{peak130}$) differed significantly between test days ($P<0.01$), but was not different between the two groups. $HR_{peak130}$ in the post-training respiratory test to fatigue (PostFResp) was significantly higher than both the pre-training respiratory test to fatigue (PreFResp; $P<0.05$) and the post-training respiratory test (PostMResp; $P<0.01$; Table 4.6) in which work was matched with PreFResp. $HR_{peak130}$ tended to be lower ($P=0.11$) in PostMResp than in PreFResp. (Although the group-by-test interaction was not significant ($P=0.25$), when analysed separately, in the T1D group, $HR_{peak130}$ was higher in PostFResp than in PreFResp, however there was no difference between PreFResp and PostMResp; whereas in the non-diabetic group, PreFResp and PostFResp did not differ, and PostMResp was lower than in PreFResp.)

Peak expired ventilation ($\dot{V}E_{peak130}$) did not differ between the two groups, but was significantly different according to test day ($P<0.001$). $\dot{V}E_{peak130}$ was significantly lower when work was matched after training in the PostMResp test ($P<0.01$). In contrast, in the PostFResp test, $\dot{V}E_{peak130}$ was significantly higher ($P<0.001$) than in both the PreFResp and the PostMResp tests.

Table 4.6 *Peak cardiorespiratory responses during the pre- (PreFResp) and post-training (PostFResp) respiratory tests conducted to fatigue at 130 % pre-training $\dot{V}O_{2\,peak}$, and the post-training respiratory test (PostMResp) in which the work performed was matched with PreFResp.*

Variable	Group	PreFResp	PostFResp	PostMResp
$HR_{peak130}$	ND	176 ± 3	177 ± 3	170 ± 2
(beats·min^{-1}) †,§§	T1D	170 ± 5	180 ± 3	168 ± 5
$\dot{V}_{E\,peak130}$	ND	104.6 ± 10.4	129.2 ± 11.2	94.3 ± 8.1
(l·min^{-1}) ††, ‡‡‡, §§§	T1D	96.1 ± 10.3	126.1 ± 13.1	79.5 ± 7.0
$\dot{V}O_{2peak130}$	ND	3.12 ± 0.28	3.36 ± 0.36	3.03 ± 0.35
(l·min^{-1}) †,§§	T1D	3.44 ± 0.33	3.48 ± 0.32	3.14 ± 0.27
$\dot{V}CO_{2\,peak130}$	ND	3.47 ± 0.42	4.11 ± 0.41	3.20 ± 0.38
(l·min^{-1}) ††, ‡‡‡, §§§	T1D	3.52 ± 0.42	4.62 ± 0.50	3.01 ± 0.30
RER ††, ‡‡‡, §§§	ND	1.17 ± 0.07	1.37 ± 0.01	1.09 ± 0.05
	T1D	1.11 ± 0.08	1.41 ± 0.07	1.00 ± 0.07
$\dot{V}_E/\dot{V}O_2$ †, ‡‡‡, §§§	ND	35.5 ± 1.8	44.6 ± 3.2	33.3 ± 2.3
	T1D	31.6 ± 2.9	40.6 ± 3.1	27.4 ± 2.5

Values are means ± SEM. n = 7, non-diabetic group (ND); 8, type 1 diabetic group (T1D). Each of the following symbol definitions refers to exercise at 130 % pre-training $\dot{V}O_{2\,peak}$. $HR_{peak130}$, peak heart rate; $\dot{V}_{E\,peak130}$, peak expired ventilation, BTPS; $\dot{V}O_{2peak130}$, peak oxygen uptake, STPD; $\dot{V}CO_{2\,peak130}$, peak carbon dioxide output, STPD; RER, peak respiratory exchange ratio during exercise; $\dot{V}_E/\dot{V}O_2$, ventilatory equivalent for oxygen. † P<0.05, †† P<0.01, PreFResp>PostMResp; ‡ P<0.05, ‡‡‡ P<0.001, PostFResp>PreFResp; §§ P<0.01, §§§ P<0.001, PostFResp>PostMResp.

Peak oxygen consumption ($\dot{V}O_{2peak130}$) differed significantly between test days (P<0.01), but did not differ significantly between groups. $\dot{V}O_{2peak130}$ was significantly lower after training when performing the same work (PostMResp; P<0.05; Table 4.6), however, was not different to PreFResp when exercise was continued to fatigue (PostFResp). The peak oxygen consumption was greater in PostFResp than in PostMResp (P<0.01). The peak

$\dot{V}_E/\dot{V}O_2$ was different between each of the three test days (P<0.001; Table 4.6), but did not differ between groups. Peak $\dot{V}_E/\dot{V}O_2$ was lower in PostMResp (P<0.05) and higher in PostFResp (P<0.001) than in PreFResp test.

Each of the respective peaks in the respiratory exchange ratio (RER) during exercise and the carbon dioxide output ($\dot{V}CO_{2\,peak130}$) was different according to test day (P<0.001). Whilst there was no difference between groups for the RER, there was a tendency (P=0.062) for a group-by-test difference in $\dot{V}CO_{2\,peak130}$. The PostMResp test was characterised by a significantly lower (P<0.01; Table 4.6) peak RER and $\dot{V}CO_{2\,peak130}$ than in PreFResp. Peak RER and $\dot{V}CO_{2\,peak130}$ in PostFResp were significantly higher (P<0.001) than their respective values in both PostMResp and PreFResp.

Accumulated and 'mean' (normalised for exercise time) values

Individual data appear in Appendix E(7-8). Significant differences (P<0.001) were found for each of the accumulated variables with respect to test day (Table 4.7). The accumulated ventilation (litres) was higher (P<0.001) for both groups when exercising to fatigue in PostFResp than in either PreFResp or PostMResp. Conversely, when the pre-training (PreFResp) work was matched after training in PostMResp, the accumulated ventilation was significantly lower (P<0.05; Table 4.7).

The accumulated oxygen uptake (litres) was 52 and 81% higher (P<0.001; Table 4.7) when exercising to fatigue after training in PostFResp, for the ND and T1D groups, respectively. In contrast, there was no difference in accumulated oxygen uptake in the matched work tests (although tending to be lower in PostMResp for the T1D group; P=0.06). When the accumulated oxygen uptake was expressed as $mmol \cdot kg^{-1}$, the tendency for the T1D group to have a lower VO_2 in PostMResp was no longer evident.

In keeping with the greater work produced when cycling to fatigue after training (P<0.001), the accumulated oxygen deficit (litres) was significantly greater in PostFResp

compared to either PreFResp or PostMResp (P=0.001; Table 4.7), with the T1D group displaying a larger increase in oxygen deficit than the ND group (P<0.05). In contrast, the accumulated oxygen deficit was not significantly different from PreFResp when work was matched after training (PostMResp) for either group. Similar results were evident when the accumulated oxygen deficit was expressed in mmol·kg^{-1}.

The accumulated carbon dioxide output was different according to test day (P<0.001). Accumulated VCO_2 was higher in PostFResp, when more work was performed, than in either PreFResp or PostMResp (P<0.001; Table 4.7), with the increase in the T1D group being greater than in the ND group (P<0.05). Even though the work performed was identical, the accumulated VCO_2 was significantly lower in PostMResp than in PreFResp (P<0.05), with no difference between groups.

Each of the accumulated respiratory variables in the 130 % $\dot{V}O_{2peak}$ tests was divided by the exercise time, and expressed as l·min^{-1} to generate a normalised 'mean' value.

Mean HR during exercise was not different after training (PostMResp) when work was matched to PreFResp, however was higher during PostFResp (P<0.01; Figure 4.6A). Separate analysis of each group demonstrated a significantly higher mean HR in PostFResp only in the T1D group, however the group-by-test interaction did not reach significance (P=0.15).

Mean $\dot{V}_E$ tended to be lower (P=0.052) in PostMResp than in PreFResp for the ND and T1D groups, respectively, and was significantly higher than both tests in PostFResp (P<0.001; Figure 4.6A). Mean $\dot{V}CO_2$ was significantly higher in PostFResp than in PreFResp (P<0.001) in both groups. In PostMResp, mean $\dot{V}CO_2$ was significantly lower than in PreFResp (P<0.05), and considerably lower than in PostFResp (P<0.001), with the T1D group tending to show a lower mean $\dot{V}CO_2$ in the latter comparison than the ND group (P=0.052; Figure 4.6B). Mean $\dot{V}_E/\dot{V}CO_2$ was not different between PreFResp,

Table 4.7 *Accumulated respiratory data during fatiguing exercise at 130 % $\dot{V}O_{2\,peak}$ before (PreFResp) and after training (PostFResp), and during matched work exercise after training (PostMResp), in the groups with (T1D) and without (ND) type 1 diabetes mellitus.*

Variable	Group	PreFResp	PostFResp	PostMResp
Accumulated V_E	ND	75.4 ± 14.1	123.3 ± 18.2	72.0 ± 12.9
(litres) ‡‡‡, §§§, †	T1D	72.0 ± 8.5	155.2 ± 25.7	63.0 ± 8.5
Accumulated Vo_2	ND	2.42 ± 0.45	3.67 ± 0.65	2.45 ± 0.45
(litres) ‡‡‡, §§§	T1D	2.75 ± 0.26	4.97 ± 0.65	2.57 ± 0.27
Accumulated Vo_2	ND	1.51 ± 0.27	2.30 ± 0.39	1.53 ± 0.27
($mmol \cdot kg^{-1}$) ‡‡‡, §§§	T1D	1.61 ± 0.19	2.87 ± 0.33	1.53 ± 0.20
Accumulated oxygen deficit	ND	1.91 ± 0.28	2.06 ± 0.26	1.88 ± 0.28
(litres) ‡‡‡, §§§	T1D	1.98 ± 0.24	2.85 ± 0.41	2.16 ± 0.29
Accumulated oxygen deficit	ND	1.19 ± 0.16	1.30 ± 0.16	1.18 ± 0.17
($mmol \cdot kg^{-1}$) ‡‡‡, §§§	T1D	1.15 ± 0.15	1.65 ± 0.23	1.25 ± 0.16
Accumulated VCo_2	ND	2.48 ± 0.55	3.94 ± 0.67	2.37 ± 0.50
(litres) ‡‡‡, §§§ †	T1D	2.57 ± 0.34	6.11 ± 0.95	2.30 ± 0.34

Values are means ± SEM. n = 7, ND; 8, T1D. ‡‡‡ P<0.001, PostFResp>PreFResp; §§§ P<0.001, PostFResp>PostMResp; † P<0.05, PreFResp>PostMResp.

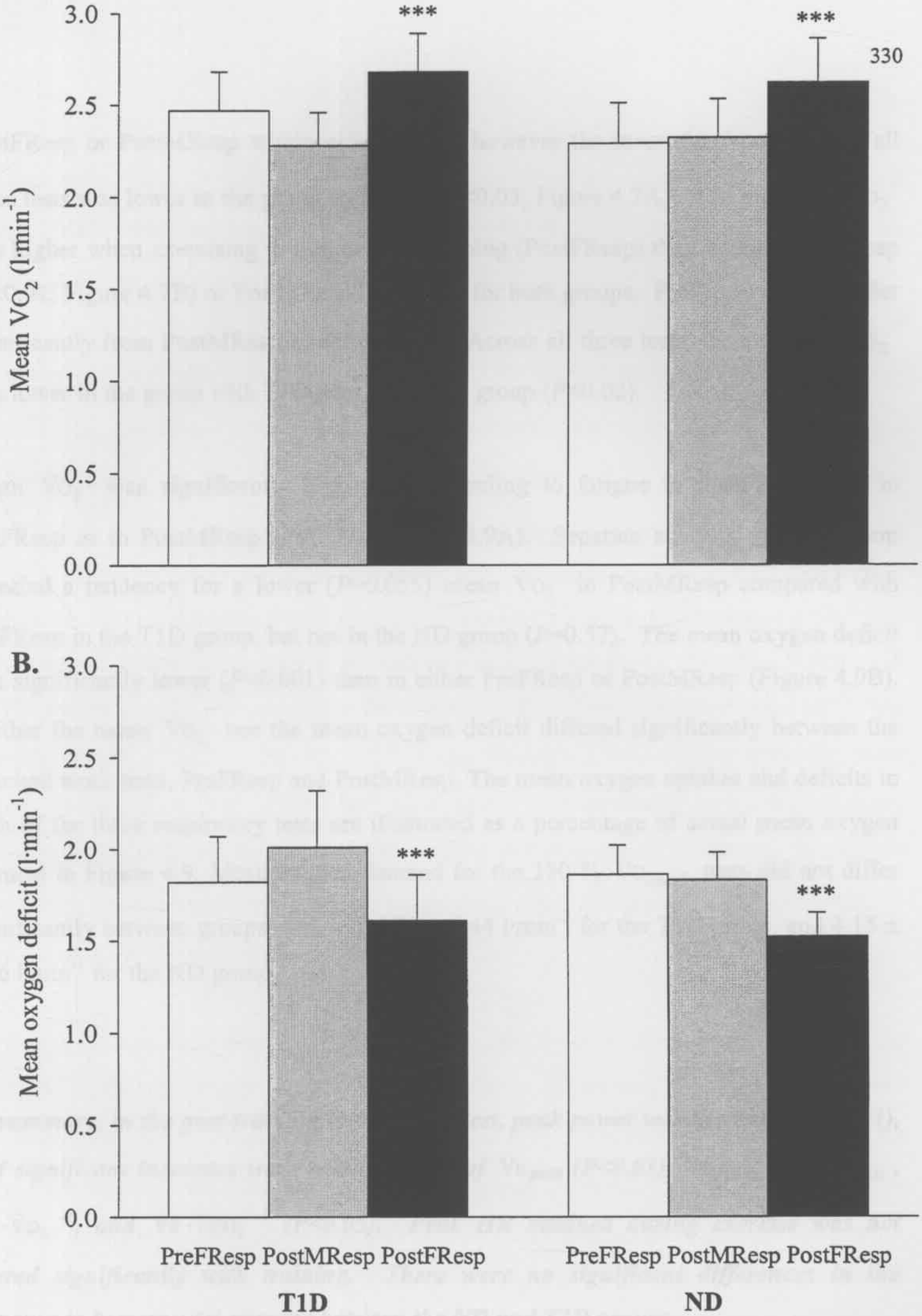


Figure 4.9 Mean (± SEM) values for (A) the oxygen uptake, and (B) the oxygen deficit, during constant power exercise at 130 % $\dot{V}O_{2peak}$, when exercising to fatigue before (PreFResp) and after training (PostFResp), and in the post-training matched work test (PostMResp), in the groups with (T1D) and without (ND) type 1 diabetes. n = 8, T1D; 7, ND. ***P<0.001, PostFResp significantly higher (mean $\dot{V}O_2$) or lower (mean oxygen deficit) than each of PreFResp and PostMResp.

PostFResp or PostMResp within either group, however the mean $\dot{V}_E/\dot{V}CO_2$ across all three tests was lower in the group with T1D ($P<0.05$; Figure 4.7A). The mean $\dot{V}_E/\dot{V}O_2$ was higher when exercising to fatigue after training (PostFResp) than in either PreFResp ($P<0.01$; Figure 4.7B) or PostMResp ($P<0.001$) for both groups. PreFResp did not differ significantly from PostMResp in either group. Across all three tests, the mean $\dot{V}_E/\dot{V}O_2$ was lower in the group with T1D than in the ND group ($P<0.02$).

Mean $\dot{V}O_2$ was significantly higher when cycling to fatigue in PostFResp than in PreFResp or in PostMResp ($P<0.001$; Figure 4.9A). Separate analysis of each group revealed a tendency for a lower ($P=0.055$) mean $\dot{V}O_2$ in PostMResp compared with PreFResp in the T1D group, but not in the ND group ($P=0.57$). The mean oxygen deficit was significantly lower ($P<0.001$) than in either PreFResp or PostMResp (Figure 4.9B). Neither the mean $\dot{V}O_2$ nor the mean oxygen deficit differed significantly between the matched work tests, PreFResp and PostMResp. The mean oxygen uptakes and deficits in each of the three respiratory tests are illustrated as a percentage of actual mean oxygen demand in Figure 4.9. Mean oxygen demand for the 130 % $\dot{V}O_{2peak}$ tests did not differ significantly between groups, and was 4.29 ± 0.44 $l \cdot min^{-1}$ for the T1D group, and 4.15 ± 0.36 $l \cdot min^{-1}$ for the ND group.

In summary, in the post-training incremental test, peak power was increased ($P<0.01$), and significant increases were noted in each of $\dot{V}E_{peak}$ ($P<0.01$), $\dot{V}O_{2peak}$, $\dot{V}CO_{2peak}$, $\dot{V}_E \cdot \dot{V}O_2^{-1}$, and $\dot{V}_E \cdot \dot{V}CO_2^{-1}$ ($P<0.05$). Peak HR attained during exercise was not altered significantly with training. There were no significant differences in the response to incremental exercise between the ND and T1D groups.

Time to fatigue ($P<0.001$) and hence total work ($P<0.001$) was increased after training in the constant power 130% $\dot{V}O_{2peak}$ test to fatigue (PostFResp). There was a tendency

for the T1D group to increase the time to fatigue (P=0.06) and total work (P=0.08) to a greater extent after training than the ND group. As a consequence of the increased $\dot{V}O_{2peak}$ *following training, the relative intensity of the 130%* $\dot{V}O_{2peak}$ *tests was slightly, but significantly reduced after training (P<0.05).*

In the respiratory tests conducted to fatigue at 130 % pre-training $\dot{V}O_{2peak}$ *(PreFResp, PostFResp), peak* $\dot{V}O_2$ *and RER were unchanged following training, whereas the respective peaks for each of HR (P<0.05),* $\dot{V}E$, $\dot{V}CO_2$, *and* $\dot{V}E \cdot \dot{V}O_2^{-1}$ *were higher (P<0.001). Accompanying these changes when exercising to fatigue after training were increases in the accumulated values of oxygen uptake, oxygen deficit, ventilation, and carbon dioxide output (P<0.001). The increase in accumulated oxygen deficit and accumulated* VCO_2 *was greater after training in the T1D group (P<0.05). When each of the variables was averaged over the duration of exercise, expressed in* $l \cdot min^{-1}$, *the means for HR (P<0.05),* $\dot{V}E \cdot \dot{V}O_2^{-1}$ *(P<0.01),* $\dot{V}E$, $\dot{V}O_2$, *and* $\dot{V}CO_2$ *(P<0.001) were higher in PostFResp than PreFResp. In contrast, and consistent with a higher mean* $\dot{V}O_2$, *was a lower mean oxygen deficit after training when exercising to fatigue (P<0.001). The relationship between mean ventilation and mean* $\dot{V}CO_2$ *(*$\dot{V}E \cdot \dot{V}CO_2^{-1}$*) was not altered as a consequence of training.*

In contrast to the results for intense exercise continued to fatigue, the post-training matched work respiratory test (PostMResp) was characterised by significantly lower peaks for $\dot{V}E$, $\dot{V}O_2$, $\dot{V}CO_2$, *and RER. The T1D group tended to display a lower peak* $\dot{V}CO_2$ *during the PostMResp test than the ND group (P=0.06). Peak HR did not differ between PreFResp and PostMResp. The accumulated and mean values for ventilation and carbon dioxide output during exercise were lower in PostMResp (P<0.05), whereas the accumulated and mean oxygen uptakes and deficits were not different to PreFResp.*

4.4.4 Blood Results

4.4.4.1 Resting measures

(i) HbA_{1c}

Glycosylated haemoglobin, A_{1c} fraction (HbA_{1c}) was 8.6 ± 0.3 % prior to training in the T1D group, and was 5.3 ± 0.1 % in the ND group ($P<0.001$). Post-training, HbA_{1c} was reduced ($P<0.05$) to 8.0 ± 0.2 % in the T1D group ($n=7$; reduced in 5 subjects, unchanged in 2 subjects), but unchanged in the ND group (Figure 4.10). One subject (JT) in the T1D group participated in the study over the Christmas period, during which she acknowledged considerable dietary indiscretion, which would have inflated her post-training HbA_{1c}. Inclusion of this subject's results (8.5% pre-training, 8.9% post-training) did not affect the group mean for the pre-training HbA_{1c}, but slightly increased the post-training mean to 8.1 ± 0.2 % and the P value to 0.09. Individual results appear in Appendix E9.

(ii) Fasting plasma glucose concentration

Fasting plasma glucose concentration ([FPG]; Appendix E9) was assayed from blood taken on a separate day to the pre-training invasive test, and primarily served to screen the ND subjects for latent diabetes. In each of the ND subjects [FPG] was below 5.4 $mmol \cdot l^{-1}$, with the means for the ND and T1D groups being 5.00 ± 0.12 $mmol \cdot l^{-1}$ and 13.26 ± 1.87 $mmol \cdot l^{-1}$, respectively ($P<0.01$). In the T1D group [FPG] did not differ from that measured in the T1D 'Resting Study' or from the resting measurement in the pre-training biopsy test (PreFB) ($P=0.87$). However, in the ND group, [FPG] tended to be lower than the resting measurement in the PreFB test ($P=0.058$), probably due to the anticipation of the biopsy procedures and the exercise test in the latter. Such an effect would also be expected in the T1D group, however possibly due to the higher [FPG] (and the factors determining such) and larger standard error, may have been masked.

A sample was also obtained after training on a separate day to the PostMB test. In the T1D group, [FPG] was similar pre- and post-training (12.52 ± 1.53 $mmol \cdot l^{-1}$, post). However, in the ND group, [FPG] was significantly higher after training (5.35 ± 0.14

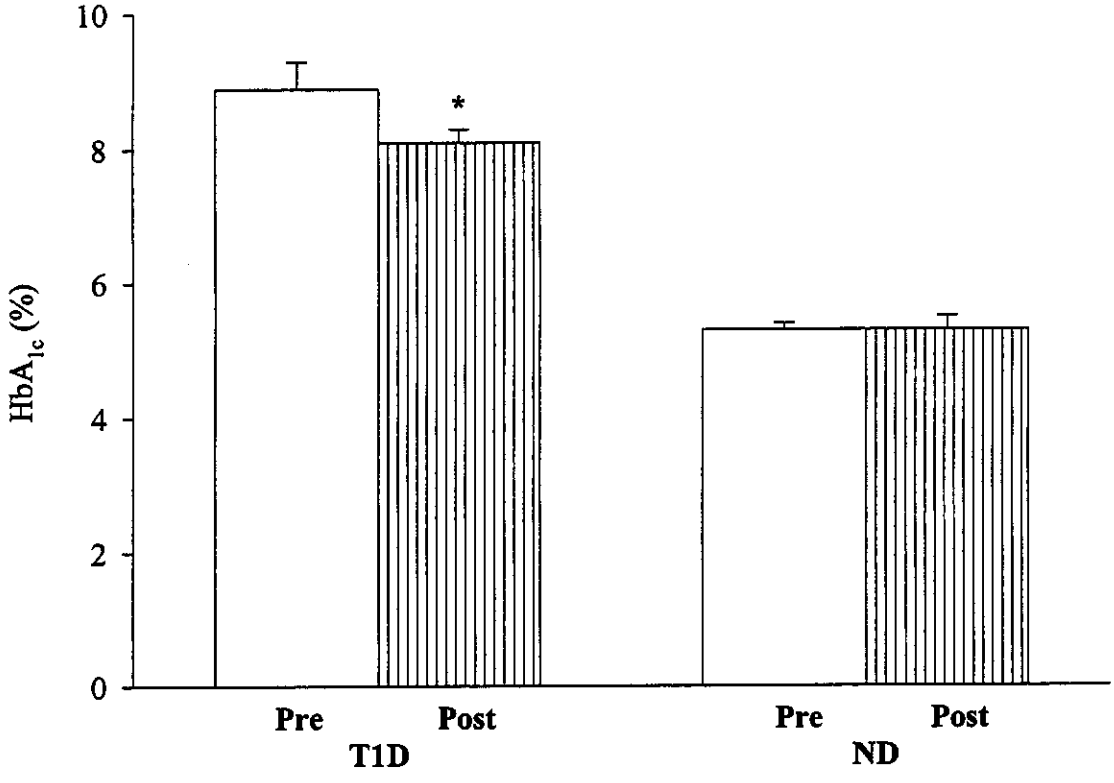


Figure 4.10 Glycosylated haemoglobin, A_{1c} fraction (HbA_{1c}, mean ± S.E.M.) in the group with type 1 diabetes (T1D, n = 7), and the non-diabetic group (ND, n = 7), measured before (Pre) and after (Post) training.
*P<0.05, Pre>Post, T1D group. Group effect, T1D>ND, P<0.001.

mmol·l^{-1}, post, $P<0.01$), although did not differ between the PreFB and PostMB tests ($P=0.83$).

(iii) Daily insulin dosage

Mean daily insulin dosage prior to training and testing, and for the seven weeks of training is illustrated in Figure 4.11A, whilst values for pre-training, weeks 3 and 7 of training, and the mean for the entire seven weeks of training are tabulated below (Table 4.8). Mean daily insulin dose was not altered by training ($P=0.76$). Individual data appear in Appendix E10.

Table 4.8 *Self-reported mean daily insulin dosage and postabsorptive blood glucose concentration: prior to training, in the third and seventh weeks of sprint training, and for the entire seven weeks of training for the group with type 1 diabetes*

		Training		
	Pre-training	Week 3	Week 7	Mean W1-7
Insulin dosage (U·day^{-1})	52.4 ± 1.5	51.6 ± 1.9	51.2 ± 1.7	51.6 ± 0.7
Fasting BGL (mmol·l^{-1})	9.9 ± 0.5	7.8 ± 0.5	8.6 ± 0.6	8.1 ± 0.2

Insulin dosage and morning fasting (postabsorptive) blood glucose level (BGL) (mean ± S.E.M.) were each averaged over one week, except the 'Mean W1-7' which represents mean values for the entire seven weeks of sprint training.

(iv) Self-monitored blood glucose concentration

For two weeks prior to commencing training or testing, and for the entire seven weeks of sprint training, blood glucose level (BGL) was monitored up to four times per day by the subjects with diabetes (Appendix E10). Of the 504 possible measurements of postabsorptive BGL (8 subjects monitoring daily for 9 weeks), 96.2% were recorded (not recorded on 8 occasions for one subject (JWH), 7 occasions for another subject (BK), 3 occasions for another (KD), and once for another subject (JT)). Postabsorptive BGL did

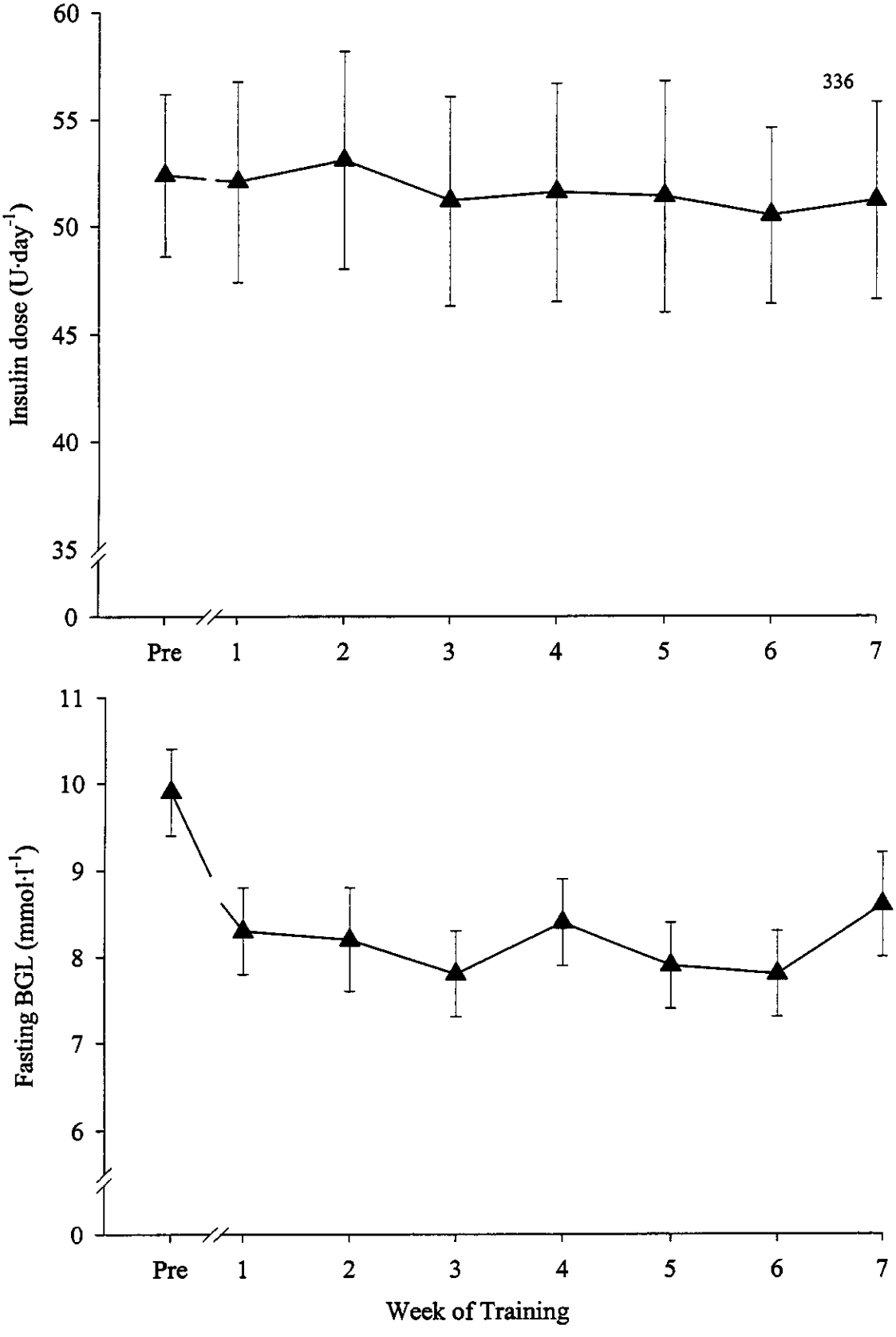


Figure 4.11 A. Average daily insulin dosage, and B. Fasting, self-monitored blood glucose level (BGL) (mean ± S.E.M.) before training or testing commenced (Pre), and for the seven weeks of sprint training. n = 8, group with type 1 diabetes. Neither daily insulin dosage nor fasting BGL differed with training, however, the latter tended to be lower (P=0.15).

not differ throughout the period of sprint training (although 13-21% lower, P=0.15; Figure 4.11B, Table 4.8). The mean postabsorptive BGL for all seven weeks of training (Table 4.8, 'Mean W1-7') tended to be lower (18%, P=0.098) than the average pre-training BGL.

The mean daily BGL (based upon 186 self-monitored BGL determinations, averaged over one week) prior to training was 9.2 ± 0.8 mmol·l^{-1} in the T1D group. The mean was 8.3 ± 0.6 mmol·l^{-1} (based on 200 determinations) by the third week of training, and 8.4 ± 0.6 mmol·l^{-1} (based on 157 determinations) in the final week of training. The difference between mean weekly values was not significant.

(v) Progesterone

Progesterone (Figure 4.12) was measured at rest in the three female subjects in each of the T1D and ND groups. In the T1D group, two of the three females were tested pre- and post-training during the follicular phase, whilst the other was tested during the luteal phase. In the ND group, two female subjects were tested during the follicular phase, however the other was tested during the follicular phase prior to training, and the luteal phase after training. Statistical procedures were not applied to the data (Appendix E9) due to the small numbers in the female sub-group.

4.4.4.2 Hormonal and metabolic response to exercise and training

(i) Insulin

Postabsorptive (overnight fasted) serum immunoreactive insulin (IRI) concentration (free insulin, T1D; total insulin, ND) did not differ between the groups, and serum IRI at rest and during exercise and recovery was not changed significantly after 7 weeks of sprint training (Appendix E11). However, the IRI response during recovery differed markedly between groups, as expected. In the ND group, total IRI concentration was slightly reduced during exercise (P<0.05), rose sharply to peak 95 % above resting values at 5 min recovery (P<0.01; Figure 4.13), then slowly declined thereafter, being similar to resting levels after 45 min recovery. In the T1D group, free IRI immediately after exercise did not differ from that of the ND group, but as expected, in marked contrast to

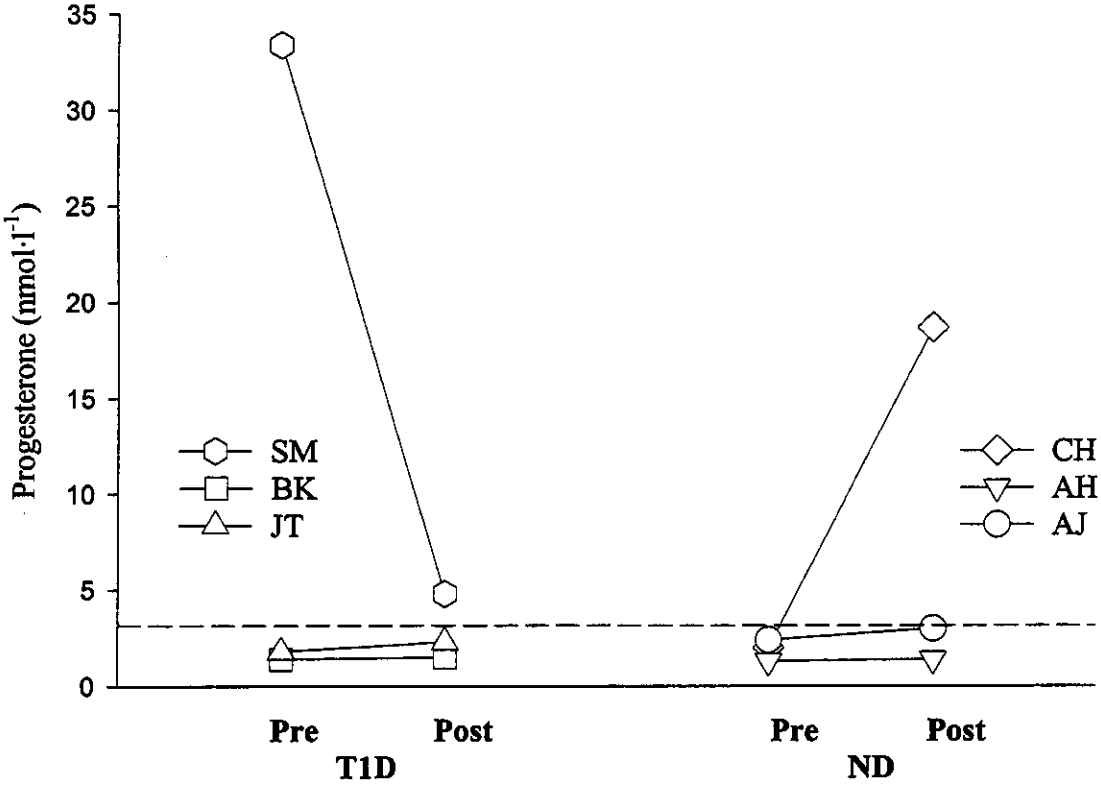


Figure 4.12 Progesterone concentration at rest, measured before and after training in the six female subjects. Three females were in the group with type 1 diabetes (T1D), and three were in the non-diabetic group (ND). The dashed reference line at 3.15 nmol·l^{-1} represents the clinical division between the progesterone concentrations in the follicular (0.6 – 3.2 nmol·l^{-1}) and luteal (3.1 – 51 nmol·l^{-1}) phases.

the non-diabetic response, IRI at 5 min recovery did not differ from resting values, then slowly fell to remain 27 % below resting values ($P<0.01$) after 60 min of recovery (Figure 4.13). Thus, the mean IRI concentration, across all times and both days, was 6.82 $mU \cdot l^{-1}$ (40.9 $pmol \cdot l^{-1}$) lower in the T1D group ($P<0.01$).

In contrast to the slow decline in free IRI concentration during recovery from the exercise tests, in the T1D Resting Study free IRI concentration did not change over time ($P=0.56$) and averaged 6.69 ± 0.30 $mU \cdot l^{-1}$ (40.1 $pmol \cdot l^{-1}$; Figure 4.14).

Within the group with type 1 diabetes, the total IRI exceeded the free IRI by a mean difference of 20.2 ± 2.3 $mU \cdot l^{-1}$ ($P<0.05$), however, the response over time and with training did not differ between total and free IRI concentrations (Appendix E11).

(ii) Glucagon

Immunoreactive glucagon (IRG) did not change significantly during exercise, however it rose by ~10 % after 5 min recovery ($P<0.05$), returned to resting levels after 20 min, then slowly rose again for the remainder of recovery, reaching a value ~17 % higher than at rest after 60 min ($P<0.05$; Figure 4.15A, B). There was no difference between the T1D and ND groups, nor was there an effect of training on the IRG response to exercise; although in PostMB there was no difference between resting and 60 min recovery values. Individual data are in Appendix E11.

(iii) Immunoreactive glucagon-to-insulin molar ratio

The molar ratio of glucagon-to-insulin (IRG/IRI; free insulin in the T1D group) increased with exercise in both groups ($P<0.05$; Figure 4.16). The IRG/IRI ratio declined in early recovery, then slowly rose towards resting values in the ND group, however, in the T1D group, the ratio remained elevated in early recovery and continued to rise in later recovery ($P<0.01$). Whilst there was a time-by-group interaction ($P<0.01$), the changes in the IRG/IRI ratio did not differ after training. Across both days and all times, the IRG/IRI was 0.71 higher in the group with type 1 diabetes ($P<0.05$).

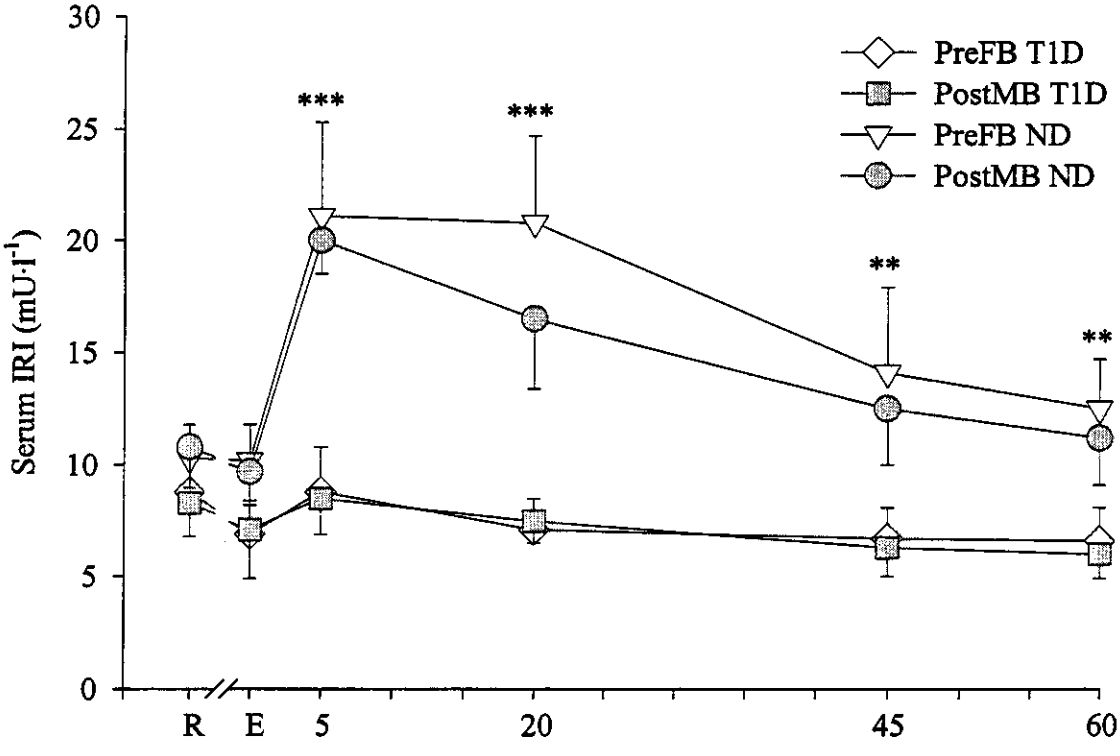


Figure 4.13 Serum immunoreactive insulin (IRI) concentration (mean ± S.E.M.) at rest (R), immediately after exercise (E), and during recovery (5, 20, 45, 60 min) in the pre-training test conducted to fatigue at 130 % $\dot{V}O_{2peak}$ (PreFB), and in the post-training matched work test (PostMB). n = 8, group with type 1 diabetes (T1D), except at E and 20 (n = 7); n = 7, non-diabetic group (ND). Resting insulin concentrations did not differ between groups, or with training. Significant main effects for time (P<0.001) and group (P<0.01) were found, and the group-by-time interaction was significant (P<0.001). ***P<0.001, **P<0.01, ND>T1D.

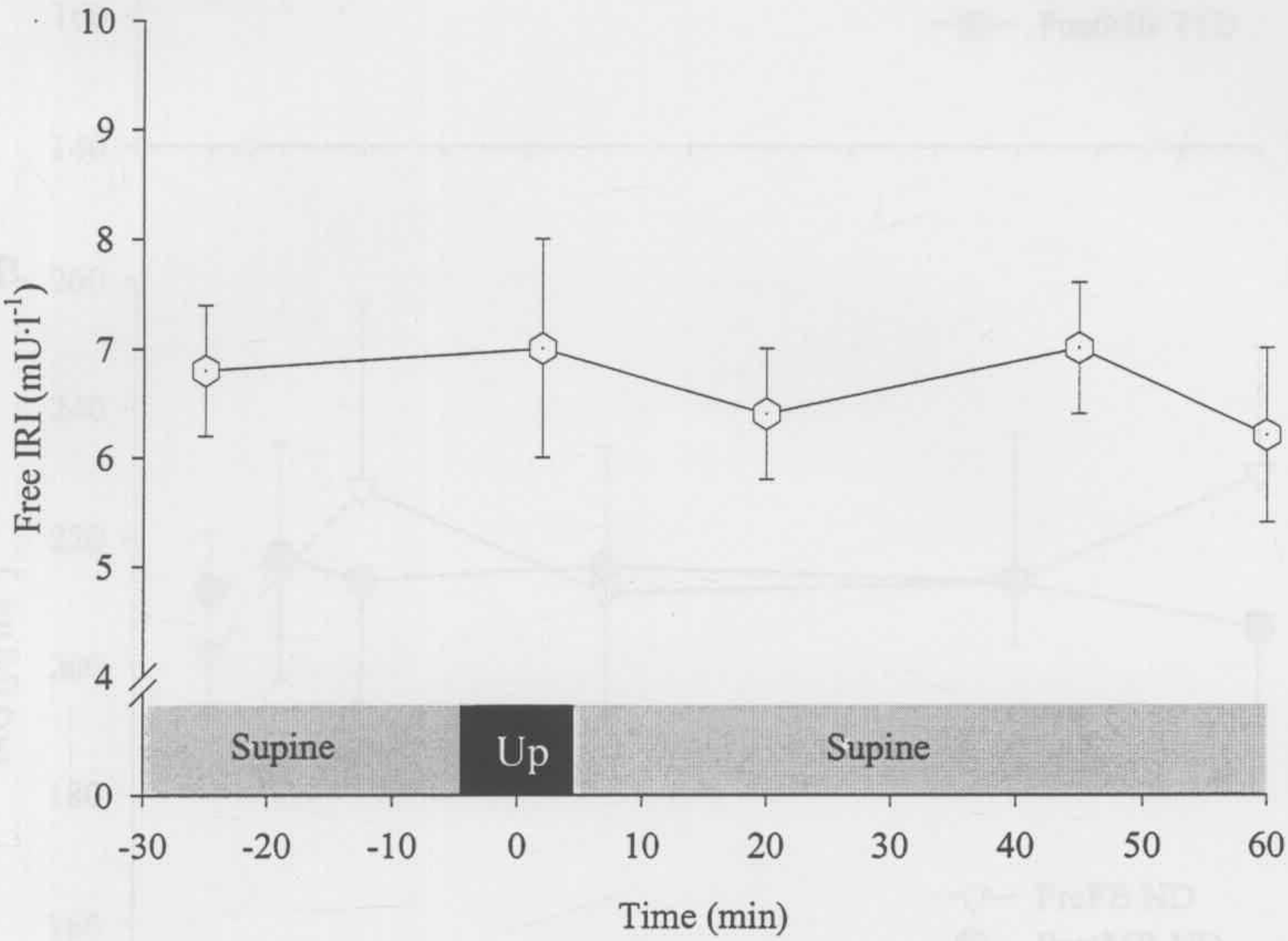


Figure 4.14 Free immunoreactive insulin (IRI) concentration (mean ± S.E.M.) in the group with type 1 diabetes (n = 8) during the 'Resting Study', in which no exercise was performed; however posture mimicked that of the invasive tests (PreFB, PostMB). Posture is indicated by the solid bars above the x-axis. Supine, lying supine on a plinth; Up, seated upright on the cycle ergometer.

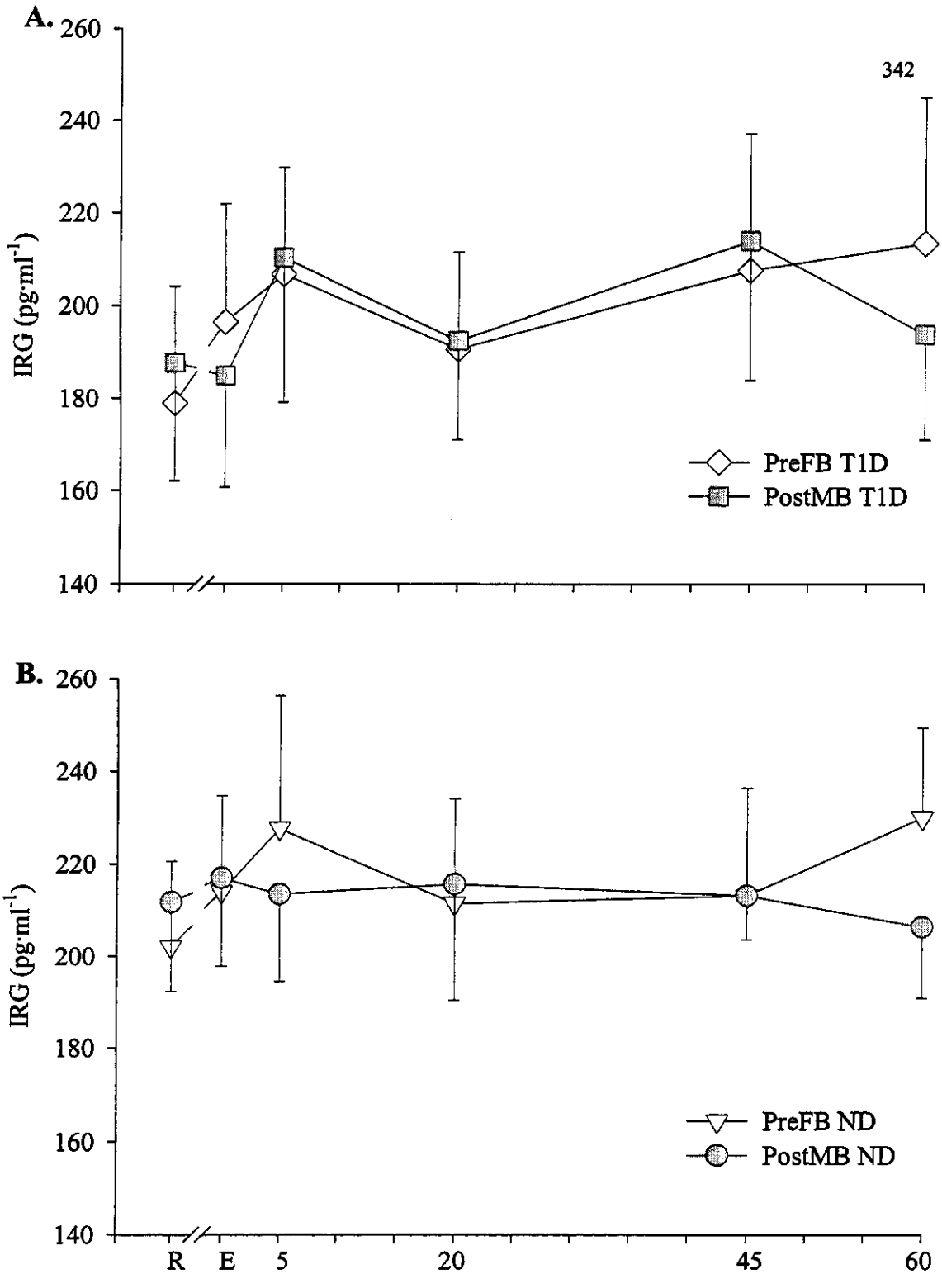


Figure 4.15 Immunoreactive glucagon (IRG) concentration (mean ± S.E.M.) at rest (R), immediately after exercise (E), and during recovery (5, 20, 45 60 min) in the pre-training invasive test conducted to fatigue at 130 % $\dot{V}O_{2peak}$ (PreFB), and in the post-training matched work test (PostMB). n = 8, group with type 1 diabetes (T1D; graph A); n = 7, non-diabetic group (ND; graph B). There was no effect of training on the IRG response to exercise, and no significant difference between groups. See text for description of the main effect of time (P=0.055).

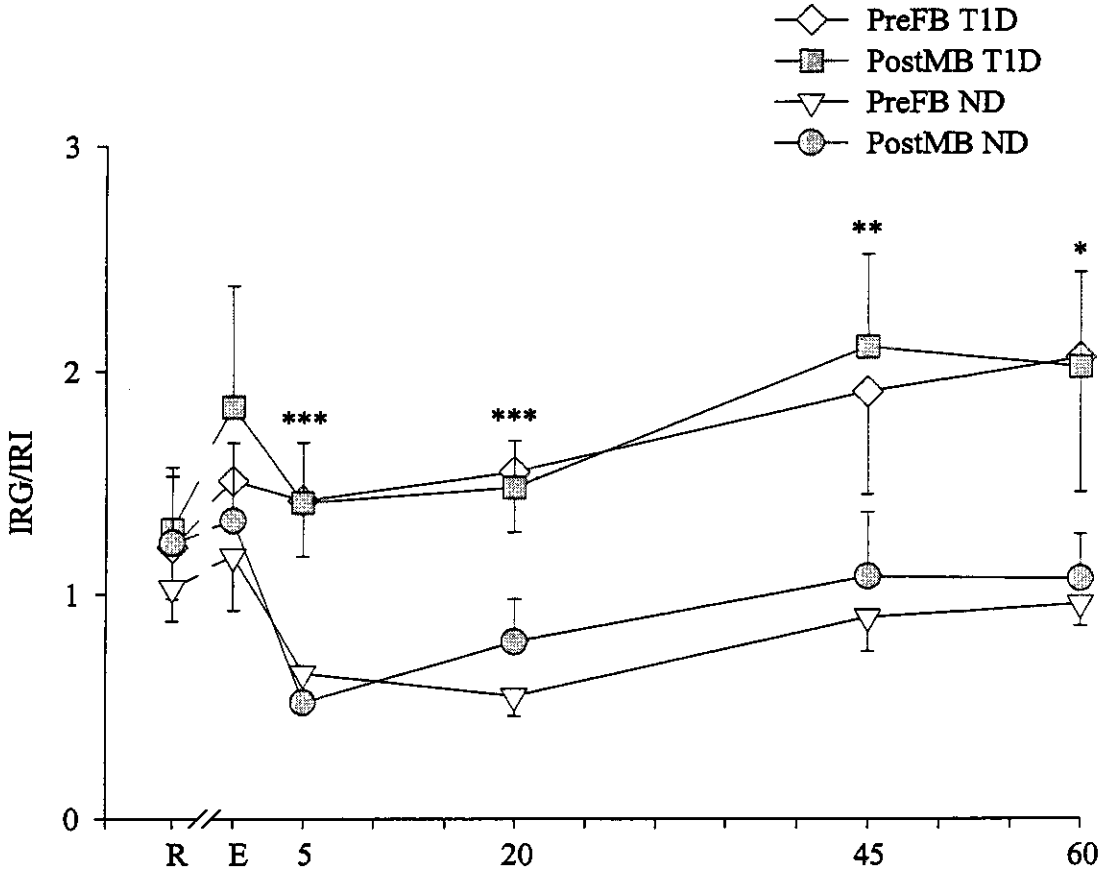


Figure 4.16 Immunoreactive glucagon-to-insulin molar ratio (IRG/IRI) (mean ± S.E.M.) at rest (R), immediately after exercise (E), and during recovery (5, 20, 45, 60 min) in the pre-training test conducted to fatigue at 130 % $\dot{V}O_{2peak}$ (PreFB), and in the post-training matched work test (PostMB). n = 8, group with type 1 diabetes (T1D), except at E and 20 (n = 7); n = 7, non-diabetic group (ND). Resting IRG/IRI did not differ between groups. Significant main effects for time (P<0.001) and group (P<0.05) were found, and the group-by-time interaction was significant (P<0.01). ***P<0.001, **P<0.01, *P<0.05, T1D>ND.

(iv) Noradrenaline

Plasma noradrenaline concentration ([NAdr]) rose sharply with exercise, peaked after 1 min recovery ($P<0.001$), then declined to resting levels after 60 min recovery (Figure 4.17A,B). After training, mean plasma [NAdr] tended to be lower (16%; $P=0.068$). Immediately after matched-work exercise, plasma [NAdr] was 7% lower, and in recovery was 13–26% lower after training. The group with type 1 diabetes (Figure 4.17A) attained a higher [NAdr] than the ND group (Figure 4.17B) immediately after exercise ($P<0.05$; group-by-time interaction) and tended to be higher at 2 min recovery ($P=0.08$); this effect was not altered by training. Individual catecholamine data appear in Appendix E11.

(v) Adrenaline

Adrenaline concentration ([Adr]) rose significantly with exercise, peaked at 1 min of recovery ($P<0.001$), then returned to resting levels by 10 min recovery (Figure 4.18A,B). Although [Adr] tended to be higher in the T1D group ($P=0.11$), there was no significant difference between the two groups, nor was there a significant effect of training on [Adr] during exercise and recovery, although peak [Adr] occurred immediately after exercise in PostMB, and a tendency for a training-by-time interaction was found ($P=0.09$), with a 31-32 % reduction in 1 and 2 min recovery concentrations after training.

(vi) Glucose

Plasma glucose concentration ([PG]) (Appendix E11) was higher at rest, and at every other measurement time in the T1D group than in the ND group ($P<0.001$; Figure 4.19), however resting concentrations did not differ within each group in the PreFB and PostMB tests.

Prior to training, in PreFB, [PG] rose sharply after exercise and peaked at 6.42 ± 0.20 $mmol \cdot l^{-1}$ after 5 min recovery, then declined for the remainder of recovery in the ND group. In the T1D group, [PG] rose sharply with exercise, and as expected, in stark contrast to the ND response ($P<0.001$), continued to rise, unabated throughout the 60 min recovery period. One subject in the T1D group (BK) had a plasma glucose response that contrasted with all other subjects (in either group). Plasma glucose slowly fell, or

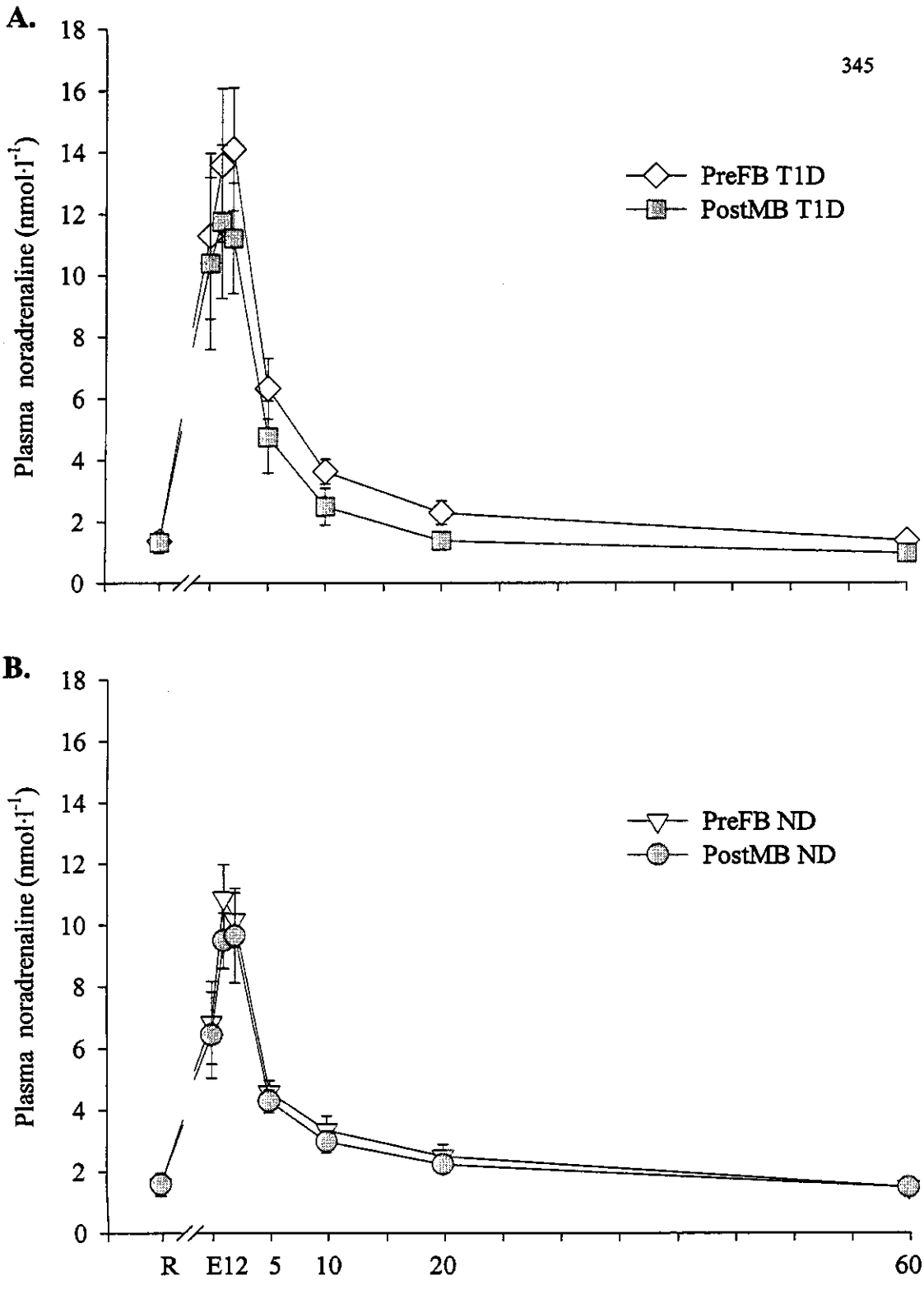


Figure 4.17 Plasma noradrenaline concentration (mean ± S.E.M.) at rest (R), immediately after exercise (E), and during recovery (1, 2, 5, 10, 20, 60 min) in the invasive 130 % $\dot{V}O_{2peak}$ pre-training test to fatigue (PreFB), and in the post-training matched-work test (PostMB), in subjects with (T1D; graph A) and without (ND; graph B) type 1 diabetes. $n = 8$, T1D, except at E, 1, 2 ($n = 7$); $n = 7$, ND, except at 1 ($n = 6$).

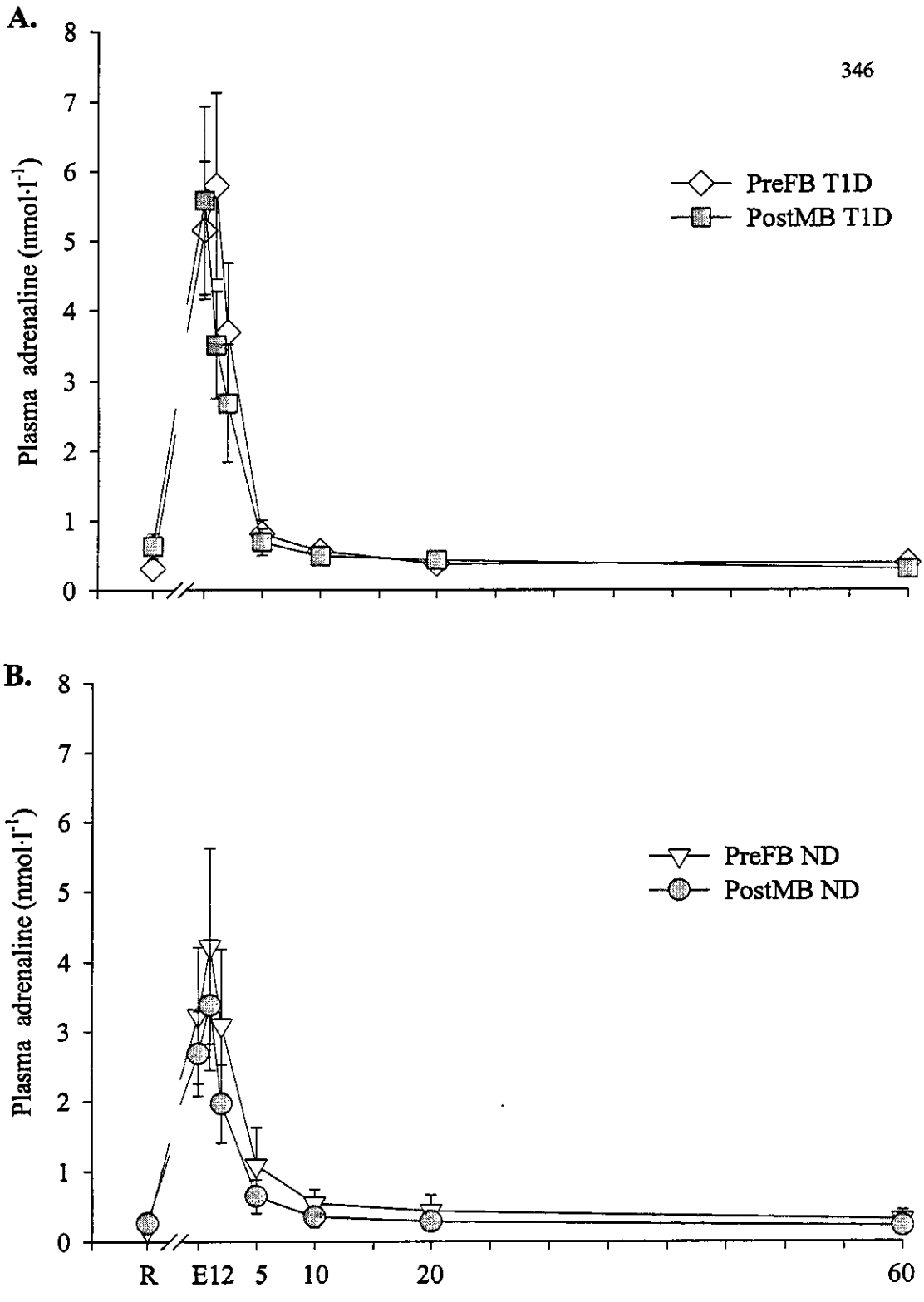


Figure 4.18 Plasma adrenaline concentration (mean ± S.E.M.) at rest (R), immediately after exercise (E), and during recovery (1, 2, 5, 10, 20, 60 min) in the pre-training 130 % $\dot{V}O_{2peak}$ invasive test to fatigue (PreFB), and in the post-training matched-work test (PostMB). (A) Group with type 1 diabetes (T1D); (B) Non-diabetic group (ND). n = 7, T1D, except at 1 (n = 5); n = 7, ND. A significant main effect for time was found (see text). No other significant differences were evident either between or within groups, although there was a tendency for a training-by-time interaction effect (P=0.09).

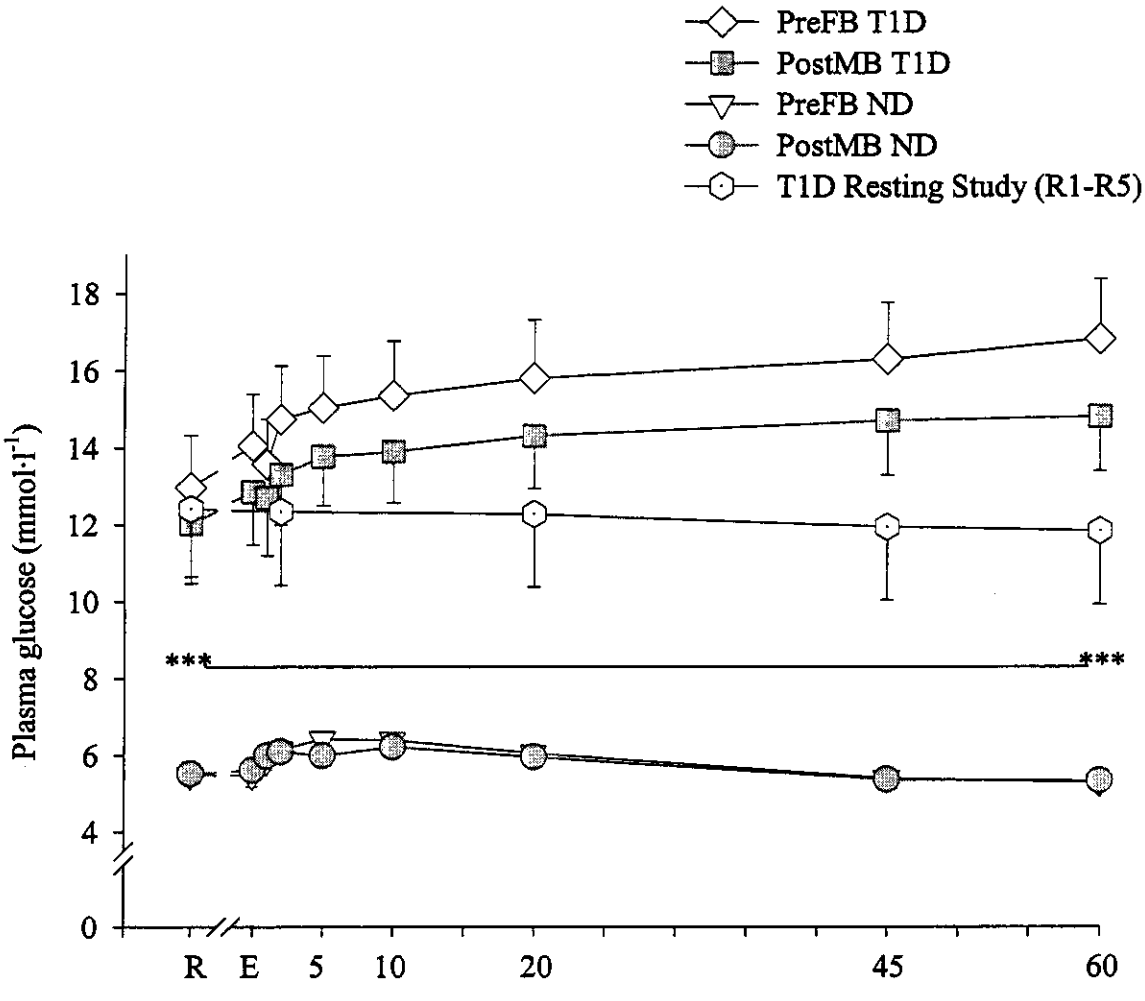


Figure 4.19 Plasma glucose concentration (mean ± S.E.M.): at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 10, 20, 45, 60 min) during the pre-training test to fatigue at 130 % $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB). n = 7, T1D (group with type 1 diabetes); n = 7, ND (non-diabetic group). A main effect for time (P<0.001) was evident (see text). The effect for group (P<0.001) and the time-by-group interaction (P<0.001) was significant. ***—***P<0.001, T1D>ND at every time point.

Also shown is the separate 'Resting Study' conducted in the T1D group. Sample times corresponded to rest (R1), 2 (R2), 20 (R3), 45 (R4), and 60 min recovery (R5) in the PreFB and PostMB tests.

remained unchanged, for the duration of the study, appearing unaffected by exercise (in keeping with a free insulin level 2-3-fold higher than that of the rest of the T1D group, an almost absent adrenaline response to exercise, and low free fatty acids). After training in the matched-work test (PostMB), the mean rise in [PG] from rest was attenuated by 26 and 22 % (mean difference, 0.10 ± 0.07 $mmol \cdot l^{-1}$, ND; 0.51 ± 0.20 $mmol \cdot l^{-1}$, T1D) in the ND and T1D groups, respectively, however this was not significantly different to pre-training (P=0.18; Figure 4.19).

Plasma glucose concentration at rest did not differ between the PreFB and PostMB tests and the T1D Resting Study. However, in marked contrast (P<0.001) to the progressive hyperglycaemia induced by exercise, [PG] fell slightly during the T1D Resting Study (P<0.05; Figure 4.19).

(vii) Free fatty acids (FFA)

Plasma FFA concentration ([FFA]) (Appendix E11) was reduced immediately after exercise and remained below resting levels (P<0.01) for the duration of recovery in the ND group, and until 45 min recovery in the T1D group (group-by-time interaction, P<0.001). Mean plasma [FFA] was 0.35 $mmol \cdot l^{-1}$ higher in the T1D group (P<0.01), and [FFA] was significantly higher at each time point compared to the ND group (Figure 4.20A). The nadir in [FFA] occurred after 5 and 45 min for the T1D and ND groups, respectively. Training did not influence the [FFA] at rest, during exercise, or in recovery. Combining pre- and post-training data, [FFA] tended to correlate inversely with plasma [Lac^-] in the T1D group (r=-0.25, P=0.08), whereas no correlation was evident in the ND group (r=-0.20, P=0.20).

Free fatty acid concentration in the T1D Resting Study was similar to that at rest in the invasive tests. However, in contrast to the [FFA] response in the PreFB and PostMB tests, in the separate resting study, [FFA] was not significantly altered during the time period of testing, although tended to fall (P=0.10; Figure 4.20B).

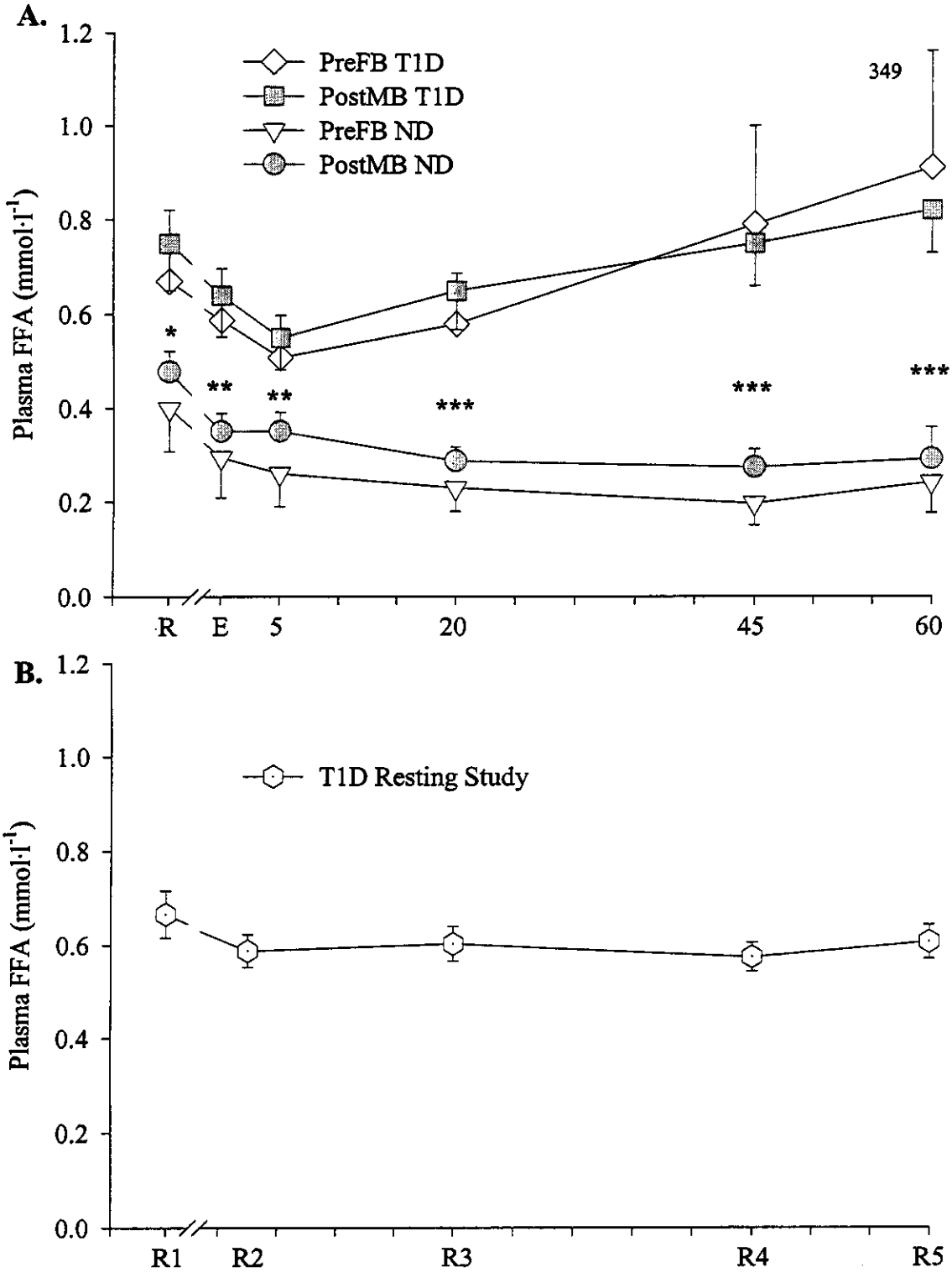


Figure 4.20 Plasma free fatty acid concentration (FFA; mean ± S.E.M.). (A) At rest (R), immediately after exercise (E), and in recovery (5, 20, 45, 60 min) during the pre-training test to fatigue at 130 % $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB). n = 8, T1D (group with type 1 diabetes); n = 7, ND (non-diabetic group). A main effect for time (P<0.001) was evident. The effect for group (P<0.01) and the time-by-group interaction (P<0.001) was significant. *P<0.05, **P<0.01, ***P<0.001, T1D>ND.

(B) During the separate 'Resting Study' conducted in the T1D group. Sample times corresponded to rest (R1), 2 (R2), 20 (R3), 45 (R4), and 60 min recovery (R5) in the PreFB and PostMB tests.

4.4.4.3 Electrolyte response to exercise and training

(i) Lactate

Plasma lactate concentration ([Lac^-]) increased ~ 5-fold with exercise, peaked at 15.85 ± 0.94 $mmol \cdot l^{-1}$ after 5 min recovery, then slowly declined thereafter, remaining above resting values at 60 min recovery ($P<0.001$; Figure 4.21). After training, in PostMB, plasma [Lac^-] was lower by a mean difference of 1.27 ± 0.23 $mmol \cdot l^{-1}$ ($P<0.02$), with no difference between groups. Plasma [Lac^-] was lower from 5 through to 60 min recovery in PostMB, compared to PreFB ($P<0.001$). Individual data appear in Appendix E12.

In the T1D Resting Study, plasma [Lac^-] increased significantly at R2 ($P<0.05$), after subjects had moved to an upright position (from the R1 supine position), then decreased thereafter (after resumption of the supine position) to be significantly below R1 at both R4 and R5 ($P<0.05$; Figure 4.21).

(ii) Potassium

Plasma [K^+] rose abruptly to peak immediately after exercise ($P<0.001$), then fell below resting values after 5 min of recovery ($P<0.05$). Plasma [K^+] then continued to rise in the PreFB test in the T1D group, to be 10% above the resting value at 60 min recovery, which differed from the ND group ($P<0.05$; Figure 4.22A, B), in whom the 60 min recovery plasma [K^+] was similar to the resting concentration. After training, during the matched-work exercise test (PostMB), plasma [K^+] was lower by a mean difference of 0.17 ± 0.03 $mmol \cdot l^{-1}$ ($P<0.02$); and was specifically lower immediately after exercise, and at 1 ($P<0.01$), 2, and 60 min of recovery in PostMB ($P<0.05$), with no difference between groups. The rise in plasma [K^+] with exercise ($\Delta[K^+]$, exercise – rest value) was reduced 25% ($P<0.01$) after training in PostMB, with no difference between groups. Individual data appear in Appendix E12.

In the T1D Resting Study, plasma [K^+] through R2 - R5 was not different to R1, and resting plasma [K^+] in the T1D group did not differ from that of the PreFB and PostMB tests (Figure 4.22B). However, comparison of plasma [K^+] in the T1D Resting Study and the pre- (PreFB) and post-training (PostMB) exercise tests, revealed a significant

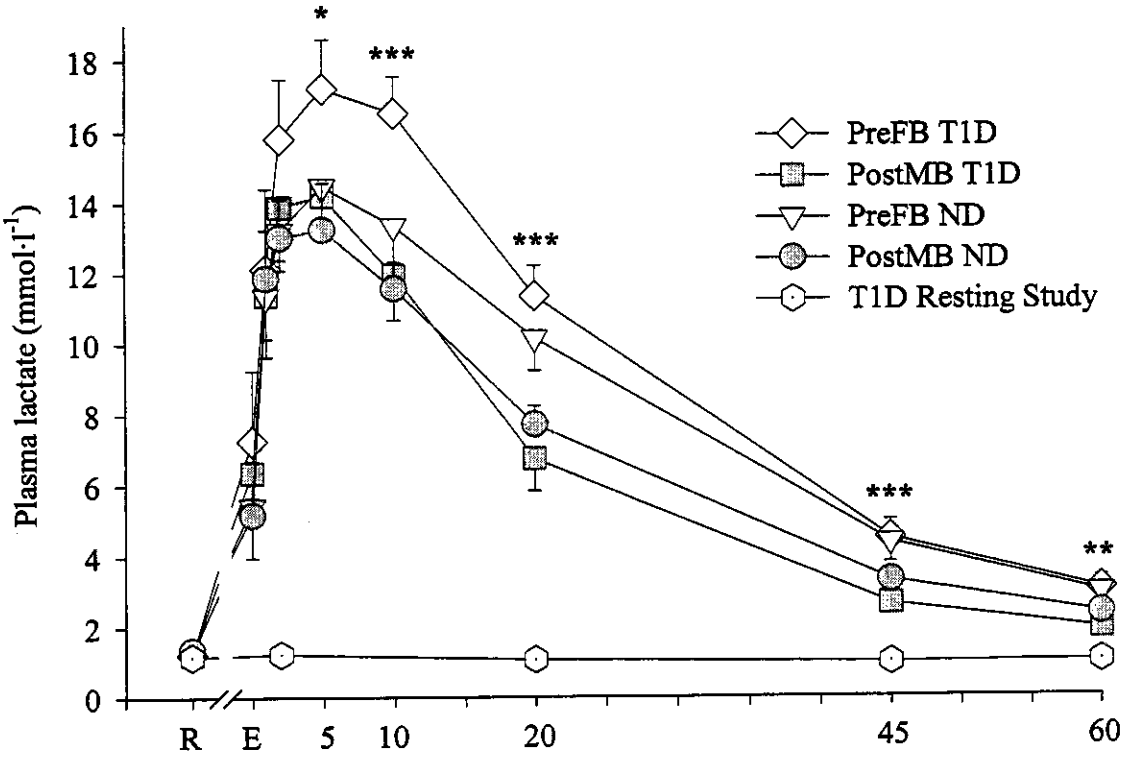


Figure 4.21 Plasma lactate concentration (mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 10, 20, 45, 60 min) during the pre-training test to fatigue at 130 % $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB). n = 8, T1D (group with type 1 diabetes); n = 7, ND (non-diabetic group). Main effects for training status (P<0.02) and time (P<0.001) were evident. *P<0.05, **P<0.01, ***P<0.001, training status-by-time interaction, PreFB>PostMB.
Also shown is plasma lactate concentration for the separate 'Resting Study' conducted in the T1D group. Sample times corresponded to rest (R1), 2 (R2), 20 (R3), 45 (R4), and 60 min recovery (R5) in the PreFB and PostMB tests.

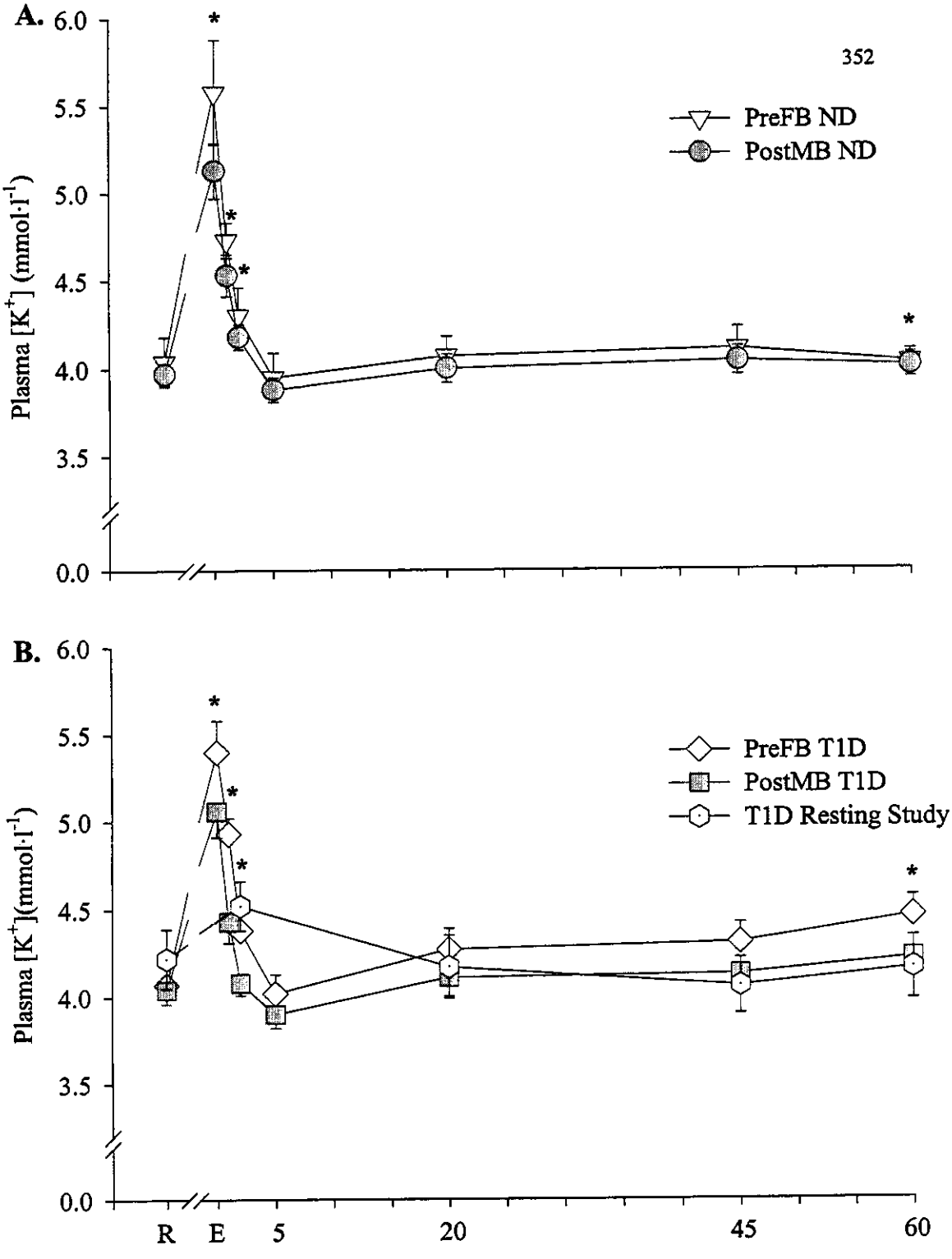


Figure 4.22 Plasma potassium concentration ([K^+]; mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 20, 45, 60 min) during the pre-training test to fatigue at 130 % $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB) in the (A) non-diabetic group (ND), n = 7 (except 2, 5, and 20 min, where n = 6); and (B) group with type 1 diabetes (T1D), n = 8 (except at 1, 2, and 20 min, where n = 7). Main effects for training status (P<0.05) and time (P<0.001) were evident. *P<0.05, training status-by-time interaction, PreFB>PostMB. No group differences were found.

Also shown on graph B is the separate 'Resting Study' for the T1D group. Sample times in the resting study corresponded to rest (R1), 2 (R2), 20 (R3), 45 (R4), and 60 min recovery (R5) in the PreFB and PostMB tests.

difference with time across the three days ($P<0.02$). Plasma [K^+] was significantly higher at 45 ($P<0.05$) and 60 min ($P<0.01$) of recovery in the PreFB test, compared to the Resting Study, however, in PostMB, the 45 and 60 min recovery values did not differ.

(iii) Sodium

Plasma sodium concentration ([Na^+]) at rest did not differ between groups or with training (Figure 4.23). In the T1D group, both before and after training, plasma [Na^+] was lower than in the ND group at 45 and 60 min of recovery ($P<0.05$). After training, in both groups, plasma [Na^+] was lower at 2 and 5 min of recovery ($P<0.05$).

In the T1D Resting Study, plasma [Na^+] did not change over time ($P=0.83$; Figure 4.23).

(iv) Chloride

Plasma chloride concentration ([Cl^-]) peaked immediately after exercise, fell below resting values by 5 min recovery ($P<0.001$) and was not different after training (Figure 4.24). In the T1D group, plasma [Cl^-] was 2.0 $mmol \cdot l^{-1}$ lower across both days and times ($P<0.02$).

4.4.4.4 Acid-base response to exercise and training

(i) Strong ion difference

The plasma strong ion difference (SID) fell following exercise to reach a nadir at 5 min recovery ($P<0.001$; Figure 4.25) and then rose to be similar to resting values by 45 min recovery. There was no difference in SID between groups or with training.

(ii) Arterialised venous carbon dioxide tension (PCO_2)

Mean PCO_2 did not differ immediately after exercise, however from 2–60 min of recovery it was lower than resting values ($P<0.001$). Mean PCO_2 across both tests was 37.1 ± 0.5 mmHg in the ND group, and was 4.6 mmHg higher in the T1D group ($P=0.01$; Figure 4.26B). A significant interaction effect for training status-by-time ($P<0.01$) was found, but no specific points were detected with *post hoc* tests.

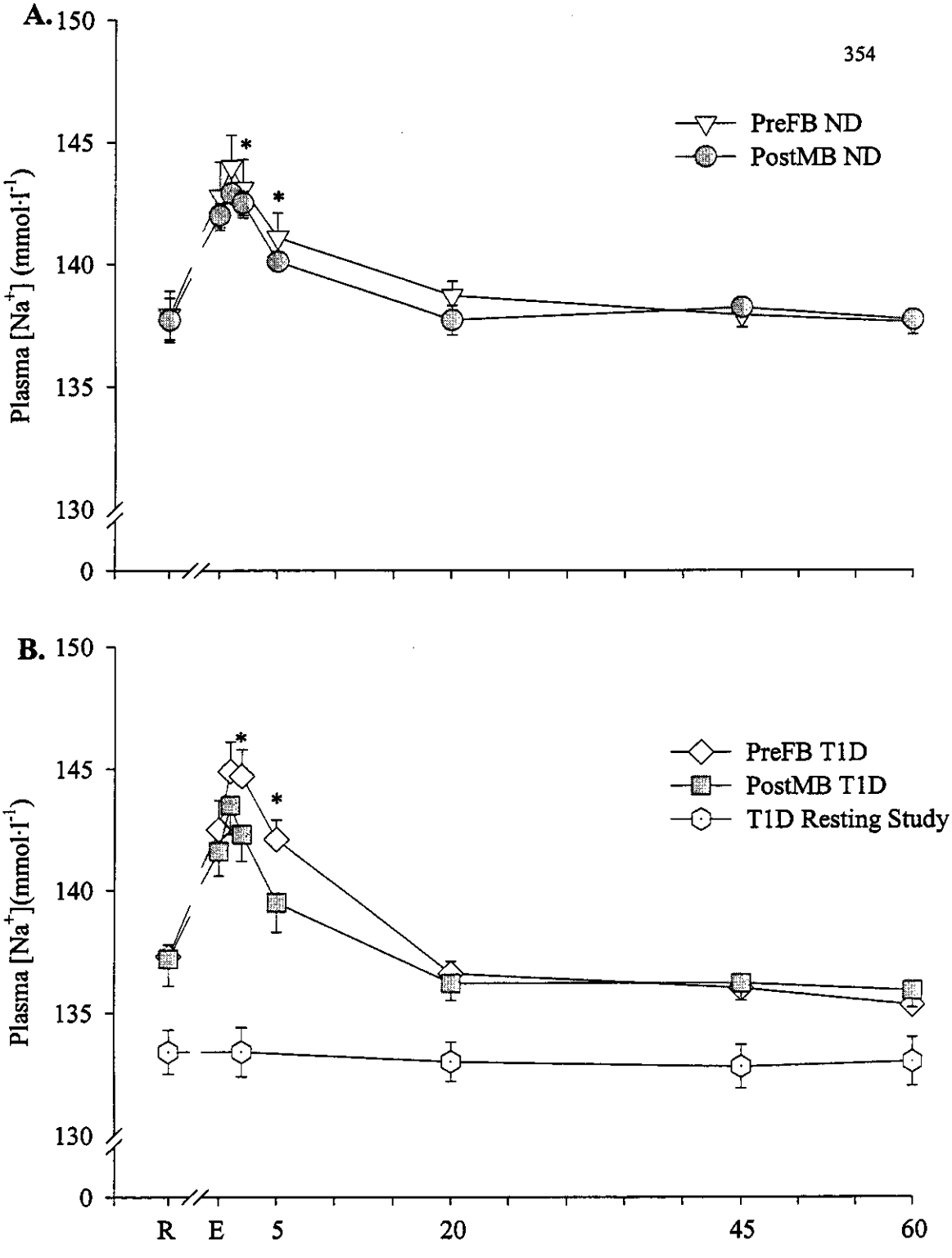


Figure 4.23 Plasma sodium concentration ($[Na^+]$; mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 20, 45, 60 min) during the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB) in the (A.) non-diabetic group (ND), $n = 6$ (except 2, 5, and 20 min, where $n = 5$); and (B.) group with type 1 diabetes (T1D), $n = 8$ (except at 1, 2, and 20 min, where $n = 7$). A main effect for time ($P<0.001$), and a training status-by-time interaction, PreFB>PostMB (*$P<0.05$) was found. Additionally, the T1D group had lower plasma $[Na^+]$ at 45 and 60 min recovery (group-by-time effect, $P<0.05$).
Also shown on graph B is the separate 'Resting Study' for the T1D group. Sample times in the resting study corresponded to rest (R1), 2 (R2), 20 (R3), 45 (R4), and 60 min recovery (R5) in the PreFB and PostMB tests.

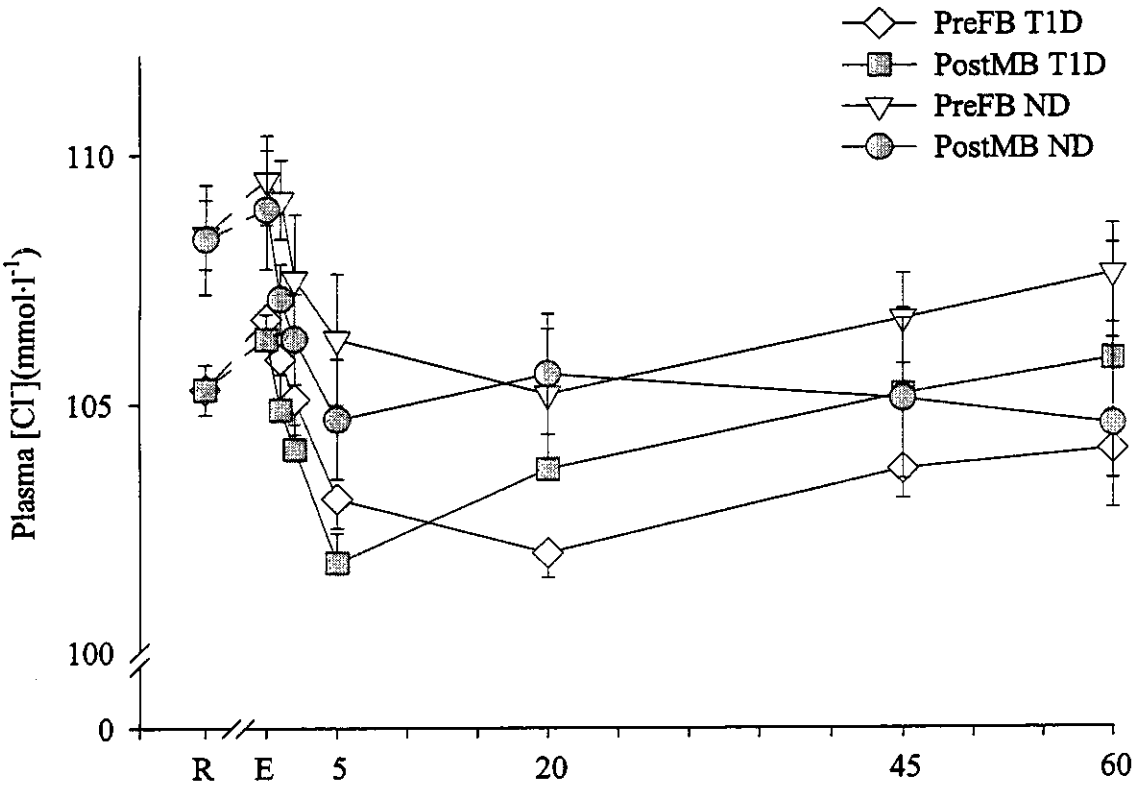


Figure 4.24 Plasma chloride concentration ([Cl^-]; mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 20, 45, 60 min) during the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB) in the non-diabetic group (ND), $n = 6$ (except 2, 5, and 20 min, where $n = 5$); and the group with type 1 diabetes (T1D), $n = 8$ (except at 1, 2, and 20 min, where $n = 7$). A main effect for time ($P<0.001$) and group (ND>T1D, $P<0.02$) was found.

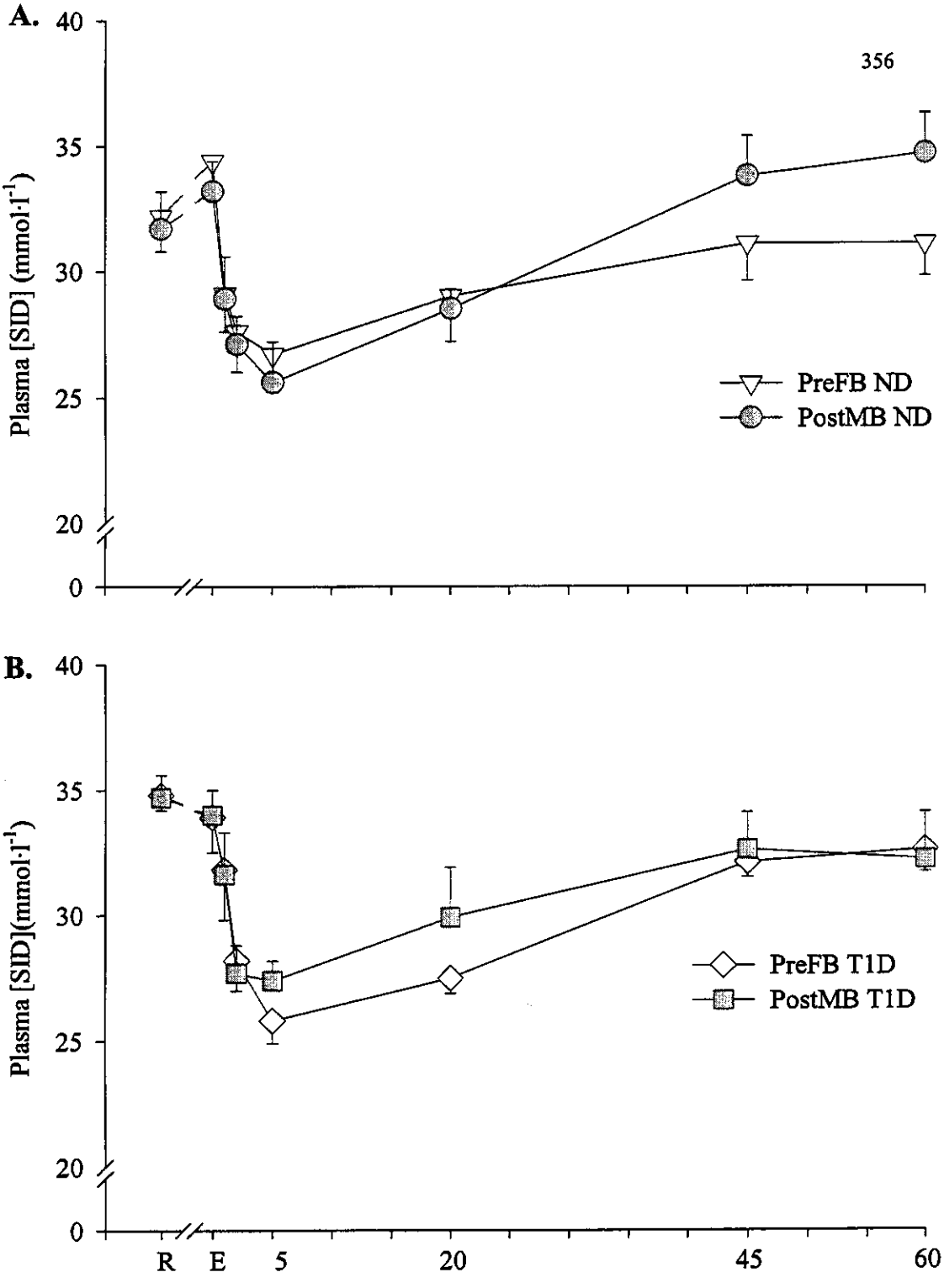


Figure 4.25 Plasma strong ion difference ([SID]; mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 20, 45, 60 min) during the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB) in the (A.) non-diabetic group (ND), $n = 6$ (except 2, 5, and 20 min, where $n = 5$); and (B.) group with type 1 diabetes (T1D), $n = 8$ (except at 1, 2, and 20 min, where $n = 7$). A main effect for time ($P<0.001$) was found.

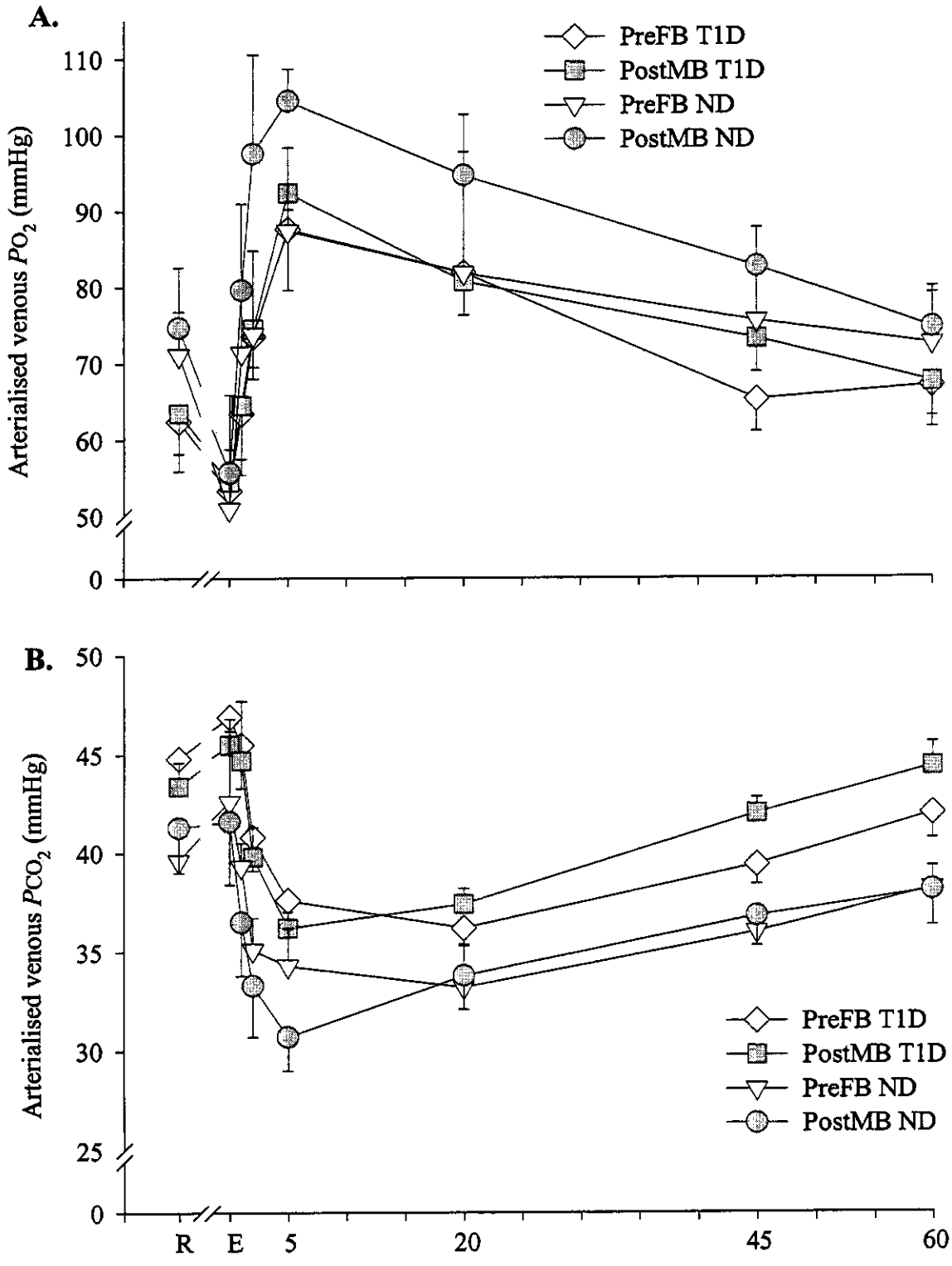


Figure 4.26 Arterialised venous oxygen (P_{O_2}; Graph A) and carbon dioxide (P_{CO_2}; Graph B) tensions (mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 20, 45, 60 min) during the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB) in the non-diabetic group (ND), n = 6 (except 2, 5, and 20 min, where n = 5); and group with type 1 diabetes (T1D), n = 8 (except at 1, 2, and 60 min, where n = 7).
Main effects for time (P<0.001) were found for both variables, and an interaction effect for training status-by-time for P_{CO_2} (P<0.01). The T1D group had significantly higher P_{CO_2} across both days and all times (P=0.01).

(iii) Plasma [H^+]

In PreFB, plasma [H^+] increased significantly with exercise, peaked at 63.9 ± 1.9 nmol·l^{-1} (pH 7.20 ± 0.01) after 5 min recovery (P<0.001) and returned to resting levels by 60 min recovery (Figure 4.27). After training, in PostMB, plasma [H^+] was lower by a mean difference of 2.48 ± 0.42 nmol·l^{-1} (P<0.001), peaked earlier (2 min, 57.7 ± 1.1 nmol·l^{-1}; pH 7.24 ± 0.01) and was specifically lower at 5, 20, 45, and 60 min recovery (P<0.01). Although there was no difference between groups, either before or after training, plasma [H^+] tended to be higher in the T1D group during exercise and recovery (P=0.12).

(iv) Plasma bicarbonate [HCO_3-]

Bicarbonate concentration fell with exercise, reached a nadir 5 min post-exercise, then slowly rose again throughout recovery, although was still below resting levels after 60 min (P<0.001; Figure 4.28). After training, recovery of [HCO_3-] was more rapid, being higher 20, 45, and 60 min post-exercise (P<0.001). Bicarbonate concentration was 1.6 mmol·l^{-1} higher in the T1D group across both days and all times (P<0.05).

4.4.4.5 Haematocrit, haemoglobin and % change in plasma volume

(i) Haematocrit

Haematocrit (Hct) increased with exercise, peaked at 1-2 min recovery, and fell below resting values by 45 min recovery (Table 4.9). There was no effect of training on Hct, however it was 2.8% higher across all times and both days in the T1D group (P<0.05).

(ii) Haemoglobin concentration

[Hb] at rest did not differ with training (14.04 ± 0.25 vs 14.02 ± 0.28 g·dl^{-1}, PreFB vs PostMB, respectively), however the rise in [Hb] from rest was reduced following matched-work exercise in PostMB (P<0.02), being significantly lower at 1, 2, and 5 min of recovery (Table 4.9). There was no difference in [Hb] between the T1D and ND groups.

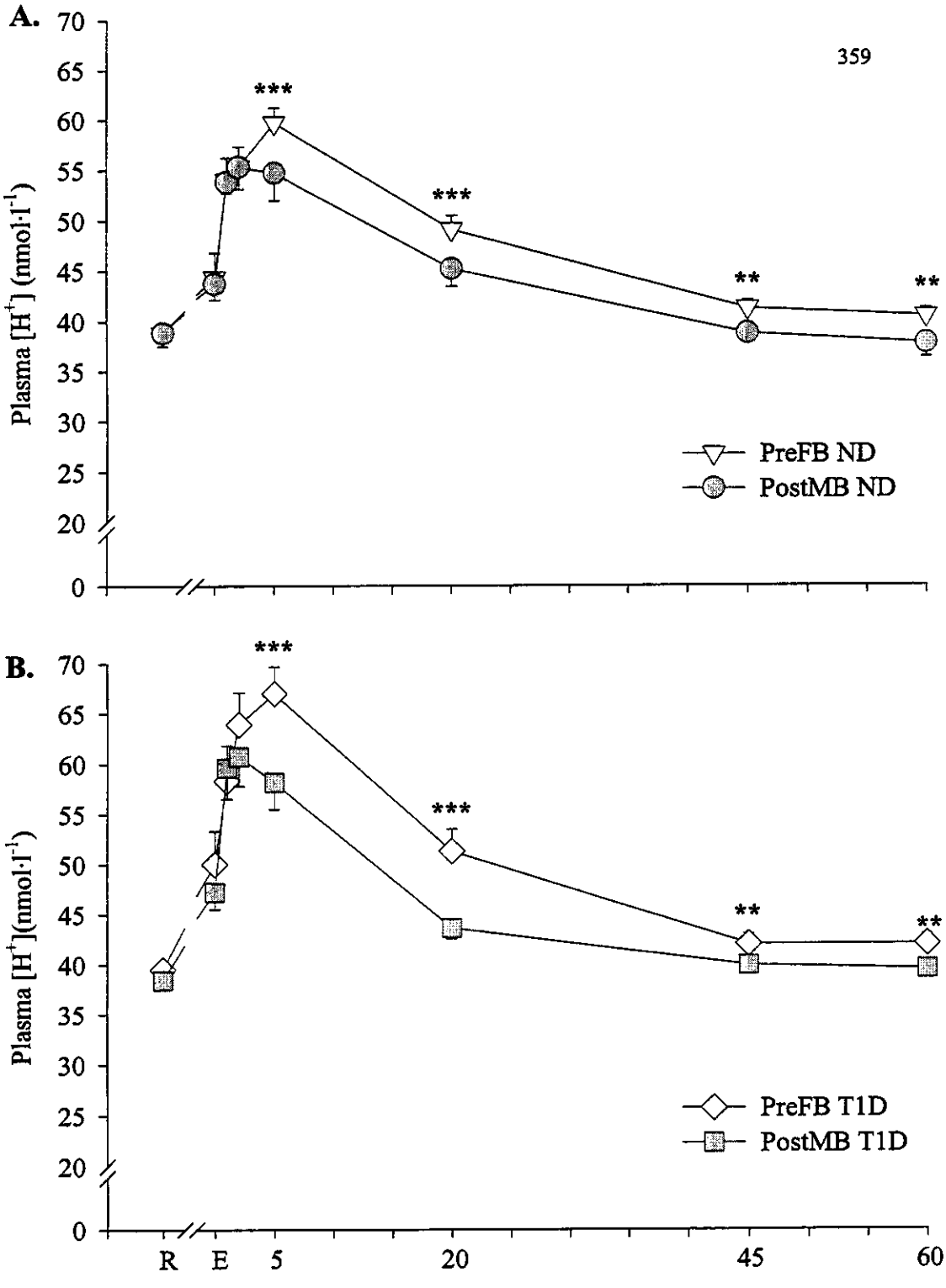


Figure 4.27 Plasma hydrogen ion concentration ($[H^+]$; mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 20, 45, 60 min) during the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB) in the (A) non-diabetic group (ND), $n = 6$ (except 2, 5, and 20 min, where $n = 5$); and (B) group with type 1 diabetes (T1D), $n = 8$ (except at 1, 2, and 60 min, where $n = 7$). Main effects for time ($P<0.001$) and training status ($P<0.001$) were found. Interaction effect for training status-by-time ($P<0.001$), whereby PreFB>PostMB, ***$P<0.001$, **$P<0.01$.

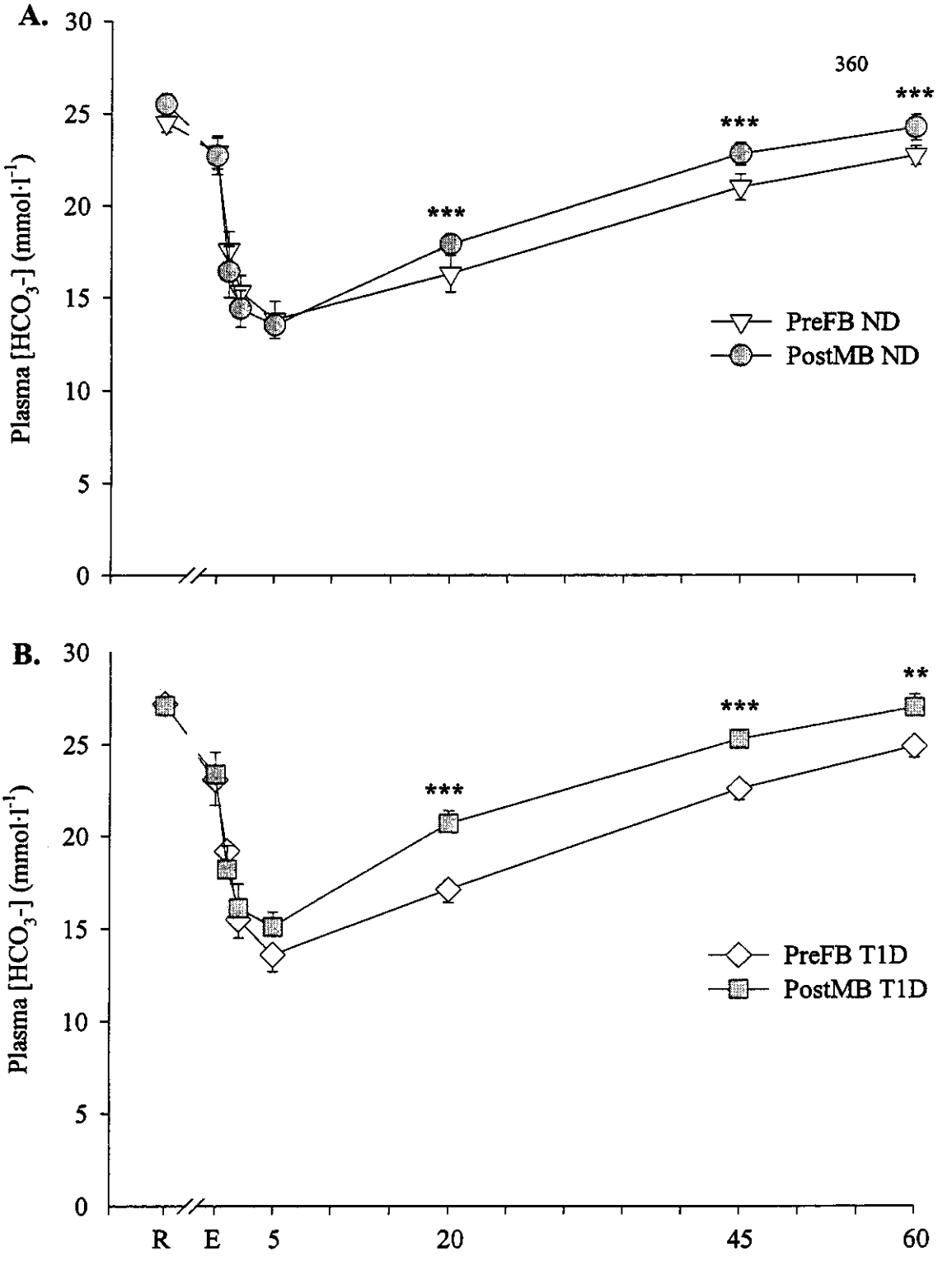


Figure 4.28 Plasma bicarbonate concentration ([HCO_3-], mean ± S.E.M.) at rest (R), immediately after exercise (E), and in recovery (1, 2, 5, 20, 45, 60 min) during the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and the post-training matched-work test (PostMB) in the (A.) non-diabetic group (ND), n = 6 (except 2, 5, and 20 min, where n = 5); and (B.) group with type 1 diabetes (T1D), n = 8 (except at 1, 2, and 60 min, where n = 7). A main effect for time (P<0.001) was found. Interaction effect (P<0.001) for training status-by-time, ***P<0.001, **P<0.01, PostMB>PreFB at 20, 45, and 60 min recovery. The T1D group had significantly higher [HCO_3-] across both days and all times (P<0.05).

(iii) Change in plasma volume

The greatest relative change (contraction) in plasma volume from rest (ΔPV) was evident at 1–2 min recovery ($P<0.001$; Table 4.9). The ΔPV was not different to rest by 20 min recovery, then expanded to be greater than rest by 45 min. After training the ΔPV was similar immediately after exercise, but significantly less at 1, 2, and 5 min of recovery ($P<0.05$). In PostMB, the ND group showed less contraction of plasma volume ($P<0.02$) relative to their pre-training values at 5 and 20 min of recovery than the T1D group.

4.4.4.6 Extent of arterialisation

(i) Arterialised venous oxygen tension (P_{O_2})

P_{O_2} fell with exercise, but recovered by 1 min post-exercise. P_{O_2} did not differ between groups and was not different after training. Across both groups, days and all times mean P_{O_2} was 74 ± 1 mmHg (Figure 4.26A).

Table 4.9 *Haematocrit, haemoglobin and the percentage change in plasma volume (relative to rest) in the pre-training test to fatigue at 130% $\dot{V}O_{2\,peak}$ (PreFB) and in the post-training matched-work test (PostMB).*

Variable	Group	Test	Rest	Ex	+1	+2	+5	+20	+45	+60
Hct % ***§	ND	PreFB	40.5 ± 1.8	44.0 ± 1.3	45.7 ± 1.0	46.5 ± 0.9	45.5 ± 0.9	41.4 ± 1.1	40.0 ± 1.3	40.6 ± 1.1
		PostMB	41.6 ± 1.5	43.9 ± 1.3	45.0 ± 1.0	44.9 ± 1.0	43.8 ± 1.6	40.3 ± 1.0	38.3 ± 1.5	40.0 ± 1.0
	T1D	PreFB	42.9 ± 1.0	46.8 ± 0.9	48.0 ± 1.0	48.5 ± 1.1	48.4 ± 1.1	43.5 ± 1.4	42.8 ± 0.9	42.8 ± 0.9
		PostMB	43.5 ± 0.8	46.9 ± 0.8	48.0 ± 0.9	47.9 ± 1.0	47.8 ± 0.8	43.5 ± 0.8	42.8 ± 0.7	42.0 ± 1.2
[Hb] $g \cdot dl^{-1}$ ***†	ND	PreFB	14.1 ± 0.4	14.9 ± 0.5	15.4 ± 0.4	15.5 ± 0.4	15.3 ± 0.5	14.4 ± 0.5	13.8 ± 0.5	13.7 ± 0.5
		PostMB	13.8 ± 0.5	14.6 ± 0.4	14.7 ± 0.4	14.5 ± 0.5	14.4 ± 0.5	13.5 ± 0.5	13.0 ± 0.3	13.0 ± 0.4
	T1D	PreFB	14.1 ± 0.3	15.3 ± 0.3	15.5 ± 0.4	15.6 ± 0.4	15.4 ± 0.3	14.1 ± 0.4	13.9 ± 0.3	13.9 ± 0.3
		PostMB	14.2 ± 0.4	15.3 ± 0.4	15.5 ± 0.4	15.3 ± 0.5	15.3 ± 0.4	14.2 ± 0.3	14.0 ± 0.3	14.2 ± 0.2
ΔPV % ***†‡	ND	PreFB	0	-10.2 ± 1.1	-16.6 ± 1.5	-17.5 ± 1.8	-16.0 ± 1.4	-4.7 ± 1.6	1.6 ± 2.2	3.5 ± 1.5
		PostMB	0	-8.9 ± 1.0	-11.5 ± 1.6	-9.8 ± 2.1	-7.2 ± 2.7	5.9 ± 4.2	13.5 ± 5.5	9.7 ± 2.9
	T1D	PreFB	0	-13.2 ± 1.3	-15.6 ± 1.5	-17.4 ± 1.3	-15.7 ± 1.3	2.7 ± 4.1	3.8 ± 0.9	3.9 ± 1.1
		PostMB	0	-11.5 ± 1.3	-14.7 ± 1.4	-13.2 ± 1.7	-13.2 ± 1.6	0.7 ± 1.4	3.7 ± 1.3	4.0 ± 1.1

All values are mean ± S.E.M. $n = 6$, T1D (group with type 1 diabetes); $n = 6$, ND (non-diabetic group). Hct, haematocrit; [Hb], haemoglobin concentration; ΔPV, change in plasma volume relative to rest. Rest, mean of supine and upright rest; Ex, exercise; +1, +2 etc, minutes of recovery post-exercise. ***$P<0.001$, main effect of time; § $P<0.05$, T1D>ND group; †$P<0.05$, interaction effect for day-by-time, PreFB>PostMB; ‡$P<0.05$, interaction effect, day-by-time-by-group.

4.4.5 Muscle Results

One female subject (CH) in the ND group elected not to have muscle biopsies, however completed all other aspects of the study. One male subject (JWH) in the T1D group experienced hypoglycaemia (and consequently consumed carbohydrate) prior to the pre-training biopsy test: however this was only discovered during the post-training test. Six months later the subject completed the entire study again, however declined to have further muscle biopsies. The blood data from the second period of training appear in Appendix E and were used for statistics. His biopsy data also appear in appendix E, however have not been included in statistical analysis. Muscle biopsy data is therefore reported for thirteen subjects (7 T1D, 6 ND) in the 130% $\dot{V}O_{2peak}$ tests, both before (PreFB) and after (PostMB) the sprint training programme.

4.4.5.1 Percentage water content of muscle

Intense exercise resulted in a significant increase in the percentage water content of the muscle (75.1 ± 0.3 *vs* 76.5 ± 0.2 %, rest *vs* exercise, respectively; $P<0.01$), which was not altered by training and was not different between groups (Appendix E16).

4.4.5.2 Total Creatine (creatine phosphate + creatine)

Total creatine (TCr) did not differ significantly with training ($P=0.44$), with time (rest *vs* exercise, $P=0.62$), or between groups ($P=0.49$) (Appendix E17). Mean TCr across all biopsies was 114.8 ± 1.8 mmol·kg^{-1} dm. Peak TCr did not differ between groups ($P=0.63$) or with training (117.6 ± 3.5 *vs* 119.5 ± 3.4 mmol·kg dm^{-1}; pre- *vs* post-training, respectively; $P=0.35$; Table 4.10).

Table 4.10 *Peak total creatine in the biopsy samples obtained before (PreFB) and after training (PostMB).*

	PreFB	PostMB
T1D	117.0 ± 4.6	116.9 ± 4.1
ND	118.4 ± 5.8	122.4 ± 5.9

n = 7, T1D (group with type 1 diabetes); n = 6, ND (non-diabetic group). All values are in mmol·kg dm^{-1} ± S.E.M.

4.4.5.3 Protein content

Muscle protein content did not differ between groups (P=0.80) or with exercise (P=0.66) or sprint training (P=0.84) (Table 4.11; Appendix E17). Mean muscle protein content for both groups, times and days was 142.2 ± 2.1 mg protein·g ww^{-1}.

Table 4.11 *Total protein content in the biopsy samples obtained before (PreFB) and after training (PostMB).*

	PreFB	PostMB
T1D	143 ± 3	143 ± 2
ND	141 ± 4	142 ± 7

n = 7, T1D (group with type 1 diabetes); n = 6, ND (non-diabetic group). All values are in mg protein·g ww^{-1} ± S.E.M.

4.4.5.4 Substrates and Metabolites

(i) Glycogen

Glycogen content was significantly reduced immediately following intense exercise (P<0.001; Table 4.12), with no difference between groups. After training, the degradation of glycogen tended to be reduced during matched-work exercise (~26% lower when both groups were combined, P=0.052). Individual results appear in Appendix E18).

Table 4.12 *Muscle glycogen content (glucosyl units) at rest and immediately after exercise in the pre-training test to fatigue at 130% $\dot{V}O_{2\,peak}$ (PreFB) and in the post-training matched-work test (PostMB).*

	ND			T1D		
	Rest	Exercise	Delta	Rest	Exercise	Delta
PreFB***	526 ± 53	347 ± 60	179 ± 24	491 ± 50	306 ± 38	185 ± 30
PostMB***#	537 ± 49	399 ± 39	138 ± 38	499 ± 61	366 ± 63	133 ± 22

n = 7, T1D (group with type 1 diabetes); n = 6, ND (non-diabetic group). All values are in mmol·kg^{-1} dm ± S.E.M. Delta, rest minus exercise glycogen content. ***P<0.001, main effect of time, i.e. rest *vs* exercise; #P=0.052, training status-by-time interaction effect, i.e. reduced degradation in PostMB.

(ii) Glucose

Muscle glucose content rose significantly with exercise ($P<0.001$; Table 4.13), was not altered after training, and was higher in the T1D group across both days and times ($P<0.01$). Individual data appear in Appendix E18.

Table 4.13 *Muscle glucose content at rest and immediately after exercise in the pre-training test to fatigue at 130 % $\dot{V}O_{2\,peak}$ (PreFB) and in the post-training matched-work test (PostMB).*

	ND		T1D $^{\Psi\Psi}$	
	Rest	Exercise	Rest	Exercise
PreFB ***	1.82 ± 0.36	3.49 ± 0.63	3.75 ± 0.74	4.88 ± 0.60
PostMB ***	1.30 ± 0.11	3.19 ± 0.34	2.93 ± 0.29	6.21 ± 1.08

n = 7, T1D (group with type 1 diabetes); n = 6, ND (non-diabetic group). All values are in mmol·kg^{-1} dm ± S.E.M. ***$P<0.001$, main effect of time, i.e. rest *vs* exercise; $^{\Psi\Psi}P<0.01$, T1D group>ND group.

(iii) Glucose 1-phosphate (G 1-P), glucose 6-phosphate (G 6-P), and fructose 6-phosphate (F 6-P)

Each of G 1-P, G 6-P and F 6-P content increased with exercise ($P<0.001$; Table 4.14), with no difference between groups. After training, the accumulation of G 6-P tended to be lower, however was not significant ($P=0.11$). There was no effect of training and no interaction effects were evident for G 1-P or F 6-P. Individual data appear in Appendix E18.

(iv) Pyruvate

Muscle pyruvate content was increased 4 to 5-fold immediately following exercise to fatigue in the PreFB test ($P<0.001$; Figure 4.29). After training, in the matched-work test (PostMB), the accumulation of pyruvate with exercise was reduced ($P<0.05$) by 0.13 ± 0.10 and 0.44 ± 0.17 mmol·kg^{-1} dm in the ND and T1D groups, respectively. In addition, the T1D group tended to display lower pyruvate content after training than the ND group ($P=0.058$). Individual data appear in Appendix E18.

Table 4.14 *Muscle G 1-P, G 6-P, and F 6-P at rest and immediately after exercise in the pre-training test to fatigue at 130% $\dot{V}O_{2\,peak}$ (PreFB) and in the post-training matched-work test (PostMB).*

		ND		T1D	
		Rest	Exercise	Rest	Exercise
G 1-P ***	PreFB	0.04 ± 0.01	0.91 ± 0.19	0.07 ± 0.02	0.81 ± 0.16
	PostMB	0.05 ± 0.01	0.56 ± 0.12	0.09 ± 0.01	0.77 ± 0.22
G 6-P ***	PreFB	1.4 ± 0.2	22.2 ± 2.7	1.5 ± 0.3	23.3 ± 1.8
	PostMB	1.2 ± 0.2	19.4 ± 1.8	1.5 ± 0.2	20.1 ± 2.2
F 6-P ***	PreFB	0.20 ± 0.02	3.24 ± 0.36	0.21 ± 0.04	3.49 ± 0.19
	PostMB	0.18 ± 0.02	3.22 ± 0.42	0.23 ± 0.03	3.12 ± 0.34

n = 7, T1D (group with type 1 diabetes); n = 6, ND (non-diabetic group). All values are in mmol·kg^{-1} dm ± S.E.M. G 1-P, glucose 6-phosphate; G 6-P, glucose 6-phosphate; F 6-P, fructose 6-phosphate. ***P<0.001, main effect of time, i.e. rest *vs* exercise.

(v) Lactate

Muscle lactate rose dramatically with exercise (P<0.001; Figure 4.30), and prior to training, in the PreFB test, accumulated to a greater extent in the T1D group than in the ND group (P<0.05). After training during matched-work exercise, muscle lactate accumulation was 12 and 34% lower in the ND and T1D groups, respectively (P=0.001).

4.4.5.5 Glycogenolytic and glycolytic rates

The rate of glycogenolysis during fatiguing exercise in the PreFB test was 1.19 ± 0.16 and 1.03 ± 0.08 mmol glucosyl units·kg^{-1} dm·s^{-1} in the ND and T1D groups, respectively. After training during matched-work exercise in PostMB, the glycogenolytic rate was significantly lower (P<0.01; Figure 4.31A), being reduced 12 and 26 % in the ND and T1D groups, respectively. Similarly, the glycolytic rate was reduced by 12 and 31 % during matched-work exercise after training (P<0.01; Figure 4.31B), in the ND and T1D groups, respectively.

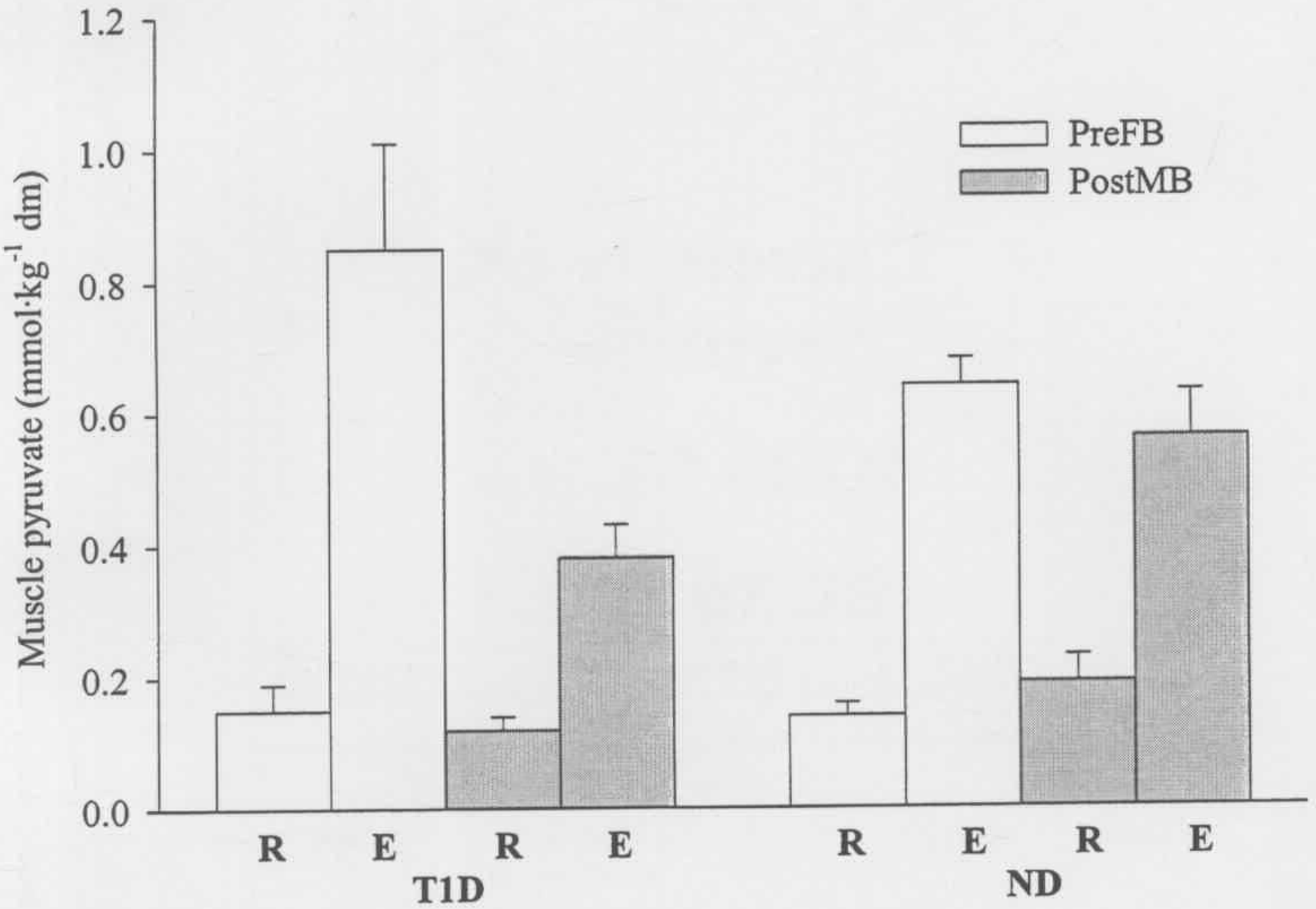


Figure 4.29 Muscle pyruvate content (± S.E.M.) at rest (R) and after exercise (E) in the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and in the post-training matched-work test (PostMB). $n = 7$, T1D (group with type 1 diabetes); $n = 6$, ND (non-diabetic group). Main effects of time ($P<0.001$) and training ($P<0.05$), and interaction effects for training status-by-time ($P<0.05$) and training status-by-group ($P=0.058$) were found, and are explained within the text.

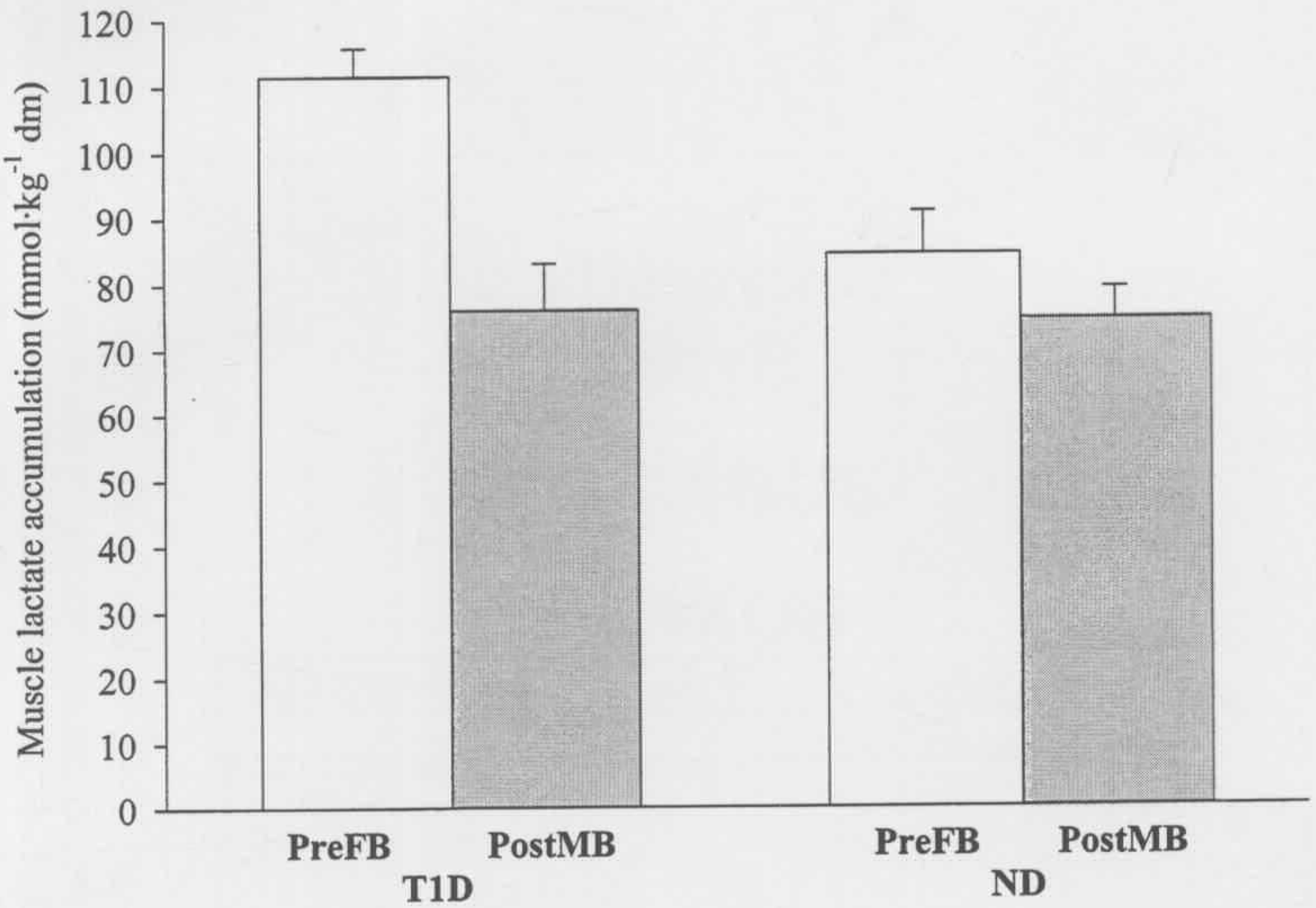


Figure 4.30 Muscle lactate accumulation (± S.E.M.) in the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and in the post-training matched-work test (PostMB). $n = 7$, T1D (group with type 1 diabetes); $n = 6$, ND (non-diabetic group). Main effects of time ($P<0.001$) and training ($P<0.01$), and interaction effects for training status-by-time ($P=0.001$), training status-by-group ($P=0.052$), time-by-group ($P<0.05$), training status-by-time-by-group ($P<0.05$) were found. Prior to training, muscle lactate accumulation was greater in the T1D group than in the ND group ($P<0.05$).

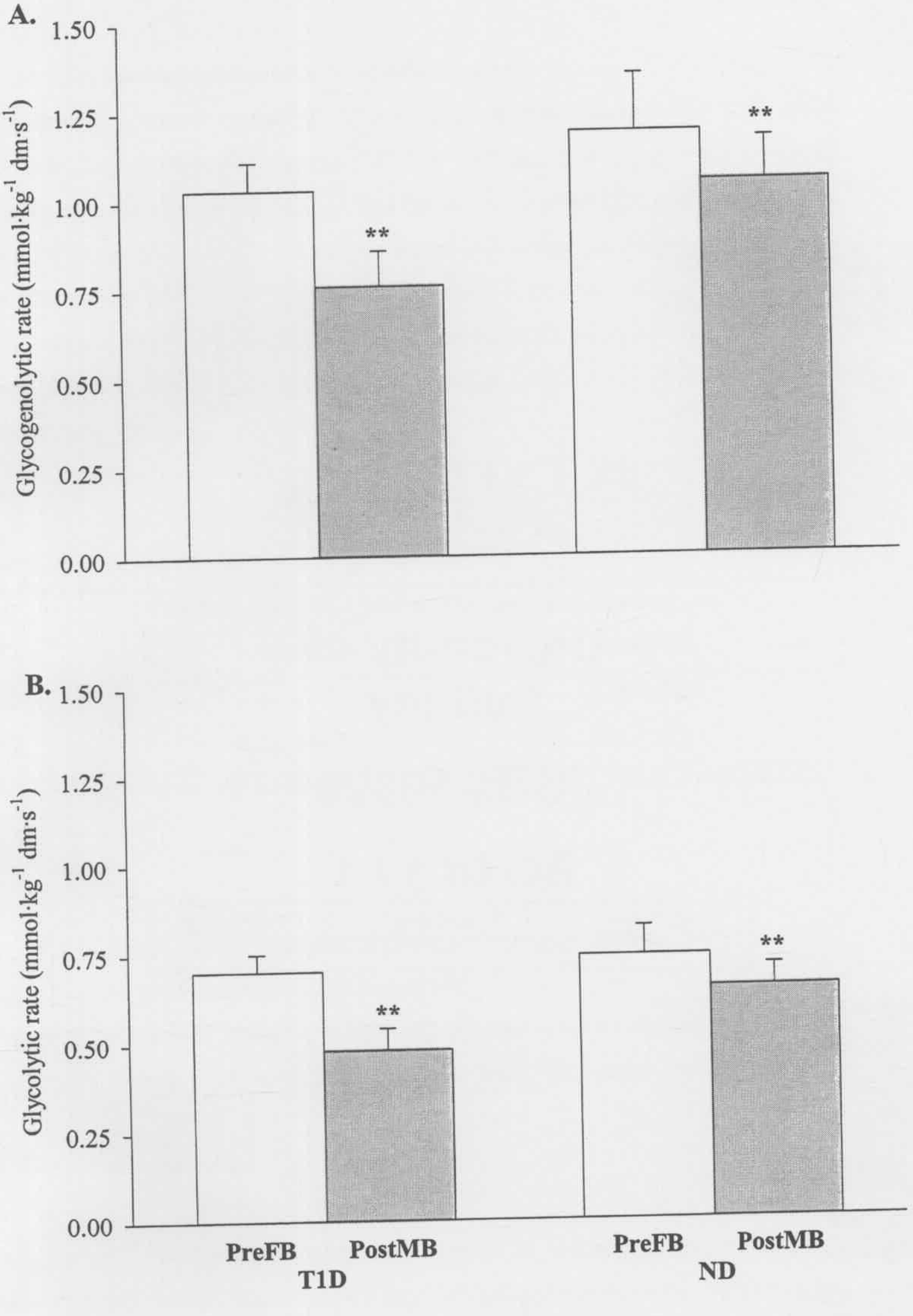


Figure 4.31 A. Glycogenolytic rate (± S.E.M.), and B. Glycolytic rate (± S.E.M.), in the pre-training test to fatigue at 130% $\dot{V}O_{2\,peak}$ (PreFB) and in the post-training matched-work test (PostMB). $n = 7$, T1D (group with type 1 diabetes); $n = 6$, ND (non-diabetic group). $^{**}P<0.01$, PreFB>PostMB.

4.4.5.6 Muscle anaerobic ATP production/ rate

Prior to training, muscle anaerobic ATP production was higher in the T1D group (240.9 ± 9.1) than in the ND group (187.9 ± 14.7 mmol ATP·kg^{-1} dm) (P<0.01), consistent with the higher muscle Lac$^-$, and the tendency (P=0.12) for a longer time to exhaustion in the T1D group. After training muscle anaerobic ATP production was lower (P<0.01) in both the ND (11%) and T1D (28%) groups. Similarly, the rate of muscle anaerobic ATP production during matched work exercise was lower after training (P<0.01), with no difference between groups (Table 4.15). Individual results appear in Appendix E20.

Table 4.15 *Muscle anaerobic ATP production rate*

	Muscle anaerobic ATP production rate (mmol·kg dm^{-1}·s^{-1})	
	PreFB	PostMB‡
T1D	3.01 ± 0.22	2.19 ± 0.25
ND	3.28 ± 0.37	2.91 ± 0.27

n = 7, T1D (group with type 1 diabetes); n = 6, ND (non-diabetic group). ‡, P<0.01, PreFB>PostMB.

4.4.5.7 High energy phosphates and degradation products

(i) Creatine phosphate (PCr) and creatine

The degradation of PCr and accumulation of creatine was not altered after training, and was not different between groups (Table 4.16). Individual results appear in Appendix E21.

(ii) ATP

Prior to training, resting ATP content was reduced 23 ± 3 and 29 ± 1 % following exercise to fatigue (P<0.001) in the ND and T1D groups, respectively. After training, resting ATP content was reduced 8% (P<0.01) when both groups were considered together, however just failed to attain statistical when each group was examined separately (ND group, 10% lower, P=0.06; T1D group, 7% lower, P=0.09). During matched-work exercise (PostMB test) after training the degradation of ATP was markedly attenuated (P<0.01) in both groups (Figure 4.32).

Table 4.16 *Muscle creatine phosphate (PCr) and creatine content at rest and after exercise in the pre-training test to fatigue (PreFB) and in the post-training matched-work test (PostMB)*

		ND		T1D	
		Rest	Exercise	Rest	Exercise
PCr ***	PreFB	75.3 ± 3.6	24.6 ± 2.7	79.9 ± 3.3	19.5 ± 2.6
	PostMB	80.4 ± 4.8	30.1 ± 5.7	79.3 ± 3.9	23.0 ± 5.7
Creatine ***	PreFB	43.1 ± 2.3	93.8 ± 6.1	37.1 ± 2.7	97.5 ± 5.4
	PostMB	42.0 ± 1.3	92.3 ± 5.8	37.6 ± 1.7	93.9 ± 3.7

n = 6, ND (non-diabetic group); n = 7, T1D (group with type 1 diabetes). All values are in mmol·kg^{-1} dm ± S.E.M. ***P<0.001, main effect of time, i.e. rest *vs* exercise.

A group-by-day effect (P<0.05) was found whereby the T1D group had higher overall ATP content (rest and exercise) after training, due to the markedly reduced ATP degradation during exercise, whereas the ND group had lower overall ATP content after than before training, due to a reduction in resting ATP content. In the ND group, although ATP degradation was reduced compared to pre-training, at the end of exercise ATP content was the same after training. Whereas in the T1D group, post-exercise ATP content was higher after training than before even though resting content was reduced – demonstrating a very marked attenuation of net ATP degradation during matched-work exercise. Individual results appear in Appendix E21.

4.4.5.8 Muscle enzymes

All muscle enzyme activities were corrected to the peak total creatine content and are expressed both as wet and as dry mass. Individual results appear in Appendix E22.

(i) Pyruvate dehydrogenase activity, active form (PDH*a*)

Prior to training (PreFB test) PDH*a* activity at rest did not differ between the ND and T1D groups (although PDH*a* tended to be lower - 24% - in the T1D group; P=0.28).

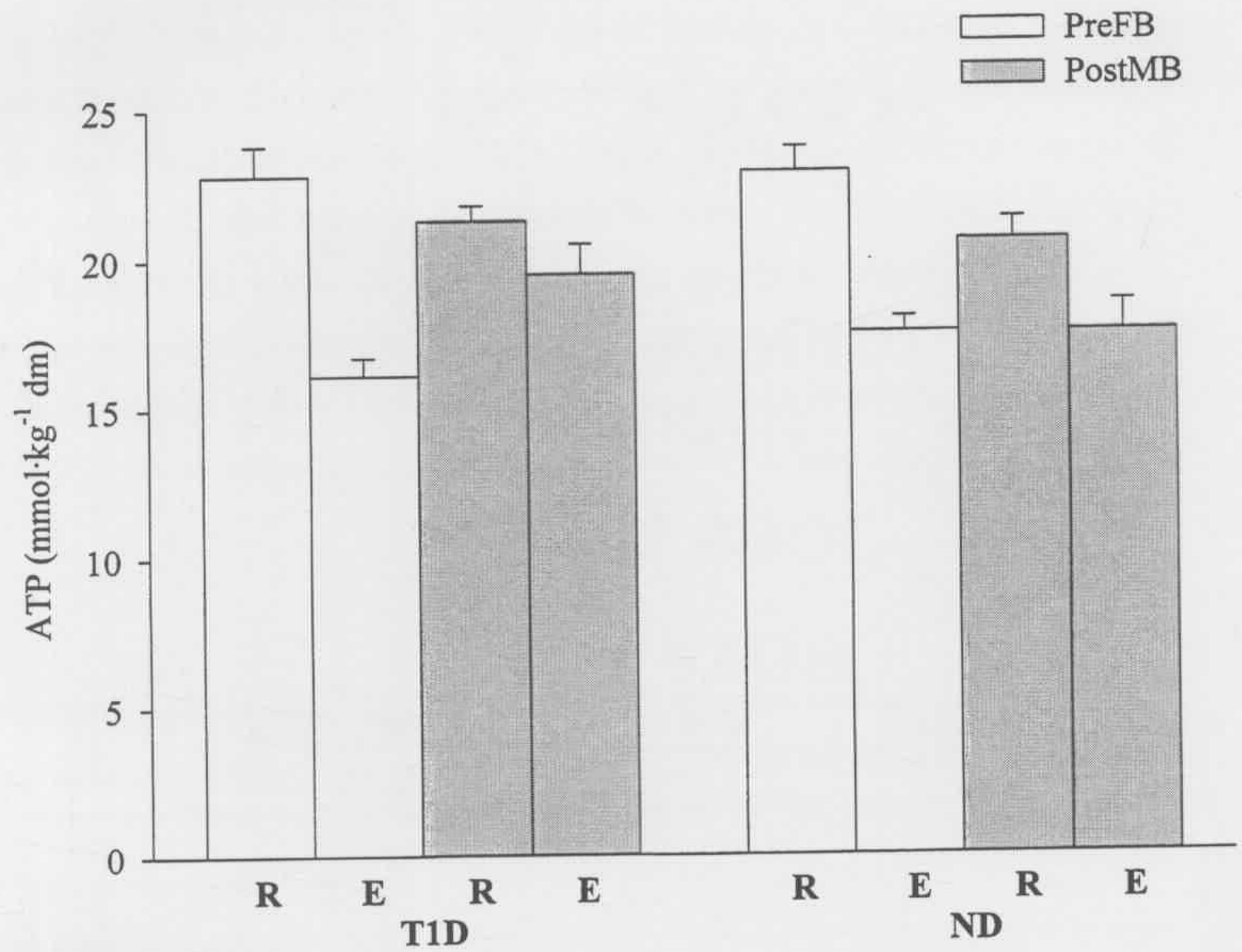


Figure 4.32 Muscle ATP content (± S.E.M.) at rest (R) and after exercise (E) in the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and in the post-training matched-work test (PostMB). $n = 7$, T1D (group with type 1 diabetes); $n = 6$, ND (non-diabetic group). A main effect of time ($P<0.001$) and interaction effects for training status-by-time ($P<0.01$) and training status-by-group ($P<0.05$) were found, and are explained within the text.

PDH*a* activity at rest was 34% lower ($P<0.05$) after training in the PostMB test (Figure 4.33), with no difference between groups.

When expressed as wet weight (Table 4.17), results for resting PDH*a* activity were similar to those for dry mass, with a 33% reduction at rest ($P<0.05$).

Values for PDH*a* after exercise do not appear in the body of the thesis, however, have been included in the Appendix (E22). Despite expert collaboration with the Biochemistry Department of The University of Sydney (see 4.3.7.2iv), the values obtained were markedly lower than those obtained for similarly intense exercise in contemporary literature (see Table 2.17). PDH*a* activity in the current study was analysed in muscle from the second post-exercise biopsy. Given that PDH*a* activity is essentially maximal within 15 s of commencing maximal exercise (Parolin et al., 1999), the initial fall post-exercise may also be very rapid (although was 2.4- to 2.9-fold above resting values after 4 min recovery from a 30-s maximal cycle bout – see section 2.19.9), and consequently peak values may have been missed in the current study.

Table 4.17 *Resting activity of pyruvate dehydrogenase, active form (PDHa), expressed as wet weight in the pre- (PreFB) and post-training (PostMB) tests.*

	ND	T1D
PreFB	0.24 ± 0.04	0.18 ± 0.03
PostMB	0.13 ± 0.03	0.15 ± 0.04

n = 6, ND (non-diabetic group); n = 7, T1D (group with type 1 diabetes).
All values are in mmol·min^{-1}·kg wet weight^{-1} ± S.E.M.

(ii) Citrate synthase activity (CS)

Resting CS activity did not differ between the ND and T1D groups in the PreFB test (67.4 ± 6.9 *vs* 65.6 ± 5.5 mmol·min^{-1}·kg dm^{-1}, respectively; Figure 4.34). However, whilst CS activity was maintained in the T1D group with exercise, it fell 20% in the ND group ($P<0.02$). In the PostMB test after training, CS activity was higher in both groups ($P<0.002$), however, the 46% increase at rest in the ND group was greater than

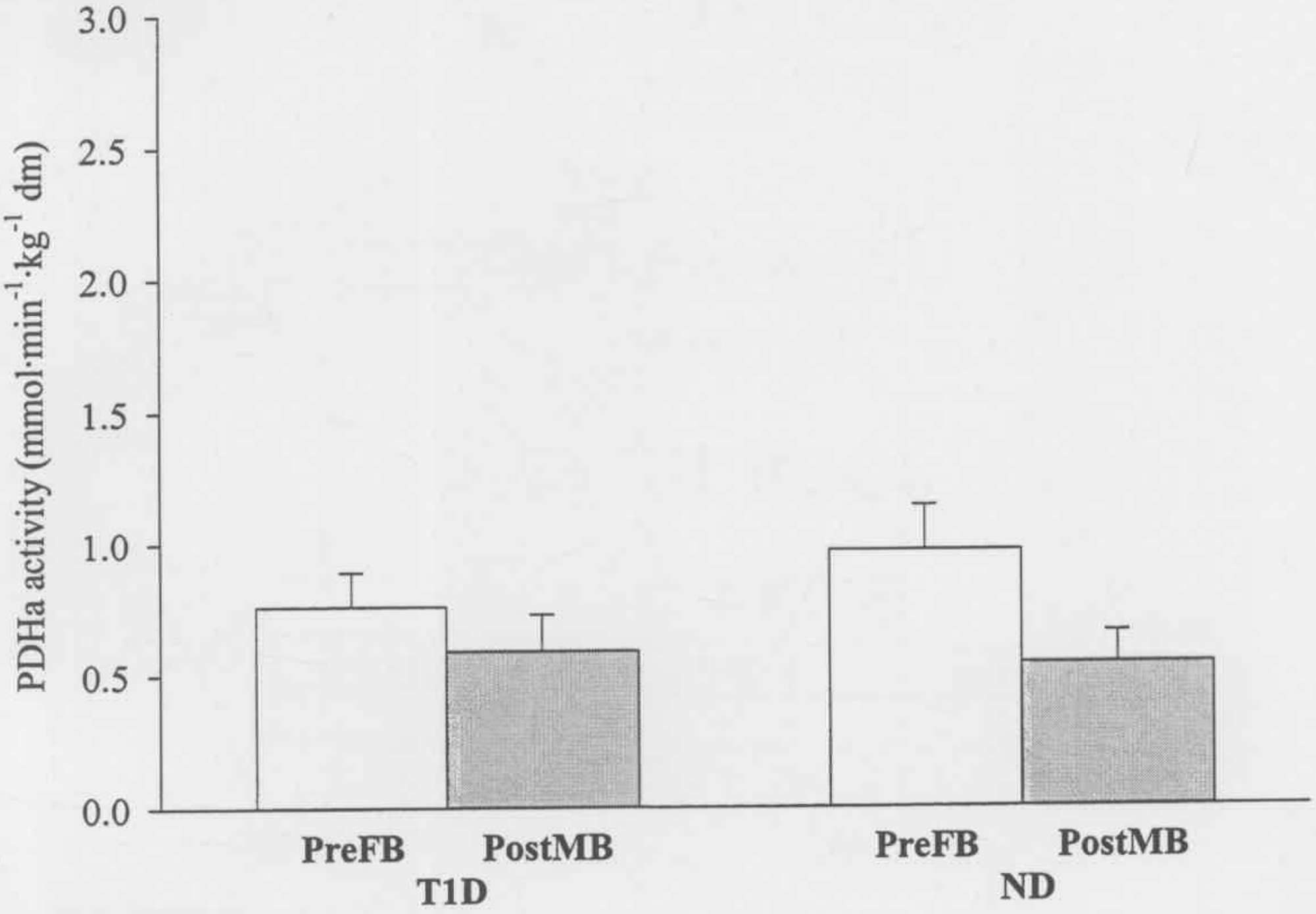


Figure 4.33 Muscle pyruvate dehydrogenase activity, active portion (PDHa, mean ± S.E.M.) at rest in the pre-training (PreFB) and post-training test (PostMB). $n = 7$, T1D (group with type 1 diabetes); $n = 6$, ND (non-diabetic group). A main effect of training status ($P<0.05$, PreFB>PostMB) was found, with no difference between groups.

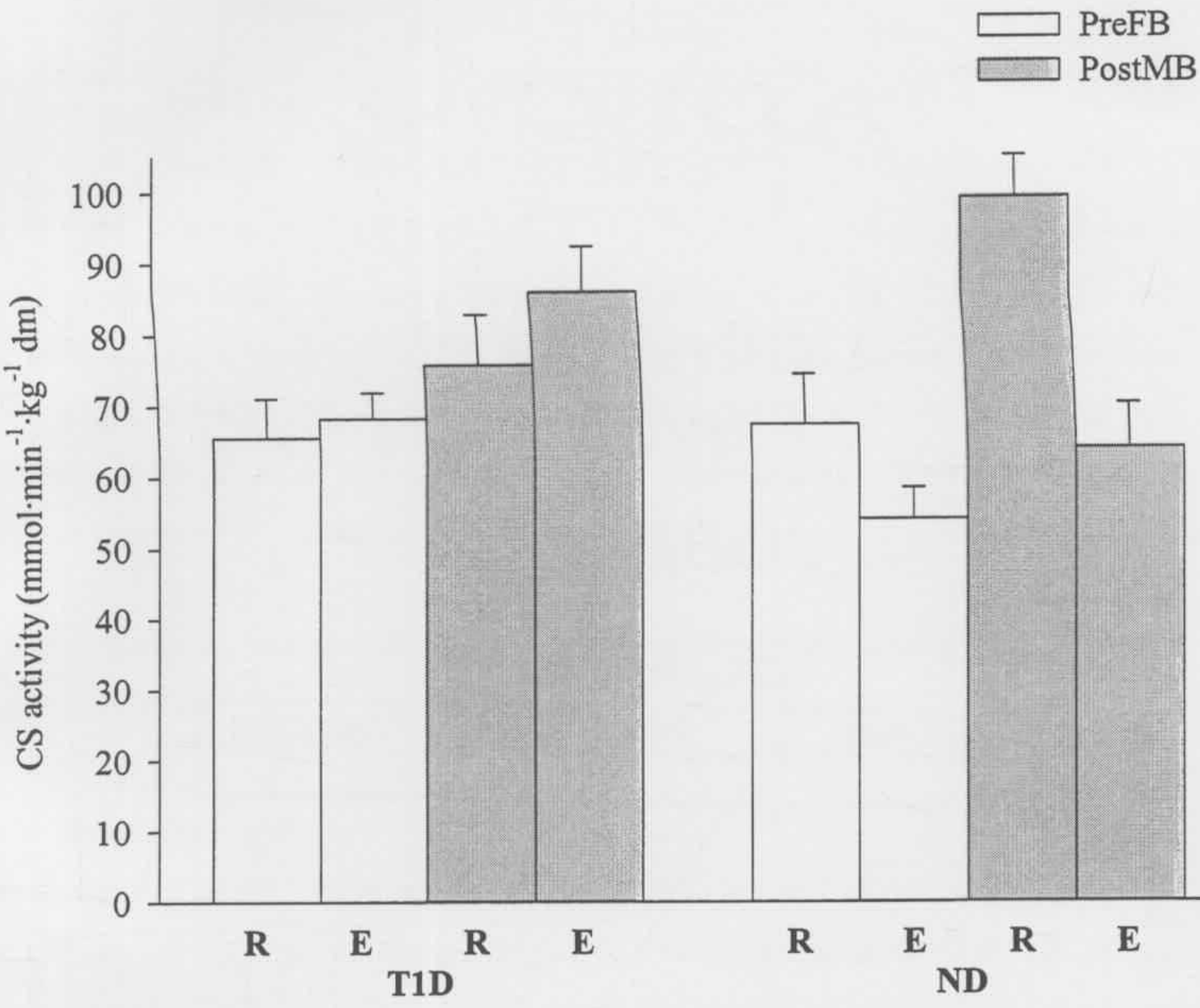


Figure 4.34 Muscle citrate synthase activity (CS, mean ± S.E.M.) at rest (R) and after exercise (E) in the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and in the post-training matched-work test (PostMB). $n = 7$, T1D (group with type 1 diabetes); $n = 6$, ND (non-diabetic group). A main effect of training status ($P<0.002$, PostMB>PreFB) was found; with the increase at rest being greater in the ND than T1D group ($P<0.05$). A time-by-group interaction effect ($P<0.02$), whereby CS activity was lower after exercise in the ND group ($P<0.01$), but not in the T1D group was evident.

the 15% increase in the T1D group ($P<0.05$). The effect of exercise on CS activity in PostMB was similar to that in PreFB, with little change in the T1D group, but a 35% decrease in activity in the ND group ($P<0.02$).

Table 4.18 *Citrate synthase (CS) and hexokinase (HK) activities, expressed as wet weight, at rest and after exercise in the pre-training test to fatigue (PreFB) and the post-training matched-work test (PostMB)*

		ND		T1D	
		Rest	Exercise	Rest	Exercise
CS *†††‡	PreFB	17.1 ± 2.2	12.8 ± 1.0	16.0 ±1.4	15.9 ± 0.9
	PostMB	25.0 ± 1.7	15.5 ± 1.4	19.0 ± 1.8	19.9 ± 1.4
HK ***†††	PreFB	2.2 ± 0.1	1.5 ± 0.1	2.2 ± 0.1	2.0 ± 0.1
ΨΨΨ##	PostMB	2.4 ± 0.1	1.8 ± 0.2	3.2 ± 0.2	2.9 ± 0.1

n = 6, ND (non-diabetic group); n = 7, T1D (group with type 1 diabetes). All values are in mmol·min^{-1}·kg wet weight^{-1} ± S.E.M. *$P<0.05$, ***$P\leq0.001$, main effect of time, i.e. rest *vs* exercise; ††$P<0.01$, †††$P<0.001$, main effect of training status; ‡$P<0.05$, time-by-group interaction; ΨΨΨ$P<0.001$, T1D>ND group; ##$P<0.01$, day-by-group interaction.

When expressed as wet weight (Table 4.18), results for CS activity were similar to those for dry mass.

(iii) Hexokinase activity

Prior to training in the PreFB test, hexokinase (HK) activity at rest, expressed as dry mass, did not differ between groups ($P=0.90$). After training in PostMB, HK activity was significantly increased ($P<0.001$; Figure 4.35). In the ND group HK activity was increased 8 ± 8% at rest, and was further increased ($P<0.02$) in the T1D group, being 49 ± 13% higher than at rest in PreFB. HK activity fell with exercise in the ND group ($P<0.01$), whereas it did not change significantly in the T1D group ($P=0.27$). HK activity was 2.53 mmol·min^{-1}·kg dm^{-1} higher in the T1D group across both days and times ($P<0.01$).

When expressed as wet weight (Table 4.18, above), results for HK activity were very similar to those for dry mass, except that the time-by-group interaction just failed to reach significance ($P=0.078$).

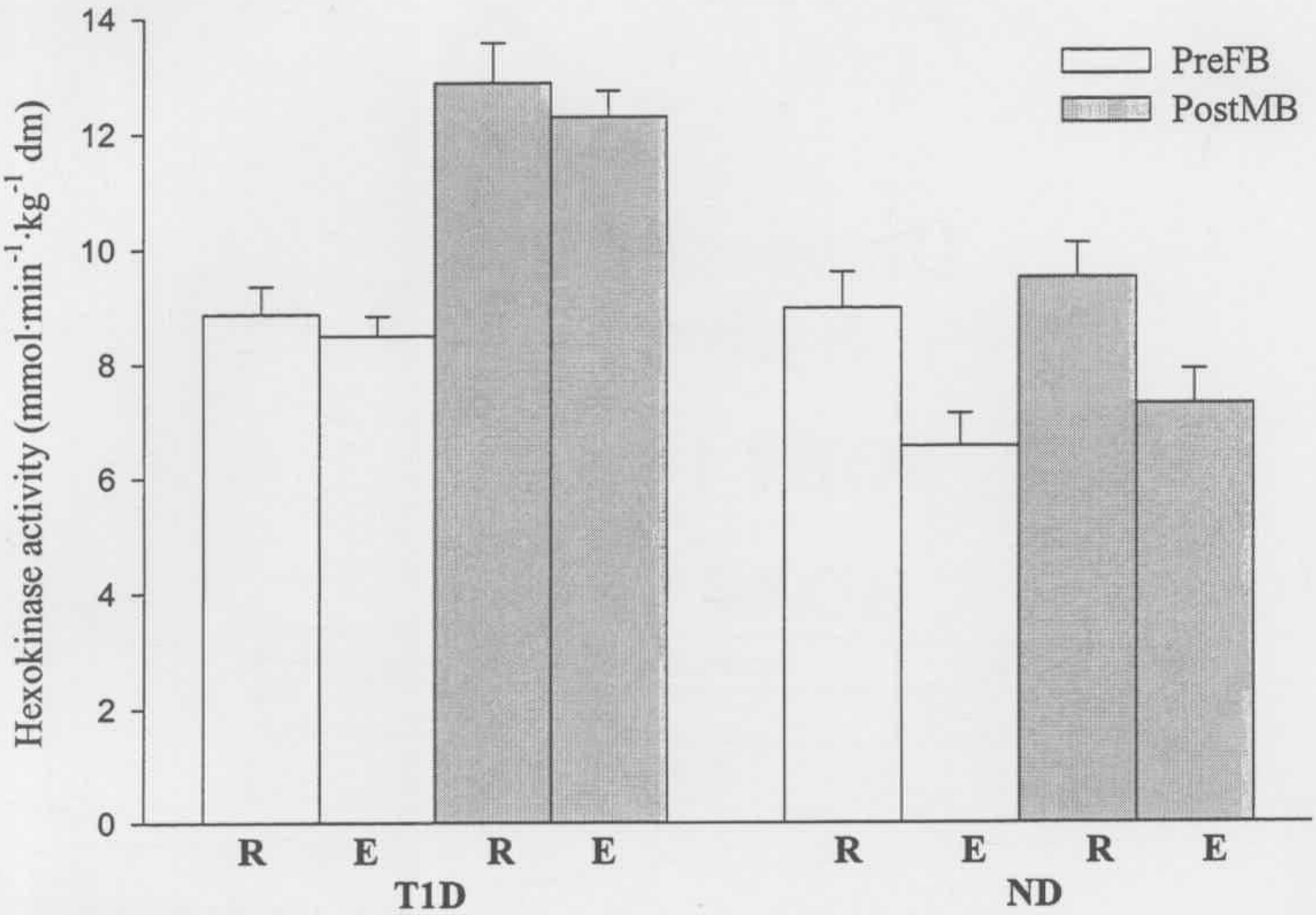


Figure 4.35 Hexokinase activity (mean ± S.E.M.) at rest (R) and immediately after exercise (E) in the pre-training test to fatigue at 130% $\dot{V}O_{2peak}$ (PreFB) and in the post-training matched-work test (PostMB) in the group with type 1 diabetes (T1D) and in the non-diabetic group (ND). Main effects were found for training status, PostMB>PreFB, $P<0.001$; time, $P<0.01$; group, T1D>ND, $P<0.01$; and interaction effects for training status-by-group, $P<0.02$; and time-by-group, $P<0.05$.

4.4.5.9 Vanadate-facilitated [^{3}H]ouabain binding site content

Muscle [^{3}H]ouabain binding site content prior to training was 321 ± 18 pmol·g ww^{-1} (Figure 4.36), with no difference between groups. After sprint training, [^{3}H]ouabain binding site content was 8.2 ± 2.2% higher (346 ± 19 pmol·g ww^{-1}; P<0.02), being increased in eleven of the thirteen subjects and unchanged in two subjects (one from each of the ND and T1D groups), with no group differences evident (P=0.90). Individual results appear in Appendix E23.

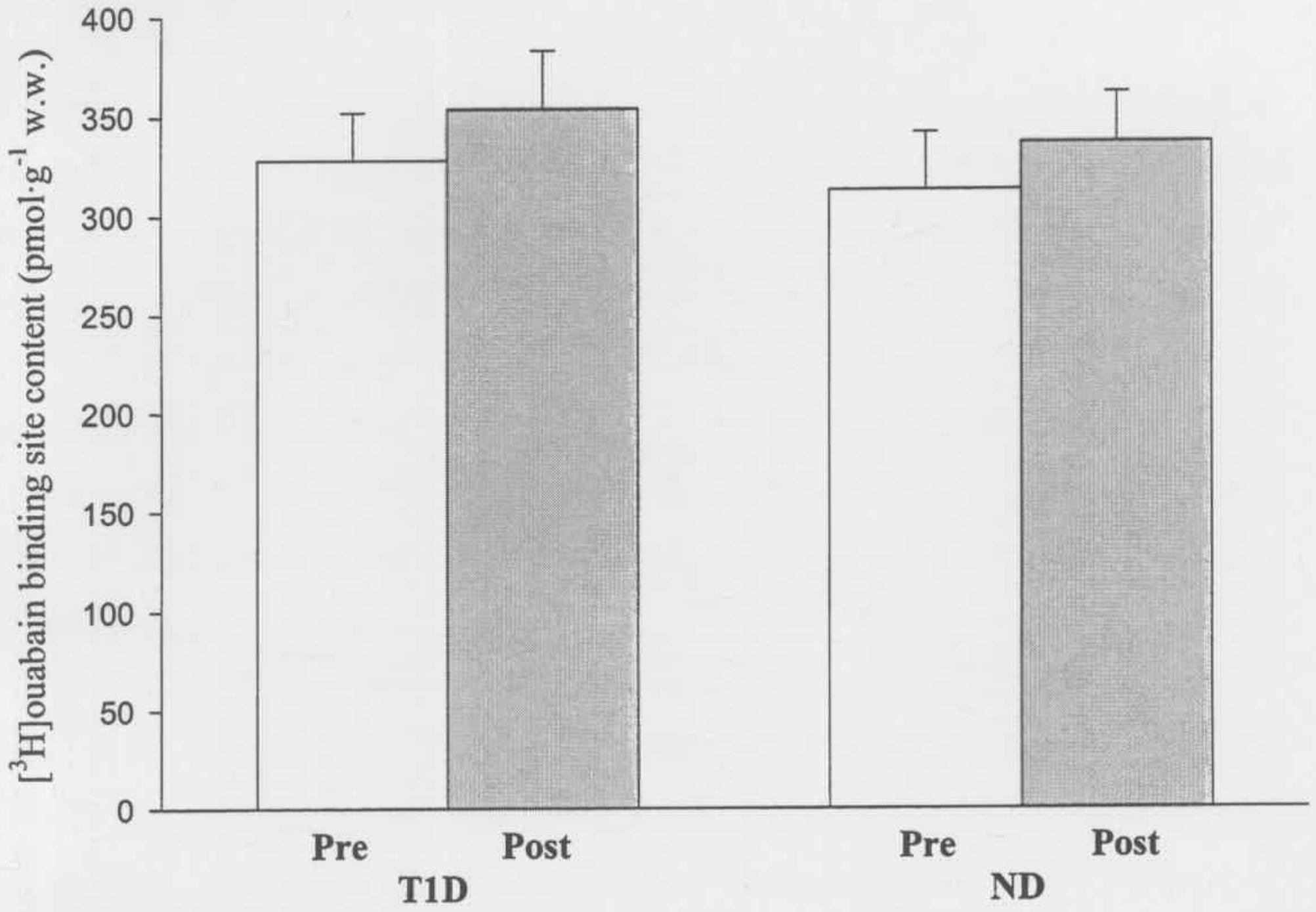


Figure 4.36 [^{3}H]ouabain binding site content (mean ± S.E.M.) before (Pre) and after (Post) training in the group with type 1 diabetes (T1D) and in the non-diabetic group (ND). A main effect was found for training status, Post>Pre, $P<0.02$, with no difference between groups.

4.5 Discussion

4.5.1 Effects of sprint training upon metabolic control

This is the first study to examine the effect of sprint training upon metabolic control in subjects with type 1 DM. Sprint training improved metabolic control, as demonstrated by the reduction in HbA_{1c} from 8.6 to 8.0%, confirming the hypothesis (see section 4.2.2.1). The tendency to lower self-monitored postabsorptive mean BGL (18% lower, P=0.098), and mean daily BGL (reduced 10%, n.s.) throughout the 7 weeks of training, as well as an unchanged daily insulin dose are consistent with improved metabolic control.

The degree of glycosylation of HbA_{1c} has been shown to respond within 1 week to heightened hyperglycaemia (Dunn *et al.*, 1979; Boden *et al.*, 1980), but to fall more slowly after restitution of lower levels of glycaemia, and therefore may be less sensitive in detecting improving metabolic conditions (Boden *et al.*, 1980). Thus, the lower HbA_{1c} evident after training in the present study may represent an underestimate of the effect of sprint training. However, consideration must be given to that fact that subjects were requested to regularly monitor their BGL (four times daily) over the duration of the study. Increased BGL monitoring may have facilitated improved control.

Results of the present study, i.e. reduced HbA_{1c} but similar daily insulin dose, are similar to those obtained after resistance training (Durak *et al.*, 1990) and combined resistance and higher intensity aerobic training (Peterson *et al.*, 1979; Jovanovic-Peterson *et al.*, 1989). In contrast, competitive athletes with type 1 DM, who trained both aerobically and anaerobically, had poorer metabolic control, and although the athletes required less insulin than sedentary subjects, insulin sensitivity was not enhanced (Ebeling *et al.*, 1995). No attempt was made in the current study to control the subjects' diet during the 7 weeks of training. It is conceivable that as a consequence of the increased energy requirements of exercise training, that subjects increased their dietary intake (Zinman *et al.*, 1984), which may have militated against finding a significant effect of training on daily insulin dosage.

Based upon the mean HbA_{1c} and the mean duration of diabetes of subjects in the current study, it is very likely that the majority of T1D subjects had a degree of peripheral insulin resistance (DeFronzo *et al.*, 1982; Yki-Järvinen & Koivisto, 1986; Nuutila *et al.*, 1993). Whether or not sprint training enhanced insulin sensitivity in the non-diabetic subjects in the present study is uncertain, however, the possibility is suggested by an improvement in HbA_{1c} in combination with an unchanged insulin dose.

Supervised sprint cycle training in the present study was undertaken at times that were most convenient to each individual subject, and was therefore not consistently related to the timing of insulin administration. High intensity exercise increased plasma glucose concentration, particularly in the subjects with type 1 DM in whom an unabated rise was observed when tested in the postabsorptive state and when relatively insulinopaenic. It is possible that subjects who undertook training in a similar state experienced higher plasma glucose concentrations, whereas those subjects who trained when insulin replete, or who injected insulin shortly after the training session, may have been exposed to lower glucose concentrations over the 7 week training period. Thus, the relationship of the timing of sprint training sessions to insulin administration may be an important determinant of the overall effect of training upon indices of metabolic control.

In summary, the present study is the first to examine the effect of sprint training in subjects with type 1 DM. HbA_{1c} was reduced, whilst daily insulin dose was unchanged. A positive effect of sprint training upon metabolic control and possibly upon insulin sensitivity is important given the deleterious effects of chronic hyperglycaemia (DCCT Research Group, 1993; DCCT Research Group, 1995) and hyperinsulinaemia (Stout, 1990) on long-term health. Although sprint training in the present study was only undertaken for a short period, preliminary indications are that such training may be undertaken with metabolic safety in young subjects without overt diabetic complications.

4.5.2 Cardiorespiratory responses and performance during maximal exercise in subjects with and without type 1 diabetes mellitus (DM)

4.5.2.1 Similar pre-training cardiorespiratory status during incremental exercise for each group

Prior to training no subject was specifically exercise trained, but most were recreationally active. Every attempt was made to match each subject with type 1 DM with a non-diabetic subject based upon reported exercise history and anthropometric variables. The similar absolute values for pre-training mean $\dot{V}O_{2peak}$, peak $\dot{V}_E$ and $\dot{V}CO_2$, and peak power output in each group attest to the success of such. None of the subjects with type 1 DM in the current study had clinical evidence of autonomic neuropathy, supported by similar maximum HR in each group.

In contrast to the pre-training results of the current study, a number of cross-sectional comparisons have demonstrated a lower $\dot{V}O_{2peak}$ in subjects with type 1 DM than in non-diabetic subjects (Larsson *et al.*, 1964; Wanke *et al.*, 1991; Baraldi *et al.*, 1992; Matthys *et al.*, 1996; Niranjan *et al.*, 1997), and inferred it to be a consequence of diabetes; however none of these studies accounted for exercise history. However, an increased oxygen cost of breathing was evident during exercise in both normo- and hyperglycaemic subjects with type 1 DM in comparison to non-diabetics (Niranjan *et al.*, 1997). Since the oxygen cost of ventilation near maximal exercise constitutes a considerable proportion of the increase in whole-body $\dot{V}O_2$ (Aaron *et al.*, 1992) and consequently compromises muscle perfusion and $\dot{V}O_2$ (Harms *et al.*, 1997; Harms *et al.*, 1998), a higher oxygen cost may exacerbate the reduction in blood flow to exercising muscles, and perhaps result in earlier fatigue. However, the almost identical peak power outputs in the incremental tests would argue against this effect being of major significance for the subjects with type 1 DM in the present study. In further support of the current study, when subjects with and without diabetes were matched for habitual exercise volume and intensity, no difference was found in $\dot{V}O_{2peak}$ or maximum HR, except in subjects with autonomic neuropathy in whom $\dot{V}O_{2peak}$ was 24% lower (Veves *et al.*, 1997). The authors concluded that although tissue glycosylation (indicated for example by reduced joint mobility) was present to

an equal degree in the three groups of patients with type 1 DM, the impairment of ventilation was not severe enough to reduce $\dot{V}O_{2max}$ (Veves *et al.*, 1997), and that exercise history and training status were of greater importance than the underlying metabolic disturbances of type 1 diabetes in determining $\dot{V}O_{2max}$.

4.5.2.2 Effects of sprint training on cardiorespiratory responses during incremental exercise

The current study is the first to report upon the effects of sprint training upon $\dot{V}O_{2peak}$ and selected cardiorespiratory variables in subjects with type 1 DM. In the incremental tests to exhaustion in the current study, peak power was increased 10% after training, with nearly identical changes in the group with type 1 DM and the non-diabetic group. Each of peak incremental $\dot{V}_E$ (10-12%), $\dot{V}O_2$ (2.5-7.2%), and $\dot{V}CO_2$ (3.4-7.2%) was increased after sprint training, with no significant difference between groups, and maximum HR was unchanged. The results for the non-diabetic group agree very closely with the results of McKenna *et al.* (1997b), the only other study to comprehensively examine the effect of sprint training on these variables. A number of previous studies have demonstrated increased $\dot{V}O_{2peak}$ after sprint training (Table 2.3).

The mechanisms by which $\dot{V}O_{2peak}$ may be increased in non-diabetics following sprint training remain to be fully elucidated. With regard to type 1 DM, the effects of probable tissue glycosylation in an asymptomatic, normotensive subject group, such as the one in the present study, may or may not be significant in terms of effects upon the ability to increase $\dot{V}O_{2peak}$ with sprint training. A similar $\dot{V}O_{2peak}$ in equally trained subjects (Veves *et al.*, 1997) and similar increases in $\dot{V}O_{2peak}$ (compared to non-diabetics) with training (Larsson *et al.*, 1964; Wallberg-Henriksson *et al.*, 1984; Zinman *et al.*, 1984) may suggest that the effects of glycosylation are not discernable in young, otherwise healthy groups with type 1 DM. However, the relative contributions of the mechanisms by which $\dot{V}O_{2peak}$ is improved may differ between subjects with and without diabetes, however have never been examined.

An incidental finding in the second study was that the $\dot{V}O_{2peak}$ and peak $\dot{V}_E$ were unchanged as a consequence of sprint training in the females, in contrast to the increases found in the male subjects (and the overall increase for the ND and T1D groups). Peak $\dot{V}_E$ attained in the incremental test was lower in the females in the current study prior to training, consistent with published data (American Thoracic Society, 1991). Whilst highly fit endurance trained females have higher peak $\dot{V}_E$ than less fit active females (McClaran *et al.*, 1998), suggesting that endurance training increases peak $\dot{V}_E$ in females, no previous studies have examined the effect of sprint training on ventilation in females. Whilst the current study was not designed to compare female with male subjects, these findings are interesting and worthy of further investigation.

4.5.2.3 Effects of sprint training on performance and cardiorespiratory responses during constant load exercise

Exercise to exhaustion

Time to exhaustion in the non-invasive respiratory test was increased 42 and 64% in the ND and T1D groups, respectively, after sprint training. The increase in time to exhaustion in the post-training respiratory test (PostFResp) tended to be greater (P=0.06) for the group with type 1 DM than in the ND group. This tendency cannot be attributed to pre-training differences since the groups were very similar.

Peak $\dot{V}O_2$ during exhausting constant load exercise was unchanged with training, although a tendency to increase was evident in the ND group (8%; P=0.095) whilst little change (1%) was evident in the T1D group (however, the groups did not differ significantly). Peak $\dot{V}CO_2$ and $\dot{V}_E$ remained closely associated after training, with a 31% increase in each variable in the T1D group and 18-24% increases in the ND group. Peak $\dot{V}CO_2$ in the test to exhaustion tended (P=0.06) to be higher in the T1D group than in the ND group, and since peak $\dot{V}O_2$ was unchanged, may have been related to the development of greater plasma acidosis, consistent with the tendency for greater production of work.

Matched-work exercise

In the matched-work test after training (PostMResp), peak and mean $\dot{V}_E$ and $\dot{V}CO_2$ were lower and peak $\dot{V}O_2$ was lower, whereas both mean HR and $\dot{V}O_2$ were similar (the latter lower in the T1D group) compared with the exhausting pre-training test (PreFResp). The present results support the suggestion that exercise training may have an indirect affect on respiratory muscle work by reducing the ventilatory response to heavy exercise (Harms & Dempsey, 1999). The finding of lower peak $\dot{V}O_2$ during matched-work exercise was contrary to the hypothesis (see section 4.2.2.4), whereas lower $\dot{V}_E$ and $\dot{V}CO_2$ confirmed the hypothesis.

A lower or similar pulmonary $\dot{V}O_2$ during exercise may ostensibly seem be at variance with the muscle results, which strongly supported enhanced oxidative metabolism after sprint training (see sections 4.5.6.2, 4.5.6.3). However, an interesting possibility has been suggested by recent work that examined the effect of altering the work of breathing on the partitioning of respiratory and exercising leg muscle $\dot{V}O_2$ and blood flow ($\dot{Q}$) during exercise at $\dot{V}O_{2max}$ (Harms *et al.*, 1997; Harms *et al.*, 1998). Respiratory muscle unloading, achieved by use of a proportional-assist ventilator, reduced the work of breathing ($\dot{V}_E$ was not altered) and hence the respiratory muscle $\dot{V}O_2$ whilst exercising at $\dot{V}O_{2max}$ (Harms *et al.*, 1997; Harms *et al.*, 1998). Consequently, whole-body $\dot{V}O_2$ and $\dot{Q}$ were reduced (Harms *et al.*, 1998), whilst leg $\dot{V}O_2$ and $\dot{Q}$ were increased (Harms *et al.*, 1997). Further, respiratory muscle unloading allowed a longer time to fatigue when exercising at $\dot{V}O_{2max}$ (Harms *et al.*, 2000). In the present study, the oxygen cost of breathing would have been reduced in the PostMResp test as a consequence of the lower peak and mean $\dot{V}_E$ (Aaron *et al.*, 1992). Therefore, the reduced work of breathing during maximal matched-work exercise may have permitted a higher blood flow and oxygen delivery to the exercising muscles, and thus facilitated higher oxidative metabolism after sprint training, even though whole-body peak and mean $\dot{V}O_2$ were similar or lower than in the PreFResp test. This intriguing possibility remains to be tested.

A further incidental finding was that the effect of sprint training on peak and mean $\dot{V}_E$ during constant-load exercise differed between the females and males in each group. In contrast to the males, after training, neither peak nor mean $\dot{V}_E$ were lower in the females in the PostMResp test. The work of breathing during moderate to strenuous exercise is higher in females than in males, due to the high prevalence of expiratory flow limitation in the former, and consequently, it has been suggested that limb blood flow may be compromised to a greater extent in females than in males during strenuous exercise (Harms & Dempsey, 1999). A relatively higher $\dot{V}_E$ (with respect to the effect in the men) and higher work of breathing suggest that the 'stealing' of $\dot{V}O_2$ by the respiratory muscles during intense exercise may have been greater in the females. If so, an effect on muscle metabolism may be anticipated. However, analysis of various muscle metabolism variables for the females versus the males (for variables for which no difference was demonstrated between the ND and T1D groups) revealed no difference. Muscle ATP degradation in the matched-work test did differ between males and males, however since sprint training effects on this variable also differed between the ND and T1D groups, no conclusions can be drawn. Further investigation, with a sprint training study designed specifically to examine differences between males and females, is warranted.

Respiratory responses across all three respiratory tests

Across the three respiratory tests (PreFResp, PostFResp, PostMResp), mean ventilatory equivalents for CO_2 ($\dot{V}_E/\dot{V}CO_2$) and O_2 ($\dot{V}_E/\dot{V}O_2$) were lower in the T1D group than in the ND group, however peak values did not differ. Neither $\dot{V}CO_2$ nor plasma $[H^+]$ (measured in the invasive test) differed between groups in the matched-work tests, and it is therefore evident that relative hypoventilation did not occur. $\dot{V}_E$ remained closely matched to $\dot{V}CO_2$. Reduced $\dot{V}_E/\dot{V}O_2$ at maximal exercise has been demonstrated in subjects with type 1 diabetes (Niranjan *et al.*, 1997), and was also evident based on calculation of the ratio from data in another study (Wanke *et al.*, 1991). In both studies a lower $\dot{V}O_{2peak}$ was evident in the groups with diabetes (Wanke *et al.*, 1991; Niranjan *et al.*, 1997). A reduced diffusing capacity, and a higher oxygen cost of breathing during exercise was measured in subjects with type 1 DM and was suggested to be related to glycosylation of lung tissue due to chronic

hyperglycaemia; perhaps explaining unequal reductions in $\dot{V}_E$ and $\dot{V}O_2$ (Niranjan *et al.*, 1997). In the present study neither peak $\dot{V}_E$ nor peak $\dot{V}O_2$ was reduced, and performance did not appear to be affected. However the consistent reduction in mean $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ during constant load exercise in the T1D group may represent an early manifestation of tissue glycosylation, however this is highly speculative.

In summary, the T1D and ND groups were well matched in terms of cardiorespiratory responses to exhausting incremental exercise prior to training. Consequently, the mean power outputs calculated to elicit 130% $\dot{V}O_{2\,peak}$, were very similar. Time to exhaustion in the constant load exercise (130% pre-training $\dot{V}O_{2\,peak}$) test was extended after sprint training in both groups, however to a greater degree in the T1D group. During matched-work exercise after sprint training in both groups, peak and mean $\dot{V}_E$ and $\dot{V}CO_2$ were reduced, as was peak $\dot{V}O_2$. Lower peak $\dot{V}O_2$ was an unexpected finding. An intriguing possibility is that the reduced the work of breathing (lower $\dot{V}_E$) during matched-work exercise may have allowed higher blood flow to the exercising muscles, as has been demonstrated in respiratory muscle unloading. However, this remains to be tested. The ventilatory equivalents for O_2 and CO_2 were lower across all three respiratory tests in the T1D group; the mechanism for such cannot be determined from the present data. However, the cardiorespiratory responses to maximal exercise were overall very similar in the T1D and ND groups.

4.5.3 The T1D 'Resting Study'

The 'Resting Study' was conducted in the postabsorptive (overnight fasted) state, at the same time of day, and over the same time period (approximately 2.5 hours) as the invasive exercise tests (PreFB, PostMB). The study was primarily designed to examine the effect of withholding insulin administration upon acute metabolic control in the subjects with type 1 DM, thus providing a non-exercise (baseline) comparison for the invasive exercise tests. In addition, the study provided a non-exercise comparison for the effects of postural change during the exercise tests. Plasma free

insulin did not differ significantly over the 2.5-hour period, indicating that subjects did not become progressively insulinopaenic to a clinically significant degree. This was reflected in the preservation, or slight lowering of resting values in several other metabolic variables, i.e. plasma glucose, [K^+], [Na^+], [Lac^-], [FFA]. Free insulin in the 'Resting Study' did not differ from free insulin rest in the invasive exercise tests.

In summary, the results of the 'Resting Study' indicate that changes observed in the invasive exercise tests (PreFB, PostMB) were not due to a natural deterioration of metabolic control due to the effect of delaying the usual morning insulin dose, but rather were due to the exercise bout per se.

4.5.4 Glucoregulation during and after maximal exercise and effects of sprint training exercise in subjects with and without type 1 diabetes mellitus (DM)

4.5.4.1 Insulin

Acute response to maximal exercise

In the ND group, serum IRI was slightly lower following intense exhausting exercise, doubled early in recovery, then slowly declined thereafter. A similar response was found following intermittent maximal exercise (Hermansen *et al.*, 1970), and intense submaximal to maximal exercise (Calles *et al.*, 1983; Mitchell *et al.*, 1988; Kjær *et al.*, 1990; Marliss *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994a; Sigal *et al.*, 1994b; Sigal *et al.*, 1996; Langfort *et al.*, 1997; Manzon *et al.*, 1998). The doubling of insulin in early recovery is secondary to the sharp rise in plasma glucose concentration that occurs consequent to a greater rate of hepatic glucose production (appearance, R_a) than peripheral glucose uptake (disappearance, R_d) (Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994b).

The values obtained for fasting, free immunoreactive insulin (IRI) concentration at rest in the T1D subjects did not differ from basal IRI in the ND group. However, as expected, in marked contrast to the ND group, free IRI was similar to resting levels at 5 min recovery, then fell slowly below resting values for the remainder of recovery in the T1D group. A similar reduction in free IRI during recovery occurred in one other study in a group with type 1 DM receiving basal level continuous subcutaneous

insulin infusion (CSII) (Mitchell *et al.*, 1988). The fall in free IRI after 5 min recovery is unlikely to be explained either by passive fluid shifts as a consequence of postural alteration, since in the T1D 'Resting Study' IRI did not change significantly with posture or over time (see section 4.5.3). Whilst C-peptide was not measured, the average duration of diabetes was approximately 7 years, and thus residual insulin secretion (which may be blunted during intense exercise and increased in recovery), would be expected to be minimal (Yki-Järvinen & Koivisto, 1986); and even if present, would increase rather than decrease plasma IRI in recovery. Splanchnic blood flow and clearance is substantially reduced during intense exercise (Rowell *et al.*, 1964; Rowell, 1993), however, in recovery, restoration of the considerable splanchnic blood flow (Bradley *et al.*, 1945; Rowell *et al.*, 1964) may enhance insulin clearance. A further factor may be that the liver may clear insulin more completely at lower concentrations (Björntorp, 1981), perhaps exacerbating the effects of blood flow redistribution.

Effects of sprint training

This is the first study to examine the effect of sprint training on basal IRI or the IRI response to intense exercise in either non-diabetics or subjects with type 1 DM. Whilst marked differences between the ND and T1D groups were evident with regard to the change in serum IRI concentration during recovery from intense exercise, sprint training did not alter basal IRI, nor did it alter responses during exercise or recovery within each group. However, whilst pre- and post-training IRI was similar, it is possible that sprint training altered insulin sensitivity. This interesting and potentially clinically valuable possibility has not been investigated.

In summary, basal IRI did not differ between the T1D and ND groups. The expected brisk IRI response to maximal exercise was evident in the ND group, whilst a slow reduction was evident in the T1D group. Thus, in the T1D group, glucoregulation during maximal exercise and recovery must be viewed in the context of a 'fixed' insulin concentration. Sprint training had no affect on serum IRI, although it is not known whether sprint training alters insulin sensitivity.

4.5.4.2 Glucagon

Acute response to maximal exercise

Plasma immunoreactive glucagon (IRG) rose by ~10% after 5 min recovery from exhausting exercise prior to training, slowly fell, then rose again in late recovery. At 5 min recovery in the ND group, peak concentrations of both plasma glucose ([PG]) and IRI occurred, and would be anticipated to inhibit glucagon secretion (Raskin *et al.*, 1975; Alford & Chisholm, 1979; Lewis *et al.*, 1996), whilst the stimulatory effect of the catecholamines (Alford & Chisholm, 1979) was rapidly diminishing. In the T1D group, [PG] was ~2-fold higher than in the ND group, but IRI was unchanged from rest, however the IRG response did not differ between groups; therefore the heightened hyperglycaemia may have compensated for the lack of physiological insulin increase. The small rise in IRG is probably explained by the haemoconcentration associated with intense exercise. Prior to training, plasma volume was reduced by ~16 % after 5 min recovery, then expanded to resting volume by 20 min and further expanded for the remainder of recovery, so that a haemodilution was evident at 45 and 60 min. Thus plasma volume contraction and re-expansion could fully account for the rise and subsequent fall in IRG; although whether or not secretion was concomitantly reduced, and was masked by the effect of haemoconcentration cannot be determined. However, in both groups IRG rose to be ~17 % above resting concentration by 60 min recovery, despite concurrent haemodilution, which suggests that secretion may have increased, or clearance decreased. The small rise in IRG in early recovery and slow return to baseline, with no difference between T1D and ND groups, is similar to findings in another study which employed high intensity exercise in untrained subjects (Purdon *et al.*, 1993).

Effects of sprint training

This is the first study to report upon IRG during maximal exercise after sprint training. Sprint training did not alter resting IRG or the change in IRG during intense matched-work exercise or recovery. During brief maximal exercise, an increase in IRG is likely to be of only minor significance, given that much of the carbohydrate used to sustain power output is derived from muscle (Spriet *et al.*, 1989), rather than liver glycogen. The lack of effect upon IRG during exercise and recovery following sprint training is consistent with the similar IRG response in moderately-trained non-diabetics versus either untrained or similarly trained subjects with type 1 DM when

exercising to exhaustion at 89-100 % $\dot{V}O_{2peak}$ (Purdon *et al.*, 1993; Sigal *et al.*, 1994b).

Effect of the IRG to IRI ratio

In the current study, the ratio of IRG to IRI increased during exercise and was not different after 7 weeks of sprint training. In recovery in the ND group, a sharp fall in the IRG to IRI ratio (IRG/IRI) at 5 and 20 min recovery was evident, and along with the rapid reduction in plasma catecholamines, may have acted respectively to enhance disappearance and reduce hepatic glucose output (HGO), thus aiding the return of plasma glucose towards resting levels. However, in recovery from maximal exercise, glucagon may be of greater importance in subjects with type 1 DM. Although IRG did not differ between groups, the relative effect of glucagon on the liver would be greater in recovery in the subjects with type 1 DM due to their lack of insulin rise (in fact, falling insulin levels). The progressively increasing IRG/IRI in the T1D group may have contributed to the progressive rise in plasma glucose concentrations, especially in late recovery; although conversely the suppressive effect of hyperglycaemia on α-cell secretion may have attenuated the effect.

In summary, a small rise was evident in IRG following maximal exercise both before and after training and was probably consequent to haemoconcentration. However, during late recovery IRG rose, despite haemodilution, suggesting that secretion may have increased. In the T1D group, the lack of rise in insulin resulted in a progressively higher IRG to IRI ratio, which may have contributed to the hyperglycaemia in recovery.

4.5.4.3 Catecholamines

Acute effect of maximal exercise

In the current study, peak [NAdr] approximated the venous threshold for glucoregulatory actions (Silverberg *et al.*, 1978), whilst [Adr] considerably exceeded both the arterial (Freyschuss *et al.*, 1986) and venous (Clutter *et al.*, 1980) glucoregulatory thresholds. The catecholamines would therefore be expected to increase the rate of appearance of glucose (glucose R_a) and reduce glucose clearance, thereby promoting an increase in plasma glucose concentration ([PG]); increase

plasma lactate concentration ([Lac$^-$]); and perhaps reduce insulin secretion (Silverberg *et al.*, 1978; Clutter *et al.*, 1980; Freyschuss *et al.*, 1986). The increase in the IRG/IRI ratio, coupled with the marked rises in [Adr] and [NAdr] explain the observed increase in [PG], which peaked after 5 min recovery in the ND group, but continued to rise, unabated, in the T1D group throughout recovery.

In the T1D group, plasma [NAdr] was higher immediately after exercise and in early recovery than in the ND group, regardless of training status; [Adr] was also higher, although it did not differ significantly (P=0.11). Since catecholamine concentrations increase in proportion to exercise duration (Galbo *et al.*, 1975; Calles *et al.*, 1983; Kraemer *et al.*, 1991), higher [NAdr] may be partly explained by a tendency for a longer time to fatigue in the invasive PreFB test than the ND group; a difference that was necessarily preserved in the post-training test (PostMB) in which work was matched to the PreFB test. Additionally, it has been suggested that subjects with type 1 DM may be more dependent on adrenergic regulation of glucose homeostasis than non-diabetics (Simonson *et al.*, 1984).

Several other studies have demonstrated no difference in catecholamine concentrations during intense submaximal exercise or recovery between non-diabetic and type 1 DM subjects who were maintained on basal insulin doses during exercise (Mitchell *et al.*, 1988; Purdon *et al.*, 1993; Raguso *et al.*, 1995). In moderately-trained subjects with type 1 DM, 2-fold lower peak [Adr], but similar [NAdr] was evident following exercise to exhaustion at 89 – 98 % $\dot{V}O_{2max}$; however, the overall catecholamine response during exercise and recovery did not differ from non-diabetic subjects (Sigal *et al.*, 1994b). The difference between these studies and the current one, may be related to exercise intensity and/or the absence of basal insulin infusion during exercise.

Effects of sprint training

The current study demonstrated an attenuated catecholamine response to matched-work maximal exercise after sprint training in both groups. In PostMB, [NAdr] was lower immediately after exercise (7%) and in recovery (13–26%) than in the pre-training test conducted to exhaustion (PreFB). Plasma adrenaline also tended to be

lower after training (P=0.09), with the peak concentration at 1 min recovery being 31% lower. These results are similar to those found in the first study of the current thesis. Similarly, in the only other sprint training study to report catecholamine concentrations, after 8 weeks of training, catecholamine concentrations after a single 2-min bout of exercise at 110 % pre-training $\dot{V}O_{2max}$ tended to be lower (13–20%) (Nevill *et al.*, 1989). However, since the first post-exercise sample was not obtained until 3 min recovery, the peak concentrations were probably missed and the effect of sprint training was not clear.

As a consequence of a higher $\dot{V}O_{2peak}$ following training in the current study, at the same absolute work load the exercise intensity was reduced ~5%, which is rather close to the 7% reduction in [NAdr] immediately after exercise in the PostMB test. This supports the expected qualitative relationship between [NAdr] and overall sympathetic nervous activity (Rowell, 1993), and the notion that the influence of training on the hormonal response is related to its effect upon $\dot{V}O_{2max}$ (Kjær, 1989).

Reduced catecholamine concentrations during matched-work exercise after sprint training may be anticipated to reduce the appearance of glucose, via reduced hepatic stimulation. Consistent with this, the rise in plasma glucose was reduced 22-26% during exercise and recovery after matched-work exercise after training, however was not statistically significant.

In summary, the increase in concentrations of the catecholamines during and after maximal exercise was of sufficient magnitude to approximate or exceed the thresholds for their glucoregulatory actions. [NAdr] was higher in the T1D group, perhaps related to the tendency for a longer time to exhaustion. After sprint training, [NAdr] and [Adr] tended to be lower during matched-work exercise, consistent with a tendency for lower plasma glucose.

4.5.4.4 Free fatty acids

Acute effect of maximal exercise

Free fatty acid concentration ([FFA]) was higher at rest in the T1D group, despite no difference in IRI between groups. This finding is consistent with reduced insulin

sensitivity in subjects with type 1 DM, which would necessitate higher free insulin levels to achieve the same degree of lipolytic suppression (Jensen *et al.*, 1989). [FFA] was reduced immediately after exercise and remained suppressed until 45 min recovery in the T1D group, and for the duration of recovery in the ND group.

The potent antilipolytic effect of insulin (Yeaman, 1990; Rebrin *et al.*, 1995; Lewis *et al.*, 1996; Saltiel, 1996; Lewis *et al.*, 1997) would have been diminished by the lack of physiological hyperinsulinaemia in the T1D group during recovery. Reduced IRI, coupled with higher catecholamine concentrations (the latter able to stimulate lipolysis even during hyperinsulinaemia in subjects with moderately-controlled type 1 DM, albeit at rest (Cohen *et al.*, 1996)) in early recovery favours lipolysis, and may explain the higher [FFA] in the T1D group. However, antagonistic to the lipolytic effect of reduced IRI and increased [NAdr] and [Adr] was the substantial accumulation of lactate, which peaked in early recovery. The similarly high [Lac^-] in both groups would be anticipated to suppress net FFA release (Boyd *et al.*, 1974; Issekutz *et al.*, 1975; Green *et al.*, 1979; Jones *et al.*, 1980) to a comparable degree. However the lack of the antilipolytic rise in insulin in recovery in the T1D group presumably allowed a more rapid recovery of FFA release towards resting levels. This notion is supported by the very similar [FFA] in recovery from exhaustive submaximal exercise (89-98% $\dot{V}O_{2max}$) in non-diabetic subjects and moderately-trained subjects with type 1 DM whose insulin infusion rate was doubled immediately after exercise (to mimic the non-diabetic response), whilst those subjects with type 1 DM who remained on a basal infusion (and were either hyper- or euglycaemic prior to exercise) tended to have higher [FFA], despite all subject groups having identical plasma [Lac^-] (Sigal *et al.*, 1994b).

Considerable accumulation of lactate occurred in the current study, with peak concentration (2-5 min recovery) approximating 16 $mmol \cdot l^{-1}$ prior to training, and 13.5 $mmol \cdot l^{-1}$ after training; well in excess of the concentrations reported to reduce net FFA release *in vitro* and *in vivo*. Although FFA R_a was not measured in the current study, plasma [Lac^-] tended to be inversely correlated ($r = -0.25$, $P=0.08$) with plasma [FFA] in the T1D group, however, no correlation was evident in the ND group (r = -

0.20, P=0.20), perhaps due to the greater suppressive effect of higher IRI in the latter group.

Effect of sprint training

This is the first sprint training study to examine FFA during maximal exercise. Sprint training did not alter the plasma FFA response during and after maximal matched-work exercise in either group.

In summary, resting FFA was higher in the T1D group. Maximal exhausting exercise reduced FFA in both the T1D and ND group. In late recovery FFA rose again in the T1D group, but remained reduced in the ND group. The physiological hyperinsulinaemia in the ND group, coupled with high [Lac$^-$] in recovery probably explains the response. In the T1D group, high [Lac$^-$] was presumably sufficient to account for the lack of insulin rise, although in late recovery when [Lac$^-$] was lower, FFA rose again. Sprint training appeared to have no affect on FFA, although based upon concentration alone, no conclusions regarding rates of release and uptake can be made.

4.5.4.5 Plasma glucose and the integrated glucoregulatory response to maximal exercise

Resting concentrations and the acute effect of maximal exercise

Mild to moderate resting hyperglycaemia was evident in the T1D subjects in the current study, although IRI was similar in T1D and ND groups. In the non-diabetic, fasting, portal vein insulin concentration is two to three times that of the peripheral veins (Blackard & Nelson, 1970; Horwitz *et al.*, 1975), and thus even when peripheral IRI concentrations are similar in subjects with type 1 DM and in non-diabetics, hepatic hypoinsulinaemia will probably exist in type 1 DM as a consequence of the peripheral route of exogenous insulin administration (Hanna *et al.*, 1980). The hyperglycaemia in the T1D group indicates that the liver was relatively hypoinsulinaemic. However, since peripheral levels of free IRI in the T1D subjects did not differ from IRI in the non-diabetics, muscle and adipose tissue would have been similarly exposed in both groups (however, possible insulin resistance may necessitate higher levels to achieve the same effect in type 1 DM).

Clinically significant hyperglycaemia ensued after only a single bout of maximal exhausting exercise (~80 s) in the T1D group in the current study, and was both sustained and progressive. Prior to training [PG] peaked ~4 mmol·l^{-1} above resting values after 60 min recovery. In marked contrast, [PG] peaked ~1 mmol·l^{-1} above resting levels, then slowly fell, returning to resting levels by 45 min recovery in the ND group. In the ND group, the transient hyperglycaemia was approximately a third to a half the magnitude of that induced by 5-15 min exhausting intense submaximal to maximal exercise or ~65% of the rise generated by an 'all out' 30-s exercise bout (see Table 2.9).

A previous report in which untrained subjects with type 1 DM cycled for a considerably longer period to exhaustion (10 min, 80% $\dot{V}O_{2max}$), with a continuous subcutaneous insulin infusion t basal levels, also found progressive hyperglycaemia, which peaked ~ 3 mmol·l^{-1} above resting levels after 120 min recovery (Mitchell *et al.*, 1988). However, in both untrained (Purdon *et al.*, 1993) and trained (Sigal *et al.*, 1994b) subjects with type 1 DM who underwent continuous i.v. insulin infusion (sufficient to maintain euglycaemia at rest) during and after exhaustive exercise at 89-98% $\dot{V}O_{2max}$, hyperglycaemia (~2.6-3 mmol·l^{-1} above baseline) was sustained throughout 120 min recovery, but was not progressive. The metabolic clearance rate (MCR) of glucose was lower in subjects with type 1 DM compared to non-diabetics and was attributable to the lack of physiological hyperinsulinaemia in recovery in the former group (Sigal *et al.*, 1994b). Thus in the current study it can be inferred that MCR was lower during recovery in the T1D group than in the ND group, especially since the present subjects were not maintained on a basal infusion of insulin.

The lower MCR for glucose, consequent to relative hypoinsulinaemia, would have directly contributed to the greater rise in [PG] in recovery in the T1D group in the current study. Indirect effects of relative hypoinsulinaemia on hepatic glucose output (Levine & Fritz, 1956; Lewis *et al.*, 1996; Mittleman *et al.*, 1997) may also have contributed to changes in [PG]. Free fatty acids were higher throughout testing in the T1D group, and may provide the liver with more energy substrate for gluconeogenesis in recovery (Ruderman *et al.*, 1969). Since the fall in glucose R_a during recovery is probably dependent more upon the fall in catecholamines than a rise in insulin

(Purdon *et al.*, 1993), the higher catecholamine concentrations in the T1D may have attenuated the fall in R_a and thus also contributed to the sustained hyperglycaemia.

The marked elevation in the catecholamines is thought to be the primary determinant of the increment in glucose R_a during intense exercise (Calles *et al.*, 1983; Kjær *et al.*, 1991; Marliss *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994b), albeit with some dispute (Kjær *et al.*, 1993), whilst the increase in the IRG/IRI ratio is of lesser significance, though may play a small role (Sigal *et al.*, 1996) (see section 4.5.4.2). One study (Kjær *et al.*, 1986) reported a 5-fold increase in R_a and a 2-fold increase in R_d, measured 60 s after 2 min non-exhausting exercise at 110% $\dot{V}O_{2max}$ (preceded by exercise at 60, then 100% $\dot{V}O_{2max}$). Although tracer estimates under such non-steady-state conditions must be viewed with caution (Marliss *et al.*, 1992; Coggan *et al.*, 1995), the changes in R_a and R_d were of a similar magnitude to those during exercise conducted at 80–85% $\dot{V}O_{2max}$ (see Table 2.9). Since in the current study, IRG/IRI rose during exercise, and [NAdr] approximated, and [Adr] considerably exceeded the arterial and venous thresholds associated with increased glucose R_a (Silverberg *et al.*, 1978; Clutter *et al.*, 1980; Freyschuss *et al.*, 1986), it can be concluded that the glucose R_a exceeded R_d and accounted for most of the exercise-induced rise in [PG] in recovery (with a small contribution from haemoconcentration) in both T1D and ND groups.

Effect of sprint training

After sprint training, resting [PG] was not different compared with pre-training, and differed between, but not within groups. With regard to the T1D group, it was important to ensure similar resting [PG] prior to commencing exercise, since poorer metabolic control, may result in an attenuated rise in [PG] in response to exhausting exercise (89–98% $\dot{V}O_{2max}$) (Sigal *et al.*, 1994b), and thus confound attempts to examine the influence of training. Consistent with the hypothesis (see section 4.2.2.1) that sprint training would attenuate the rise in plasma glucose during matched-work exercise, in the PostMB test (matched-work), the rise in [PG] was 22–26% lower in both groups, however did not attain statistical significance. This is the first study to demonstrate a reduction in exercise-induced hyperglycaemia after a programme of sprint training.

One previous sprint training study (Nevill *et al.*, 1989) reported less rise in blood glucose in early but not later recovery after a 2-min run at 110% pre-training $\dot{V}O_{2max}$, but no change in catecholamines; however, since peak concentrations were probably missed, the relationship remained unclear. In the current study after training, IRG/IRI was unchanged but catecholamines were lower immediately after exercise and in recovery, and thus may be anticipated to have stimulated hepatic glucose output to a lesser degree, and thus presumably reduced R_a. No longitudinal studies have examined the change in R_a and R_d during maximal exercise at the same absolute work load. Since the catecholamines also act to restrain R_d (Marliss *et al.*, 1991; Marliss *et al.*, 1992; Purdon *et al.*, 1993; Sigal *et al.*, 1994b), lower catecholamine concentrations during exercise at the same absolute work load after training may result in a slightly higher R_d (especially since the IRG/IRI ratio did not differ) which may also have contributed to the reduction in hyperglycaemia in both T1D and ND groups.

The change in concentration of FFA during exercise and recovery did not differ after training in either group in the current study, so is unlikely to have made a difference to the reduction in hyperglycaemia. Plasma [Lac^-] was lower in both T1D and ND groups after training, and since high plasma [Lac^-] has been postulated to contribute to hepatic glucose output following intense exercise (Green & Newsholme, 1979), a reduction post-training may have reduced the lactate contribution to gluconeogenesis during recovery, and perhaps contributed to the lessening of hyperglycaemia.

Another possibility is that insulin sensitivity was enhanced in both groups following sprint training, however the present study did not address this issue.

The T1D 'Resting Study'

In the T1D 'Resting Study', [PG] was similar to resting values in the invasive exercise tests, and was slowly reduced over time. Thus metabolic deterioration did not occur in the time period employed in the current study, and therefore would not have contributed to the progressive hyperglycaemia evident in the T1D group during the maximal exercise tests.

In summary, plasma glucose concentration ([PG]) was higher in the T1D group than the ND group at all times as a consequence of hepatic hypoinsulinaemia. However, within each group, [PG] did not differ at rest before and after training. Acute maximal exhausting exercise resulted in a transient rise in [PG] in the ND group, but a sustained, progressive rise in the T1D group. The latter was in marked contrast to the slow reduction in [PG] over time in the T1D 'Resting Study'. The unabated hyperglycaemia in recovery in the T1D group highlights the importance of insulin (or lack thereof) in glucoregulation after maximal exercise. Higher plasma [NAdr] in the T1D group, would have contributed to the marked hyperglycaemia in early recovery, whilst the IRG to IRI ratio, higher plasma FFA and reduced glucose clearance would be anticipated to have contributed to the progressive increase in later recovery.

The rise in [PG] was attenuated to a similar degree in both groups during maximal matched-work exercise after sprint training, however was not statistically significant. Lower catecholamines and [Lac^-] after training may have contributed to the lessening of hyperglycaemia. It is also possible that insulin sensitivity was enhanced, however this has not been tested.

4.5.5 Ion regulation during maximal exercise in subjects with and without type 1 DM and effects of sprint training

4.5.5.1 Plasma [K^+] and [Na^+]

Rest and acute exercise

The current study is the first to report upon the effect of maximal exercise on plasma [K^+] and [Na^+] in subjects with type 1 DM. Prior to training, in the PreFB test (pre-training test to exhaustion), the plasma [K^+] and [Na^+] at rest, immediately after exercise, and in early recovery were very similar in the ND and T1D groups. However, from 45-60 min recovery plasma [Na^+] was lower, and after 60 min of recovery from exercise, plasma [K^+] was higher in the T1D group than in the ND group.

The finding of similar resting plasma [K^+] and [Na^+] in both groups (both before and after training), despite mean resting plasma glucose concentration being >10.5 mM in the T1D group, did not confirm the hypothesis (section 4.2.2.3), which was based upon findings in a previous study (McNair *et al.*, 1982). (See below in T1D 'Resting Study' for a discussion of resting values in relation to McNair *et al.*, 1982.) However, the finding of higher plasma [K^+] (and lower [Na^+]) in late recovery did confirm another hypothesis (section 4.2.2.3), although occurred somewhat later than hypothesised.

Higher plasma [K^+] and lower plasma [Na^+] late in recovery in PreFB in the T1D group may have been due in part to the exercise-induced hyperglycaemia, which progressively increased throughout recovery, and/or to the significant reduction in free insulin after 60 min recovery. The mechanism by which hyperglycaemia may induce hyperkalaemia and reduce [Na^+] in subjects with type 1 DM has been thought to be due to the ensuing hyperosmolality, rather than hyperglycaemia *per se* (Viberti, 1978). The hyperglycaemia evident in type 1 DM is due to a relative lack of insulin; thus when both glucose and insulin were infused in subjects with type 1 DM, plasma [K^+] fell (Farber *et al.*, 1951). Thus in the current study, the exercise-induced hyperglycaemia, which progressively increased throughout recovery, would have progressively increased plasma osmolality and combined with a fall in free insulin, contributed to higher plasma [K^+] and lower [Na^+] late in recovery. In contrast, in the ND group, increased insulin concentration in response to exercise-induced hyperglycaemia caused a rapid decline in plasma glucose, and hence rapidly reduced exercise-induced hyperosmolality.

With regard to the effect of maximal exercise, in the present study, there was no difference between groups in peak plasma [K^+] or in concentrations in early recovery. No previous studies had investigated the plasma K^+ response to intense exercise (which results in marked, but short-lived increases in plasma K^+ in non-diabetic subjects - see Table 2.10 and sections 2.10.2 and 2.13.1) in subjects with type 1 DM, despite reports of dysregulation of potassium metabolism at rest. Dysregulation was manifested variously by resting or spontaneous hyperkalaemia, hyperkalaemia in response to an oral glucose load, reduced whole-body K^+, and reduced muscle K^+ content (Walsh *et al.*, 1974; Viberti, 1978; Nicolis *et al.*, 1981; McNair *et al.*, 1982;

Sjögren *et al.*, 1986; Shalwitz *et al.*, 1991). The present study demonstrates that in a single bout of brief maximal exercise, insulin appears to have little effect on acute regulation of hyperkalaemia, probably being overridden by effects of muscle contraction and actions of the catecholamines.

Effects of sprint training

This is the first report of a significant reduction ($P<0.05$) in exercise-induced hyperkalaemia when work was matched after training, in a group with type 1 DM. In support of the first study of this thesis, reduced exercise-induced hyperkalaemia was also evident in the non-diabetic group during matched-work exercise after sprint training. Sprint training also significantly reduced plasma [Na^+] in both T1D and ND groups ($P<0.05$).

In the post-training matched-work exercise test (PostMB), plasma free insulin concentration did not differ from PreFB, and although plasma glucose concentration was 22% lower in the T1D group, it was not significant. In addition to the mechanisms proposed to contribute to improved K^+ regulation following sprint training in non-diabetic subjects (see section 3.5.2.1), the trend to reduced hyperglycaemia in the T1D group after training may have contributed to the lower recovery plasma [K^+]. It is also possible that insulin sensitivity may have increased following training, however it is not known if insulin resistance affects the skeletal muscle Na^+, K^+ pump.

The lower plasma [Na^+] evident in the T1D group at 45 and 60 min of recovery in the PreFB test, was also evident after training in the PostMB test, and [Na^+] was lower in the T1D group across both tests. The latter finding agrees with the results of McKenna *et al.* (1997a), who reported lower arterial and femoral venous [Na^+] after sprint training.

The T1D 'Resting Study'

In the current study, arterialised venous plasma [K^+], measured at rest in the subjects with type 1 DM in the 'Resting study', did not differ (except briefly with upright posture) over time, and averaged 4.23 ± 0.07 mmol·l^{-1}. This value was lower than that

found in 14 patients with type 1 DM, from whom insulin had been withheld for a longer period (24 hours), and in whom mean resting venous plasma [K^+] was 4.6 mmol·l^{-1}, which did not differ from levels in 70 non-diabetic subjects (4.4 mmol·l^{-1}) (Farber *et al.*, 1951). However, another study (McNair *et al.*, 1982), in which a much larger number of patients (who had fasted overnight) with type 1 DM was studied, reported a graded phenomena with regard to serum [K^+] at rest. A positive correlation was found between resting [PG] and [K^+] (McNair *et al.*, 1982). Normal serum [K^+] (4.35–4.40 mmol·l^{-1}) occurred when blood glucose level (BGL) was between 2.5-10.5 mmol·l^{-1}, but progressively higher [K^+] was evident when BGL was in the range of 11.0–29.5 mmol·l^{-1}, with the mean of the latter range being 4.60 mmol·l^{-1} (McNair *et al.*, 1982). Plasma glucose in the T1D 'Resting Study', and at rest in the pre- and post-training exercise tests (PreFB, PostMB), ranged between 12–13 mmol·l^{-1}, however plasma [K^+] at rest did not differ between T1D and ND groups, nor was there any correlation between plasma glucose and [K^+] for the T1D group alone. This may be due to the younger age of subjects in the current study, their lack of clinical complications, and the relatively small *n*. However, the correlation between resting plasma [K^+] and resting plasma glucose level in both ND and T1D groups combined, just failed to attain significance ($r = 0.31$; $P=0.06$), and was such that for each 1 mmol·l^{-1} increment in plasma glucose, a 0.02 mmol·l^{-1} rise in plasma [K^+] was evident. No correlation was found between resting plasma [K^+] and HbA_{1c}, similar to the finding in the only other study to report the relationship (Sjögren *et al.*, 1986).

The improved K^+ regulation following training resulted in there being no difference between the T1D 'Resting Study' and PostMB at 45 or 60 min recovery (in contrast to the PreFB test).

4.5.5.2 [^{3}H]ouabain binding site content (Na^+,K^+-ATPase content)

Pre-training

The mean value for vastus lateralis [^{3}H]ouabain binding site content obtained prior to training in the non-diabetic group in the current study (313 ± 29 pmol·g^{-1} w.w.) was

very similar to the previously reported means for healthy, untrained human subjects, which encompassed a range of 223 - 360 pmol·g^{-1} w.w (Table 4.19).

Table 4.19 *[^{3}H]ouabain binding site content (mean ± SEM) in vastus lateralis muscle from healthy, untrained human subjects*

Reference	Subjects *n*	[^{3}H]ouabain binding site content (pmol·g^{-1} w.w.)
(Nørgaard *et al.*, 1984)	20	278 ± 15
(Dørup *et al.*, 1988a)	6	258 ± 16
(Dørup *et al.*, 1988b)	18	251 ± 15
(Klitgaard & Clausen, 1989)	6	276 ± 19
	6	~ 237
(Kjeldsen *et al.*, 1990)	15	308 ± 13
(Benders *et al.*, 1992)	5	360 ± 70
(Green *et al.*, 1993)	9	339 ± 16
(McKenna *et al.*, 1993)	6	333 ± 19
	3	311 ± 14
(Leivseth & Reikeras, 1994)	6	306 ± 68*
(Schmidt *et al.*, 1994)	8	223 ± 13
(Gullestad *et al.*, 1995)	7	~ 260
(Haller *et al.*, 1998)	6	317 ± 37
(Green *et al.*, 1999)	9	326 ± 17
(Green *et al.*, 2000)	6	348 ± 12

* Mean ± SD.

The current study demonstrated no difference in [^{3}H]ouabain binding site content between the T1D and ND groups. This is only the second report of [^{3}H]ouabain binding site content in humans with type 1 DM. The mean value obtained for [^{3}H]ouabain binding site content in the T1D group in the current study (328 ± 24 pmol·g^{-1} w.w.) fell well within the range reported for healthy, untrained subjects (Table 4.19).

In contrast to these findings, untrained insulin-treated subjects with type 1 DM had 22% greater vastus lateralis [^{3}H]ouabain binding site content than a control group of non-diabetics (Schmidt *et al.*, 1994). The mean value for the human type 1 DM group (273 ± 15 pmol·g^{-1} w.w.) in Schmidt *et al.* (1994) was also within the range reported for healthy untrained subjects. Interestingly, the values reported for the controls in the same study (Schmidt *et al.*, 1994) represented the lowest of the range reported for healthy, untrained subjects (Table 6.19), and were comparable to values reported for patients with McArdle's disease (Haller *et al.*, 1998), in whom exercise tolerance was markedly diminished.

The subjects with type 1 DM in the current study were approximately 15 years younger than those in Schmidt *et al.* (1994), however adult age does not affect [^{3}H]ouabain binding site content (Nørgaard *et al.*, 1984). Both groups of subjects in the current study fasted overnight, as did the subjects in Schmidt *et al.* (1994); thus any acute effects of fasting (Nishida *et al.*, 1992) should have been similar. One explanation may be related to the prevailing plasma insulin concentration in the two respective type 1 DM populations. Insulin is an acute stimulator of skeletal muscle Na^+-K^+-ATPase activity (Gavryck *et al.*, 1975; Clausen & Kohn, 1977), and may increase pump activity by the translocation of pump subunits from an intracellular pool to the plasma membrane (Grinstein & Erlij, 1974; Erlij & Grinstein, 1976; Erlij & Schoen, 1981; Omatsu-Kanbe & Kitasato, 1990; Hundal *et al.*, 1992; Marette *et al.*, 1993; Lavoie *et al.*, 1996). In the current study the T1D subjects delayed the administration of their morning insulin dose until after testing, and their basal free insulin concentrations did not differ from those of the ND group, whereas in Schmidt *et al.* (Schmidt *et al.*, 1994), plasma insulin was three-fold higher in the type 1 DM subjects than in controls. Thus, in the Schmidt *et al.* study (1994), an acute effect of insulin stimulation may explain the increased Na^+,K^+ pump content, however this is only speculative since no studies have investigated whether insulin induces translocation of pump subunits in human muscle.

Effect of training

After sprint training in the current study, vastus lateralis [^{3}H]ouabain binding site content at rest was increased (P<0.02) 9% in the ND group and 8% in the T1D group,

with no difference between groups, and confirmed the hypothesised effect of training (section 4.2.2.3). The current study is the first to examine the effect of sprint training, indeed the effect of any form of exercise training, on the [^{3}H]ouabain binding site content in skeletal muscle of human subjects with type 1 diabetes.

The increase in vastus lateralis Na^+-K^+-ATPase concentration in the ND group supports the only previous report to measure [^{3}H]ouabain binding site content after sprint training (McKenna *et al.*, 1993), in which a 16% increase was found after an identical sprint training programme in untrained men.

No previous studies have examined the effect of sprint training on [^{3}H]ouabain binding site content in subjects with type 1 DM. Schmidt *et al.* (1994) demonstrated that 10 weeks of endurance training in mildly to moderately diabetic rats, not treated with supplemental insulin, could prevent the diabetes-induced decline in [^{3}H]ouabain binding site content; thus demonstrating the potential for exercise to up-regulate Na^+,K^+ pump number in diabetes. The current study demonstrated that intense exercise training up-regulates [^{3}H]ouabain binding site content in human subjects in whom type 1 diabetes is in good to moderate control. This response to training did not differ from that of non-diabetic subjects.

Both the T1D and ND groups extended their time to exhaustion in the post-training non-invasive test (PostFResp). Potassium (K^+) loss from the exercising muscle may impair the excitability of the membrane and thereby contractility, and is considered a major factor in the development of muscle fatigue during intense muscle contraction in humans (Bigland-Ritchie *et al.*, 1979; Jones, 1981; Hermansen *et al.*, 1984; Sjøgaard, 1986; Sjøgaard, 1991; Lindinger *et al.*, 1995; McKenna *et al.*, 1997a; McKenna *et al.*, 1997b; Juel *et al.*, 2000b). However, there was no correlation between [^{3}H]ouabain binding site content and any of peak plasma [K^+], the rise in plasma [K^+] with exercise, or the time to exhaustion following training in the current study. Although since the change in [^{3}H]ouabain binding site content was only small, detecting a relationship may have been difficult. However, McKenna *et al.* (1993) similarly found no relationship between [^{3}H]ouabain binding site content and the ratio of the rise in plasma [K^+] to work after sprint training. Another study (Kjeldsen *et al.*, 1990) in which exercise-induced hyperkalaemia was reduced following moderate

endurance training, despite no change in [^{3}H]ouabain binding site content, also failed to find a significant relationship between the variables. Haller *et al.* (1998) compared patients with McArdle disease with matched controls during a 20 min bout of exercise, and despite lower [^{3}H]ouabain binding site content and higher exercise-induced peak plasma K^+ in the patients, no relationship was found between the two variables. In contrast to these findings, two other studies found significant positive correlations between [^{3}H]ouabain binding site content and maximal isometric strength (Klitgaard & Clausen, 1989) and treadmill performance (Evertsen *et al.*, 1997). The lack of consistent agreement in these studies probably highlights the multiplicity of factors that may influence plasma [K^+] during exercise and recovery after a training programme.

One such factor is Na^+,K^+ ATPase activity, which was not measured in the present study. However it is likely to have been an important factor in enhanced K^+ regulation (McKenna *et al.*, 1997a), and perhaps significant in allowing an extended time to exhaustion during maximal exercise after sprint training.

In summary, this is the first study to report upon the effect of maximal exercise on plasma [K^+] and [Na^+] responses in subjects with type 1 DM. Plasma [K^+] and [Na^+] at rest and during maximal exhausting exercise did not differ between the ND and T1D groups. However, in late recovery, when marked hyperglycaemia was evident in the T1D group, plasma [K^+] was higher and plasma [Na^+] lower than in the ND group. This was probably due to the osmotic effect of the hyperglycaemia and to falling free insulin. Sprint training enhanced K^+ regulation during intense matched-work exercise in both the ND and T1D groups. The present study confirmed the reduction in exercise-induced hyperkalaemia during maximal matched-work exercise that was found after training in the first study of this thesis, and extended this to demonstrate a similar adaptation in a group with type 1 diabetes. Sprint training attenuated the late-recovery [K^+] elevation in the T1D group. Further, the present study confirmed the findings of McKenna et al. (1993), demonstrating a sprint training-induced upregulation of [^{3}H]ouabain binding site content, and extended this to find a similar adaptation in subjects with type 1 diabetes. Improved K^+ regulation may have contributed to the significantly longer

time to exhaustion in maximal exercise after sprint training, possibly via an enhanced defence of the muscle membrane potential.

4.5.6 Muscle metabolism during maximal exercise in subjects with and without type 1 DM and effects of sprint training

4.5.6.1 Muscle glucose, and hexokinase activity

Muscle glucose

Muscle glucose content was higher across both days and times in the T1D group. In the ND group, muscle glucose content at rest, and the 1.9-fold rise with exhausting exercise at 130% $\dot{V}O_{2peak}$ was similar to previous reports (Spriet *et al.*, 1987; Nevill *et al.*, 1989; Gaitanos *et al.*, 1993; Greenhaff *et al.*, 1994; Bogdanis *et al.*, 1995; Bogdanis *et al.*, 1996; Howlett *et al.*, 1999b; Parolin *et al.*, 1999). Supporting the findings of the only other sprint training study to examine muscle glucose (Nevill *et al.*, 1989), training had no affect upon muscle glucose at rest or upon the increase following intense exercise. Similarly, sprint training did not significantly change muscle glucose in the T1D group.

Hexokinase activity at rest

Prior to training, total maximal *in vitro* hexokinase (HK) activity at rest did not differ between the ND and T1D groups and was similar to previously reported values (Costill *et al.*, 1979; Wallberg-Henriksson *et al.*, 1982). In contrast to the results of the present study and that of Costill *et al.* (1979), two other studies found lower (or a trend for lower) HK activity in men with type 1 DM (Saltin *et al.*, 1979; Wallberg-Henriksson *et al.*, 1984). HbA_{1c} was similar in the subjects with type 1 DM in the aforementioned studies and the present study, and subjects were well matched with nondiabetics for such variables as $\dot{V}O_{2max}$ and body mass index in both the present and one of the other studies (Wallberg-Henriksson *et al.*, 1984). Duration of diabetes in the latter (Wallberg-Henriksson *et al.*, 1984) was twice that of the present study, and daily insulin dose was lower than in the present study. It is possible that either or both of these factors may account for the tendency for lower HK activity in the group with type 1 DM in that study. Whilst details of the non-diabetic men were not presented in Saltin *et al.* (1979), the subjects with type 1 DM had been without insulin

for 24 hours prior to testing, which probably explains the difference between that and the present study (in which only the morning insulin dose was delayed).

Resting HK activity was increased by sprint training in both groups, however was increased to a greater extent in the T1D group. This is the first report of the effect of sprint training on HK activity in subjects with type 1 DM.

The results of the present study are consistent with those of a previous study which demonstrated an increase in HK activity at rest after sprint training in non-diabetics (MacDougall *et al.*, 1998). It was suggested that increased HK activity after training was consistent with the high plasma glucose induced by intense exercise, and may facilitate greater glucose use during exercise and recovery intervals in intermittent exercise (MacDougall *et al.*, 1998). In addition, increase in HKII mRNA and activity is induced both by insulin (Mandarino *et al.*, 1995; Kruszynska *et al.*, 1998; Vogt *et al.*, 2000) and exercise in humans (Koval *et al.*, 1998). The present study demonstrated a rise in plasma insulin after intense exercise that was sustained above resting levels for ~45 min in the ND group, but not in the T1D group, as expected. The effect of repeated sprint bouts during training, both in terms of the post-exercise rise in plasma insulin and the exercise *per se*, probably induced the increase in resting HK activity in the present study. Interestingly, the increase in resting HK activity was considerably greater in the T1D group, in whom the post-exercise rise in plasma insulin was absent. However, the 7-week training programme was conducted under varying conditions of insulinaemia, and thus plasma insulin concentration during exercise and recovery may have been higher in the T1D subjects than in the ND subjects. Insulin sensitivity may also have been altered by the sprint training programme, which may possibly contribute to increased HK responsiveness to insulin, since in patients with type 2 DM (with characteristic marked insulin-resistance) HKII activity was not increased by hyperinsulinaemia (Kruszynska *et al.*, 1998).

Effect of acute exercise on hexokinase activity

A novel finding in the present study was the significant reduction in HK activity after exercise in the ND, but not the T1D, group, regardless of training status.

Reduced maximal *in vitro* activity of HK (Boström *et al.*, 1974) and mitochondrial enzymes (Ji *et al.*, 1988) has been reported after acute exhausting exercise (swimming or running) in rats. It was suggested that free radicals and/or peroxides generated in the mitochondria during the course of respiration altered essential thiol groups in the mitochondrial enzymes (Ji *et al.*, 1988). Structural deviations were evident in muscle fibres from rested elite male sprinters, and marked structural alterations, including extensive Z-band streaming and bizarrely-shaped mitochondria, and autophagic vacuoles containing degradation products, were identified by electron microscopy in the vastus lateralis of elite male sprinters 2 hours after repeated exhausting sprint running bouts (Fridén *et al.*, 1988). It is possible that alterations in the chemical structure of HK and/or acute muscle damage occurred in the present study, which may explain the reduced *in vitro* HK activity after maximal cycling in the ND group.

In contrast to the ~32-35% reduction in the ND group, HK activity was not reduced after exercise in the T1D group. It is tempting to speculate that the presumed increase in enzyme glycosylation consequent to the effects of diabetes in the T1D group may have structurally modified HK and prevented down-regulation with high intensity exercise, however this hypothesis has never been tested.

There was no apparent difference in the reduction in maximal *in vitro* HK activity with exercise in the ND group when performing matched exercise after sprint training in the present study. The lower glycogenolytic rate in both groups during maximal exercise after training in the present study (see section 4.5.6.3) tended to reduce G 6-P accumulation (P=0.11), and hence would be anticipated to reduce, rather than increase the *in vivo* inhibition of HK activity. Further, since resting HK activity was elevated after sprint training and the relative reduction in activity after exercise was similar, the higher absolute activity would have permitted greater glucose entry into the glycolytic pathway, albeit remaining a very minor carbohydrate source during a single maximal exercise bout. However, in subsequent exercise bouts (as occurred during the course of sprint training), glucose may constitute a somewhat more significant carbohydrate source, perhaps to a greater extent in the T1D group.

In summary, the increase in muscle glucose concentration during maximal exercise was unaffected by sprint training, however the capacity for glucose entry into

glycolysis was increased, with increased resting HK activity. The stimuli for increased HK activity at rest after sprint training may include the high glucose and insulin fluxes (the latter only in nondiabetics) associated with recovery from maximal exercise during training, and perhaps also an effect of muscle contraction per se. In the ND group only, a single bout of maximal exercise resulted in a significant reduction in HK activity, both before and after training, however since absolute HK activity was higher after training, the potential for glucose flux into glycolysis was enhanced. Although the contribution of glucose to ATP generation is minor in intense exercise, an increased capacity for glucose use may be of more importance in repeated sprint bouts in which the glycogenolytic rate is progressively reduced. In contrast to the ND group, HK activity was not significantly reduced after exercise in the T1D group, either before or after training. Although the mechanism cannot be determined from the present study, it is possible that enzyme glycosylation may have modified HK and prevented down-regulation during high-intensity exercise, although this has never been examined.

4.5.6.2 Muscle high-energy phosphates

Pre-training

Resting contents of ATP and PCr in the vastus lateralis did not differ between the T1D group, who had delayed the administration of the morning insulin dose, and the ND group, and were similar to the results of Study 1. These results support and extend two previous studies in which subjects with moderately- (Yki-Järvinen *et al.*, 1990) or poorly-controlled type 1 DM (Cline *et al.*, 1997) who were rendered normoglycaemic prior to testing, had similar ATP and/or PCr at rest to non-diabetics.

The present study is the first to report on the effect of intense exercise on muscle ATP and PCr contents in human subjects with type 1 DM. Prior to training, the fall in ATP and PCr content with exhausting exercise was similar in the T1D and ND groups.

Effects of sprint training on muscle ATP and PCr contents

After 7 weeks of sprint training, resting PCr content was not affected, but resting ATP content in the combined groups was reduced 8% ($P<0.01$). Overall, this result was similar to the findings in the first study in this thesis and to three previous sprint training studies (Green *et al.*, 1987b; Hellsten-Westing *et al.*, 1993; Stathis *et al.*,

1994), however the reduction in resting content was ~50% less than in each of these studies. When each group was considered separately, resting ATP content was lower after training, but just failed to reach statistical significance in each group (ND, P=0.06; T1D, P=0.09). The test in which the post-training resting biopsy was obtained was conducted ~6 days after the final training session (as compared with 3 or 5 days in the first study of this thesis); the additional time may therefore have permitted greater net resynthesis of ATP. The mechanism by which resting ATP content may be reduced after sprint training is thought to be related to the loss (and relatively much slower repletion) of adenine nucleotides from skeletal muscle consequent to repeated, severe sprints (Green *et al.*, 1987b; Hellsten-Westing *et al.*, 1993; Stathis *et al.*, 1994), or perhaps, though unlikely, to downregulation of ATP content (Stathis *et al.*, 1994), as discussed for study 1 (see section 3.5.3.1).

During matched-work exercise after sprint training, PCr degradation and creatine accumulation were unchanged in comparison to fatiguing exercise prior to training, similar to the findings of the first study of this thesis (see section 3.5.3.1). Net ATP degradation was reduced after training when matched-work exercise was performed, supporting the hypothesis (section 4.2.2.2) and similar to study 1 of this thesis and two other studies (Green *et al.*, 1987b; Stathis *et al.*, 1994) (see section 3.5.3.1).

The present study is the first to examine the effect of sprint training on high-energy phosphate regulation during intense exercise in subjects with type 1 DM. PCr degradation did not differ between the ND and T1D groups, and did not differ during matched-work exercise after training. After training net ATP degradation after matched-work exercise was markedly attenuated in both the T1D (8% post-training *vs* 29% pre-training) and ND groups (15% post-training *vs* 23% pre-training). However, a group-by-day effect reflected a higher overall ATP content in the T1D group and a lower overall ATP content in the ND group after sprint training. The absolute amount of work performed in the matched-work test did not differ significantly between T1D and ND groups (actually was 30% higher in the T1D group), and so cannot explain the result. The extent of ATP degradation during exercise in the T1D group is very similar to that of the subjects in the first study of this thesis. Therefore in the present study, rather than the difference between groups being due to an effect of diabetes, it may reflect a difference in the ND group response. One subject in the ND group had

higher resting ATP content and greater net degradation of ATP with exercise after sprint training. When this subject was excluded from the analysis the group-by-day interaction just failed to attain significance.

The improved balance between ATP degradation and resynthesis (hence anticipated lower IMP accumulation) evident in both subject groups during matched-work exercise is consistent with the significantly extended time to exhaustion after sprint training in the non-invasive respiratory test (PostFResp) and enhanced oxidative metabolism during maximal exercise.

In summary, the resting content, and the net fall in muscle ATP and PCr during exhausting exercise was similar in the ND and T1D groups, and was similar to the findings in non-diabetics in the first study of this thesis. This is the first study to report upon the effect of maximal exercise on muscle high-energy phosphate contents in subjects with type 1 DM. Delaying the morning insulin dose in subjects with type 1 DM in moderate to good control had no affect upon muscle high-energy phosphate contents and did not affect the fall in such during exhausting maximal exercise. Sprint training resulted in a small reduction in the resting content of ATP in both groups. This result supports the first study of this thesis and three previous studies, however is of approximately half the magnitude, probably related to the timing of the post-training biopsies. During matched-work exercise after sprint training, net ATP degradation was markedly attenuated in both groups, supporting the results of Study 1. Sprint training therefore also seems to enhance muscle respiratory control in subjects with type 1 DM.

4.5.6.3 Muscle glycogenolytic and glycolytic rates, and anaerobic ATP production

Acute exercise

This is the first study to report upon the effect of maximal exercise on muscle metabolism in subjects with type 1 DM. Glycogen degradation and the rates of glycogenolysis and glycolysis during a single bout of maximal exhausting exercise did not differ between the T1D and ND groups. However, both muscle Lac^- accumulation and muscle anaerobic ATP production were significantly higher in the T1D group prior to training.

Time to exhaustion was slightly longer and resting and exercise PDH*a* slightly lower in the T1D group, however neither differed significantly from the ND group. Another possibility that has not been investigated is that Lac^- transport is lower in those with type 1 DM, possibly associated with basement membrane thickening (see sections 2. 2.1, 2.2.2).

Effects of sprint training

As hypothesised (section 4.2.2.2), sprint training resulted in a reduction in each of pyruvate and Lac^- accumulation, glycogenolytic and glycolytic rates, and the rate of anaerobic ATP production, during maximal matched-work exercise in both groups. There was also a tendency for a reduction in glycogen degradation (P=0.052) during PostMB. These results, coupled with tighter energy coupling (an improved balance between ATP degradation and resynthesis), and increased CS activity, provide strong metabolic evidence that energy supply during maximal exercise after sprint training is accomplished more oxidatively. The respiratory results may also support these findings if $\dot{V}O_2$ is partitioned differently during maximal exercise after sprint training. These results extend those of Study 1 and also provide new information on the effects of sprint training on muscle metabolism in a group with type 1 DM.

Control of glycogenolysis is very complex, with flux being determined by both transformational and post-transformational factors, as illustrated by recent work (Parolin *et al.*, 1999). In the studies of this thesis, sprint training reduced net ATP degradation and IMP accumulation during maximal exercise, and hence would have reduced the accumulation of AMP_f. AMP_f activates GP*a* and is thought to also activate GP*b* during intense exercise, and the rise in IMP late in intense exercise may also be sufficient to activate GP*b* (Connett & Sahlin, 1996). Since the rate of muscle glycogenolysis is closely related to the rate of ATP turnover (Ren & Hultman, 1990), improved cellular energy balance may primarily account for the reduced rate of glycogenolysis evident during maximal exercise after sprint training. Additionally, during the matched-work tests [NAdr] tended to be lower after training, which may be expected to reduce activation of GP.

One consideration which may weaken the conclusions of this study, is the possibility that sprint training enhanced muscle lactate transport, as was discussed for Study 1 (section 3.5.3.2). Enhanced lactate removal during exercise after sprint training would be expected to lessen the accumulation of muscle lactate and pyruvate to some degree and increase plasma [Lac^-] (although other tissues may also increase their ability to transport and clear circulating lactate), which would then result in an underestimation of muscle anaerobic ATP production and the rates of glycogenolysis and glycolysis. Plasma [Lac^-] whilst lower in the PostMB test after sprint training, was on post hoc testing, only lower from 5 to 60 min of recovery. This suggests that either lactate transport may have improved, or that there was a greater flux of pyruvate through the PDH complex.

A lower rate of presentation of pyruvate to PDH*a*, or greater PDH*a* after training, may have increased the proportion of carbohydrate that was metabolised oxidatively during intense matched-work exercise after sprint training, as was discussed for Study 1 (section 3.5.3.2). Considering the near significance (P=0.052) of the decrease in glycogen degradation in Study 2, it is likely that a higher proportion of pyruvate was oxidised during matched-work exercise. Although it cannot be determined from the present study, it is possible (given the lower glycogenolytic and glycolytic rates) that greater oxidation of fatty acids occurred during maximal exercise after training, however, this remains to be examined.

4.5.6.4 Muscle PDHa

PDHa at rest

Prior to training, after an overnight fast, the amount of pyruvate dehydrogenase complex in the active form (PDH*a*) at rest in the present study was similar to values previously reported in postabsorptive subjects (Ward *et al.*, 1982; Kruszynska *et al.*, 1986; Mandarino *et al.*, 1987; Constantin-Teodosiu *et al.*, 1992). The effect of fasting versus the fed state on PDH*a* at rest has not been examined in human subjects. A high-fat, low-carbohydrate diet (LCD), which has a similar effect to fasting in terms of augmenting fat metabolism, instituted for 3-6 days, has been demonstrated to reduce PDH*a* in comparison to a mixed (Peters *et al.*, 1998; St. Amand *et al.*, 2000), or low-fat, high-carbohydrate diet (HCD) (Putman *et al.*, 1993), and to increase PDK activity in humans (Peters *et al.*, 1998). Resting PDH*a* activity in the present study

was very similar to PDH*a* values obtained after high-fat diets (Putman *et al.*, 1993; Peters *et al.*, 1998; St. Amand *et al.*, 2000).

Prior to training, PDH*a* at rest in the T1D group was 24% lower than in the ND group (which tended to confirm the hypothesis - section 4.2.2.2), however there was no statistical difference. This finding is similar to the that of the only previous study to report PDH*a* in human subjects with type 1 DM, in whom a non-significant 34% reduction was evident (even with an overnight insulin infusion) (Kruszynska *et al.*, 1986). The slightly lower PDH*a* in the T1D group (whose last insulin dose was administered 10-12 hours previously) in the present study is consistent with studies of rodents with alloxan- or STZ-induced diabetes, in whom deprivation of insulin for a longer period (24-72 hr) reduced PDH*a* at rest by 40-80% compared with controls (Hennig *et al.*, 1975; Hagg *et al.*, 1976; Caterson *et al.*, 1982; Fuller & Randle, 1984; Feldhoff *et al.*, 1993), and elevated PDK activity (Feldhoff *et al.*, 1993; Wu *et al.*, 1999). PDH phosphatase activity is acutely activated by calcium (Denton *et al.*, 1972; Fuller & Randle, 1984) and insulin (Feldhoff *et al.*, 1993), but is not affected by insulin deficiency or fasting (Fuller & Randle, 1984). Thus, in the present study, the non-significant reduction in resting PDH*a* in the T1D group is consistent with slightly higher PDK activity consequent to relative insulinopaenia.

Total PDH activity (PDH*t*) was not measured in the present study, however has previously been demonstrated to be unaffected by exercise, brief or protracted periods of endurance or strength training, a high-fat diet (see Tables 2.17, 2.18) or diabetes mellitus (Kruszynska *et al.*, 1986) or insulin administration in humans (Kruszynska *et al.*, 1986; Mandarino *et al.*, 1987; Mandarino *et al.*, 1990; Kelley *et al.*, 1993).

Effects of sprint training on PDHa at rest

The present study is the first to examine the effect of sprint training upon PDH*a*. After 7 weeks of sprint training, vastus lateralis PDH*a* was reduced 34% at rest ($P<0.05$) with no significant difference between ND and T1D groups. Subjects were postabsorptive both before and after sprint training, thus reduction in PDH*a* at rest may be attributed to an effect of training (or perhaps to the cumulative effect of repeated acute intense exercise bouts), rather than to differing nutritional states.

Few studies, human or animal, have examined the effect of other forms of exercise training upon the PDH complex. Seven days of endurance training did not alter PDH*a* at rest in humans (Putman *et al.*, 1998), although PDH*a* was 3-27% lower during exercise. Similar to the present study, PDH*a* at rest was reduced by 44% in fed rats after 8 weeks of endurance training (Nakai *et al.*, 1999). This was accompanied by increased PDK and β-hydroxyacyl-CoA dehydrogenase (HAD; an enzyme involved in the β–oxidation of fatty acids) activities, increased acetyl CoA (although no change in the ratio of acetyl CoA to CoA), and a reduction in muscle pyruvate (Nakai *et al.*, 1999). In the present study muscle pyruvate was not reduced at rest after sprint training, and acetyl CoA and HAD were not measured. The lower PDH*a* at rest after training in the present study is consistent with increased PDK activity, possibly mediated via increased HAD activity, which would increase the supply of acetyl CoA and NADH, and perhaps lead to an upregulation of PDK protein expression. This mechanism has been proposed to explain the stable upregulation of PDK activity in diabetes, starvation (Wu *et al.*, 1999), and after high-fat feeding in rats (Holness *et al.*, 2000). Reduced PDH*a* activity in the rested state would be expected to be advantageous in terms of protecting carbohydrate stores, and thus seems teleologically sound.

In contrast to the present study, fasting for 24 hours reduced PDH*a* similarly in 8-week endurance trained and sedentary rats (Nakai *et al.*, 1999). The duration of fasting in the rats may have overshadowed the effect of training to reduce PDH*a* and may explain the difference from the present study. It is also possible that the adaptation to sprint training differs from that due to endurance training. In further contrast to the present study, three other studies reported increased resting PDH*a*: after 7 days of endurance training in rats (Brozinick *et al.*, 1988), 5 months of strength training in humans (Ward *et al.*, 1986), and in endurance trained athletes (Ward *et al.*, 1982). The reason for the difference between these studies and the present study is unclear, however the training protocols were quite different.

Effect of sprint training on resting PDHa in type 1 diabetes mellitus

The present study is the first to examine the effect of sprint training on subjects with type 1 DM. Resting PDH*a* was reduced 17 and 46% after sprint training in the T1D

and ND groups, respectively, with absolute values for PDH*a* being very similar between groups after training. These results may suggest that PDK activity is upregulated to an optimal level after sprint training. The results also indicate that young subjects with type 1 DM who are in moderate to good metabolic control adapt to sprint training similarly to non-diabetic subjects, and when fasted after training have a similar capacity for pyruvate flux through the PDH complex.

The effect of sprint training on insulin sensitivity is unknown, however based upon the improvement in HbA_{1c}, without an increased insulin dosage, in the subjects in the T1D group, it may be increased. No human studies have investigated the effect of acutely raising insulin concentration on PDH*a* after exercise training. In 8-week endurance trained rats the increase in PDH*a* in response to insulin stimulation at rest was nearly twice as great as that in sedentary rats (Nakai *et al.*, 1999). The authors suggested that exercise training may have increased PDHP (Nakai *et al.*, 1999); however it is also possible that insulin sensitivity was enhanced. Whether or not either of these possibilities occurred in the present study cannot be determined.

In summary, the present study is the first to examine the effect of sprint training upon PDHa. PDHa in subjects with type 1 DM in moderate to good metabolic control who had delayed their morning insulin dose and fasted overnight was slightly lower (24%, not significant) at rest than in the non-diabetics prior to training. Seven weeks of sprint training reduced PDHa at rest, with a similar effect in subjects with and without type 1 DM. This may be related to an effect of training to alter the relative proportions of carbohydrate- and fat-derived fuel at rest, which may effect an upregulation of PDK activity; however this remains to be investigated.

4.5.6.5 Muscle citrate synthase activity

Muscle CS activity at rest did not differ between the T1D and ND groups prior to training. Sprint training increased resting CS activity in both groups, however the affect was more pronounced in the ND group. However, similar to results for HK, CS activity was significantly reduced in the ND, but not the T1D group after exhausting maximal exercise, both before and after sprint training.

The increase in resting CS activity would be consistent with an increased mitochondrial density after sprint training, and supports the muscle metabolism results which point to greater oxidative energy production during maximal exercise after sprint training (section 4.5.6.3). As discussed above (section 4.5.6.1), exhausting exercise in rats led to reduced maximal activity of mitochondrial enzymes, postulated to be due to free radical or peroxide enzyme modification (Ji *et al.*, 1988). The reason for the lack of reduction in activity with maximal exercise in the T1D group is not clear, but may be related to structural alterations in enzymes consequent to glycosylation, however this was not examined and remains speculative.

4.5.7 Study 2 Conclusions

In postabsorptive subjects with type 1 DM who had delayed their morning insulin dose, the acute response to exhausting maximal exercise was similar to that of non-diabetic subjects, although several differences were evident. Marked, progressive hyperglycemia occurred in recovery from maximal exercise in the T1D group, and plasma $[K^+]$ was increased during late recovery. Muscle Lac^- accumulation was greater and anaerobic ATP production therefore higher during maximal exhausting exercise. However, since the rates of glycolysis, glycogenolysis, and anaerobic ATP production did not differ between groups, it may be suggested that removal of Lac^- was lower in the T1D group. Consistent with this, PDH*a* was slightly lower at rest and during exercise in the T1D group, however, the difference was not significant.

Seven weeks of sprint training improved metabolic control in the T1D group, and may have also improved insulin sensitivity given that HbA_{1c} was reduced without an increase in insulin dosage. The metabolic and ionic adaptations to sprint training were similar in the T1D and ND groups, and in support of the first study of this thesis, indicate that sprint training results in improved performance and probably in increased oxidative metabolism during maximal matched-work exercise. The rates of muscle anaerobic ATP production and glycogenolysis and glycolysis were lower, and net ATP degradation was attenuated during matched-work maximal exercise after sprint training. Although not examined in this thesis, changes in muscle lactate transport may have contributed to the lower muscle lactate accumulation. However, adding support to the conclusion that oxidative metabolism was enhanced was the finding of

increased CS activity after training. In plasma, peak [K^+], and peak and mean [H^+] were reduced, and although non-significant, the exercise-induced hyperglycaemia tended to be lower during matched-work exercise after sprint training. The mechanism of the proposed enhancement of oxidative metabolism may be via a slower rate of pyruvate presentation, thus allowing a greater proportion to be oxidized. Fat metabolism may also be enhanced during maximal exercise, however this was not examined.

The current study indicates that sprint training may be undertaken with metabolic safety in young subjects with type 1 DM who have no overt complications of diabetes, and that the adaptations to training are very similar to those of non-diabetics, i.e. that sprint training results in improved performance and increased oxidative ATP generation during maximal exercise.