UNIVERSITÉ DE MONTRÉAL

Study of the mechanisms by which carbohydrate administration during prolonged muscle contractions increases performance

(Étude des mécanismes par lesquels l'administration de glucides améliore la performance durant des contractions prolongées du muscle)

by

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This thesis entitled:

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Summary

The review of the literature examines the effects of carbohydrate administration on muscle performance during prolonged muscle contractions. In general, the administration of carbohydrates increases muscle performance during prolonged exercise. However, the mechanism(s), which could mediate improvements in exercise performance, associated with carbohydrate administration remain(s) unclear. In particular, it is not known if carbohydrate administration attenuates fatigue through a central effect or through a direct improvement of peripheral neuromuscular function, or both. In fact, no data appear to be currently available on possible improvements of peripheral muscular function associated with glucose administration during prolonged submaximal stimulation. The purpose of this work was to enrich our limited understanding of how carbohydrate administration during exercise increases muscle performance in an *in situ* model. Study 1 verified if glucose infusion could attenuate muscle fatigue during prolonged muscle contractions. Peak dynamic force and M-wave characteristics of the plantaris muscle was measured in anaesthetized rat during prolonged nerve electrical stimulation *in situ*, with and without glucose infusion. Results showed that glucose infusion attenuates fatigue in rat plantaris muscle stimulated *in situ*, and this is associated with a better maintenance of electrical properties of the fiber membrane.

Study 2 investigated if elevated plasma insulin concentration due to glucose infusion rather than high plasma glucose concentration *per se* could attenuate muscle fatigue during prolonged indirect electrical stimulation *in situ*. The results

indicate that the increase in insulin concentration is not responsible for the increase in muscle performance observed following the elevation of circulating glucose.

The purpose of the third study was to further investigate the effect of lactate infusion on the electrical properties of the muscle fiber membrane and the development of muscle fatigue. Results showed that the beneficial effect of lactate infusion on muscle force during prolonged stimulation was associated with a better maintenance of M-wave characteristics compared to control.

Study 4 determined if glucose infusion would attenuate the decrease in resting membrane potential observed immediately after prolonged muscle contractions. This study showed that resting membrane potential can be attenuated with glucose infusion however it cannot explain the better maintenance of muscle force and M-wave peak-to-peak.

Study 5 examined if the infusion of glucose could possibly have an effect on different metabolites such as ATP, PCr and lactate concentrations during prolonged indirect stimulation of the rat plantaris muscle *in situ*. The results show that glucose infusion attenuated the decline in PCr degradation suggesting that the phosphate potential could be better maintained.

Collectively, the findings of these studies shared the common goal of furthering our understanding of the mechanisms through which carbohydrate administration increases muscle performance during prolonged contractions. These findings may help clarify the role of muscle fatigue in the regulation of metabolism and muscle function.

Sommaire

L'administration de glucides augmente la performance dans les épreuves d'endurance, cependant, les mécanismes qui pourraient expliquer ce phénomène ne sont pas clairs. En particulier, il n'est pas établi si l'administration de glucides atténue la fatigue par un effet central ou par une amélioration directe de la fonction neuromusculaire périphérique. Aucune donnée ne semble être disponible sur l'effet de l'administration de glucose sur la fonction musculaire périphérique au cours d'une stimulation sous maximale prolongée. Le but de ce travail était d'étudier cette question.

Dans une première étude la force dynamique submaximale et les caractéristiques de l'onde M de l'EMG de surface du muscle plantaire ont été mesurées chez le rat anesthésié pendant une stimulation électrique prolongée du nerf *in situ*, avec et sans l'infusion de glucose. Les résultats montrent que l'infusion de glucose atténue le développement de la fatigue, et ceci est associé à un meilleur entretien des propriétés électriques de la membrane musculaire.

Dans la deuxième étude, les observations ont été faites dans deux situations où l'insulinémie était haute mais la glycémie était basse ou élevée. Les résultats indiquent que la fatigue n'est atténuée que si la glycémie est haute, ce qui suggère que l'augmentation de la concentration de l'insuline n'est pas responsable de l'augmentation de la performance du muscle observée lorsque du glucose est infusé.

Dans la troisième étude l'hypothèse faite est que lorsque du glucose est infusé, l'amélioration de la performance du plantaire et le meilleur maintien des

propriétés électriques de la membrane sont dus à la fourniture d'ATP glycolytique aux pompes membranaires. Les résultats montrent que bien que le lactate ne puisse fournir d'ATP glycolytique, son administration se traduit par un meilleur maintien des caractéristiques de l'onde M de l'EMG et de la force. Ceci peut suggèrer que le glucose n'exerce pas ses effets via la fourniture d'ATP glycolytique.

La quatrième étude avait pour but de déterminer si l'infusion de glucose limitait la réduction du potentiel de repos de membrane qui est observé juste après une période de contractions prolongées induisant un certain niveau de fatigue. Les résultats montrent que la réduction du potentiel de repos de membrane peut être atténué par l'infusion de glucose. Toutefois, ceci n'est pas lié de façon étroite au meilleur maintien de la force musculaire ou des caractéristiques de l'onde M de l'EMG.

Finalement la cinquième étude a examiné si l'infusion du glucose pendant la stimulation indirecte prolongée du muscle plantaire du rat *in situ* pouvait modifier les variations des contenus de différents métabolites du muscle, qui pourraient être elles-mêmes liées au développement de la fatigue : ATP, créatine phosphate, et lactate. Les résultats indiquent que l'infusion de glucose a atténué la diminution de la dégradation de la phosphocréatine, suggérant qu'elle permet un meilleur maintien du potentiel phosphate. Toutefois, les mécanismes par lesquels le glucose peut contribuer au maintien du potentiel phosphate, et les mécanismes par lesquels un meilleur potentiel phosphate peut contribuer à l'atténuation de la fatigue, demeurent obscurs.

Keywords

- Muscle fatigue
- Electromyography
- M-wave
- Muscle force
- Glucose
- Lactate
- Insulin
- Resting Membrane Potential
- Phosphocreatine
- In situ

Mots Clés

- Fatigue musculaire
- Electromyographie
- Courbe-M
- Force Musculaire
- Glucose
- Lactate
- Insuline
- Potentiel Membranaire de Repos
- Phosphocreatine
- In situ

Table of Contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Abbreviations

ANOVA:	analysis of variance
CHO:	carbohydrates
RMP:	resting membrane potential
L_o:	muscle length for optimal twitch tension development
EMG:	electromyography
ATP:	adenosine triphosphate
ADP:	adenosine diphosphate
IMP:	ionosine monophosphate
PCr:	phosphocreatine
Pi:	inorganic phosphate
Na+/K+:	sodium/potassium pump
HE:	hyperinsulinemic-euglycemic clamp
HH:	hyperinsulinemic-hyperglycemic clamp

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Chapter 1

INTRODUCTION

1.0 Introductory statement

Circulating glucose and muscle glycogen are types of carbohydrates (CHO) that can readily be oxidized by skeletal muscle for energy production in exercising humans during submaximal exercise (Coggan 1991). The availability of CHO as a substrate for muscle metabolism is a critical factor in the performance of prolonged aerobic exercise (Coggan and Coyle 1991). As such, strategies to maintain or enhance CHO availability, such as the ingestion of glucose before, during and after exercise, are beneficial to the performance of a variety of sports events, and are a key recommendation in current exercise physiology guidelines (Hargreaves 1999).

2.0 Carbohydrate ingestion and performance during exercise

Historical perspectives

The first study to show the effects of CHO administration on performance appears to have been done by Levine et al. (1924). The authors recognized the importance of the body's CHO stores for the maintenance of CHO metabolism during prolonged exercise when lowered plasma glucose concentrations were observed in many runners after the Boston marathon (<3 mmol/l). In that study, some runners showed signs of neuroglucopenia (e.g. muscular twitches and unconsciousness). When CHO were ingested on the day before and during the race, blood glucose concentrations (~5 mmol/l) were maintained in these same runners on the following year. In addition, mean course time significantly

decreased from 3 h 5 min 48 sec to 2 h 57 min 12 sec and no neurological symptoms were observed.

In another study, Dill et al. (1932) observed that feeding one dog 20 g of CHO every hour during prolonged exercise maintained blood glucose levels and enabled the animal to run at 124 m/min up a 17.6 % grade for at least 13 hours without fatiguing (Figure 1). When the animal was administered with water alone during exercise, blood glucose concentrations significantly decreased and the animal was able to exercise at this intensity for 3 to 6.5 hours before exhaustion. In another experiment the dog ran for 4.25 hours in the fasted state, which resulted in a decrease in blood glucose concentration to 2.6 mmol/l. The animal was then fed 40 g of CHO during an 8-min rest period, which increased blood glucose to >6 mmol/l and enabled the dog to run for another 1.5 h, at which time he was still not exhausted. These results led Dill et al. (1932) to suggest that the limiting factor in the performance of prolonged exercise was the quantity of easily available fuel in the form of circulating glucose. Ingestion of CHO during exercise was therefore thought to delay fatigue by maintaining the availability of this important CHO source for oxidation by the exercising muscles.

Christensen and Hansen (1939) used an experimental protocol similar to that employed by Dill et al. (1932). In that study, two subjects performed prolonged exercise on a cycle ergometer at an intensity of 65 % of VO_{2max} after following a diet low on CHO for three days. After the first period of exercise of 130 or 160 min (subject 1 and 2, respectively), the subjects were exhausted and their blood glucose levels had decreased from 5 to 3.3 mmol/l and from 4.4 to 2.8

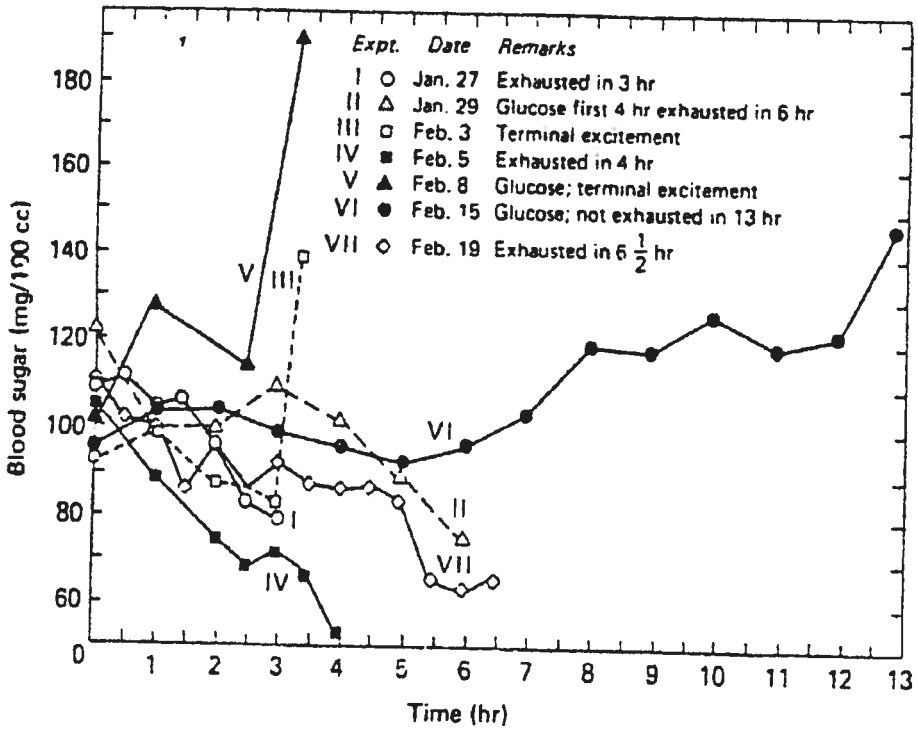

Fig. 1. Blood glucose levels in one dog and the duration of exercise (Dill 1932).

mmol/l, respectively. Thereafter, the subjects ingested 200 g of glucose in solution during a 15-min period of rest. Blood glucose concentrations in both subjects rapidly increased above 5.5 mmol/l and this allowed them to continue to exercise at the same intensity for another 45 min.

Since then, it is well established that CHO administration increases performance during prolonged exercise in man (Coggan et al. 1991; Maughan 1991; Coyle 1997; el-Sayed et al. 1997; Hargreaves 1999) and in animal (Dill 1932; Bagby et al. 1978; Slentz et al. 1990). This phenomenon has been extensively reviewed and will not be further summarized or discussed in this thesis. Furthermore, the ability to perform resistance exercise could also increase when CHO is administered (see the review by Haff et al. 2003). For example, Lambert et al. (1991) showed that total number of sets and repetitions tend to increase when subjects ingested 125 g of CHO while performing leg extensions at 80 % of their previously determined 10 repetition maximums, with three min of rest between sets. In another study, by Haff et al. (2001) subjects were required to perform 16 sets of 10 repetitions of leg extension/flexions at 120^0/s on a cybex isokinetic dynamometer, with three min of rest between sets with and without CHO ingestion. Results show that total work performed significantly increased from 38.1 to 41.1 kJ when subjects ingested 240 g of CHO.

However, the mechanism(s), which could mediate improvements in exercise performance associated with CHO administration remain(s) unclear. This review of the literature focuses on the possible underlying mechanisms that could explain the increase in muscle performance observed with the

administration of CHO during prolonged muscle contractions in humans and animals. The beneficial effect of CHO ingestion on performance during prolonged exercise and on the ability to increase work with resistance exercise could be due to several factors including: 1) a better maintenance of blood glucose concentration, which could delay central fatigue and has been shown to improve cognitive performance; 2) an increased contribution of plasma glucose oxidation to the energy yield; 3) muscle glycogen sparing; 4) a better maintenance of muscle metabolites, and 5) a better maintenance in muscle force production.

3.0 Glycemia and performance during exercise with CHO ingestion

Circulating glucose is the only energy source for the brain and a continuous systemic supply is essential for optimal function (Pardridge 1983). In addition, there is evidence to support that impaired performance could be associated with hypoglycaemia (Koslowski et al. 1981; Amiel 1998; Collardeau et al. 2001; Nybo 2003). For example, Amiel (1998) showed that a decrease in blood glucose concentration (<3 mmol/l) is associated with cognitive dysfunction as indicated by impaired performance in neuropsychological tests. Interestingly, it appears that only one study has examined the effect of prolonged exercise on cognitive performance with and without CHO administration (Collardeau et al. 2001). In that study, cognitive tasks were performed before and after a 100-min run at 64 % VO_{2max}. Blood glucose concentration significantly increased from 5.2 to 5.6 mmol/l when subjects ingested 118 g of glucose during exercise, whereas when subjects ingested a placebo blood glucose concentration decreased from

5.1 to 4.7 mmol/l. Immediately after exercise several cognitive tasks were performed and this resulted in a significant decrease of choice reaction time from 688.5 to 654 ms in the CHO group, whereas in the control group choice reaction time remained unchanged (688 ms vs. 676 ms). In addition, no significant differences in the rating of perceived exertion were observed in the CHO, while the rating of perceived exertion significantly increased from 11 to 16 in the control group (Figure 2). These results suggest that glucose ingestion could increase cognitive function, but further research is needed to investigate the different mechanisms that could support a causal link between physiological processes and cognition. In addition, it appears that only two studies have examined the effect of CHO ingestion on mental function during prolonged exercise (Keith et al. 1991; Kreider et al. 1995). CHO administration improved mood states during prolonged exercise in elite female cyclists (Keith et al. 1991), and field hockey players (Kreider et al. 1995).

The effect of exercise-induced hypoglycaemia on the ability of the central nervous system (CNS) in activating skeletal muscle has not been investigated. However, the idea that CHO availability for the brain is important in maintaining an adequate neural drive to the muscles is supported by the finding that glucose infusion (1.5 mmol/l kg^{-1} min^{-1}) directly in the carotid artery, which increased central blood glucose concentration from 4 to 10 mmol/l and maintained peripheral blood glucose concentration at 4.5 mmol/l, can delay fatigue in dogs exercising until exhaustion on a treadmill with a slope of 12^{0} and a speed ranging from 1.2 to 1.8 m sec^{-1} (Koslowski et al. 1981). Interestingly, Nybo (2003)

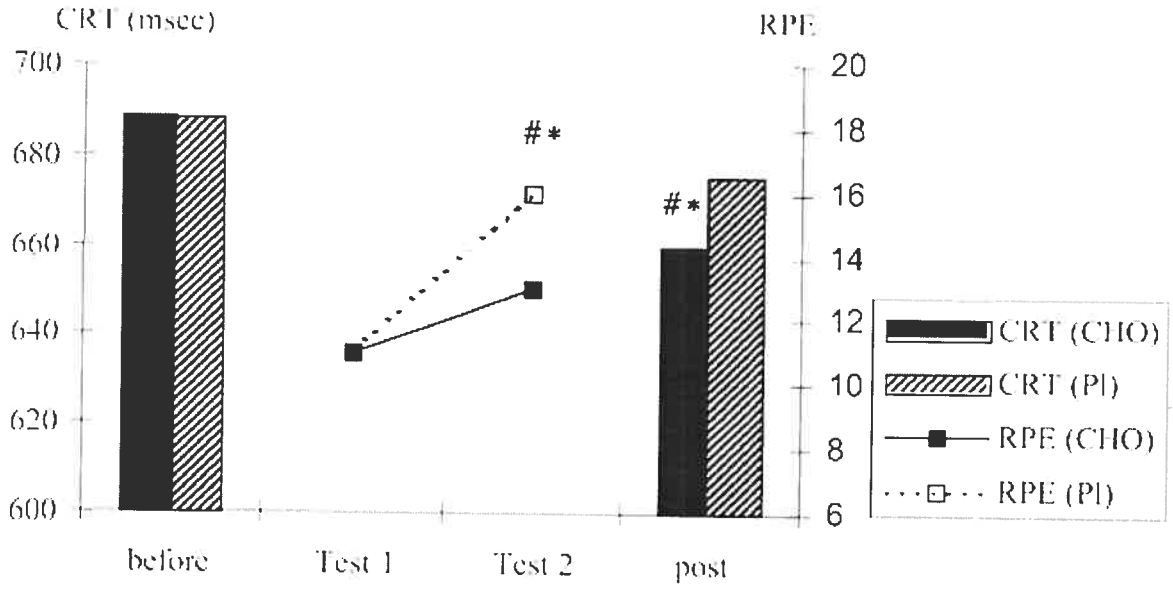

Fig. 2. Choice reaction time performance (left ordinate) and ratings of perceived exertion (right ordinate) during the experimental protocol for both the CHO and placebo groups (Collardeau et al. 2001).

showed that the average force production during a sustained maximal muscle contraction was decreased after 3 hours of exercise at 60 % VO_{2max} in endurance-trained subjects, in which blood glucose concentration significantly decreased from 4.5 to 3 mmol/l after exercise. The reduced force development in this study was associated with a diminished activation drive from the CNS. This central fatigue was reversed when euglycemia (4.5 mmol/l) was maintained with the ingestion of 200 g of CHO. In addition, it was easier for the subjects to retain power output at the end of prolonged exercise when hypoglycemia was prevented.

Newsholme et al. (1987) first proposed the central fatigue hypothesis, which suggests that higher serotonin levels could potentially influence the development of central fatigue by affecting arousal and mood that could be linked to altered perceptions of effort and muscle fatigue. In support of this hypothesis, it has been shown that serotonin levels in the brain are increased during prolonged exercise in rats (Bequet et al. 2001). Furthermore, tryptophan is the precursor for the synthesis of serotonin, and an increased plasma concentration of free tryptophan could increase the cerebral tryptophan uptake and enhance serotonin production in the brain (Davis et al. 1997). During prolonged exercise low levels of plasma insulin are observed, which favours the release of free fatty acids (FFA) from adipose tissue. This will result in an increased plasma concentration of both FFA and free tryptophan, as FFA binds to albumin and displaces some of the albumin-bound tryptophan (Curzon et al. 1973). Glucose ingestion stimulates the secretion of insulin and blunts the exercise-induced rise

in both plasma FFA and free tryptophan (Davis et al. 1997). Therefore, this could counteract the development of central fatigue by attenuating the rise in brain serotonin. This hypothesis was tested in the study of Davis et al. (1992). In that study, plasma free tryptophan increased by ~7 fold, which was associated with an increase in plasma FFA, when subjects performed prolonged exercise for 200 min at 68 % VO_{2max}, which reduced blood glucose levels from 5 to 4 mmol/l in the control situation. When subjects ingested 268 g of CHO, blood glucose concentration was maintained at 5.5 mmol/l and plasma free tryptophan as well as plasma FFA was significantly attenuated and fatigue was delayed by ~1 hour.

During prolonged exercise at a moderate intensity, without the ingestion of CHO, glycemia may remain stable or tends to decrease in a progressive way (Coyle et al. 1983). Moreover, several studies have shown that when CHO are ingested 30-45 min before the start of exercise, glycemia could decrease during exercise (Foster et al. 1979; Brouns et al. 1989; Jentjens et al. 2002). For instance, Foster et al. (1979) reported that when subjects ingested 75 g of glucose 30 min before exercise, blood glucose levels increased to 6.7 mmol/l at the start of exercise and thereafter decreased to 4 mmol/l after 50 min of exercise at 80 % VO_{2max}. When CHO are ingested immediately before or during exercise, the final glycemia observed is higher than the value of glycemia observed at the same time during exercise without the ingestion of CHO. For example, in the study of Coyle et al. (1983), when subjects ingested a placebo during exercise at 75 % VO_{2max} until exhaustion, blood glucose levels decrease from 5 to 3 mmol/l. However, blood glucose levels increased from 5 to 7 mmol/l

when subjects ingested CHO and this was associated with an increase in time to exhaustion. Therefore, these observations has led Coggan and Coyle to hypothesized that the increase in performance could be due to a better maintenance in blood glucose levels (Coggan et al. 1987; Coggan et al. 1991).

Coggan & Coyle (1987) hypothesized that, if a decline in plasma glucose during prolonged exercise contributes to fatigue, it should be possible to reverse fatigue late in exercise by restoring euglycemia. To test this hypothesis, trained cyclists first exercised to fatigue at 70 % VO_{2max} after an overnight fast. This required about 170 min and resulted in a significant decrease in plasma glucose concentration (3-3.5 mmol/l) at fatigue (Exercise Bout 1, Figure 3). The subjects attempted to perform further exercise at the same intensity after a 20-min rest period when one of three treatments was conducted (Exercise Bout 2, Figure 3). In one trial, the subjects ingested a placebo solution at the end of Exercise Bout 1. Plasma glucose concentration increased slightly (~4 mmol/l) during the rest period, but then decreased again during further exercise (~3 mmol/l). In that trial, subjects were able to tolerate only 10 ± 1 min of additional exercise (Figure 3). During a second trial, the subjects ingested ~210 g of CHO at the end of Exercise Bout 1. This initially increased plasma glucose concentration (~5 mmol/l) during Exercise Bout 2 above the levels observed at fatigue in Exercise Bout 1 (Figure 3). Plasma glucose concentration were not maintained, however, declining progressively until the subjects again fatigued (3.7 mmol/l), which occurred after 26 ± 4 min (Figure 3). During a third trial, glucose was infused

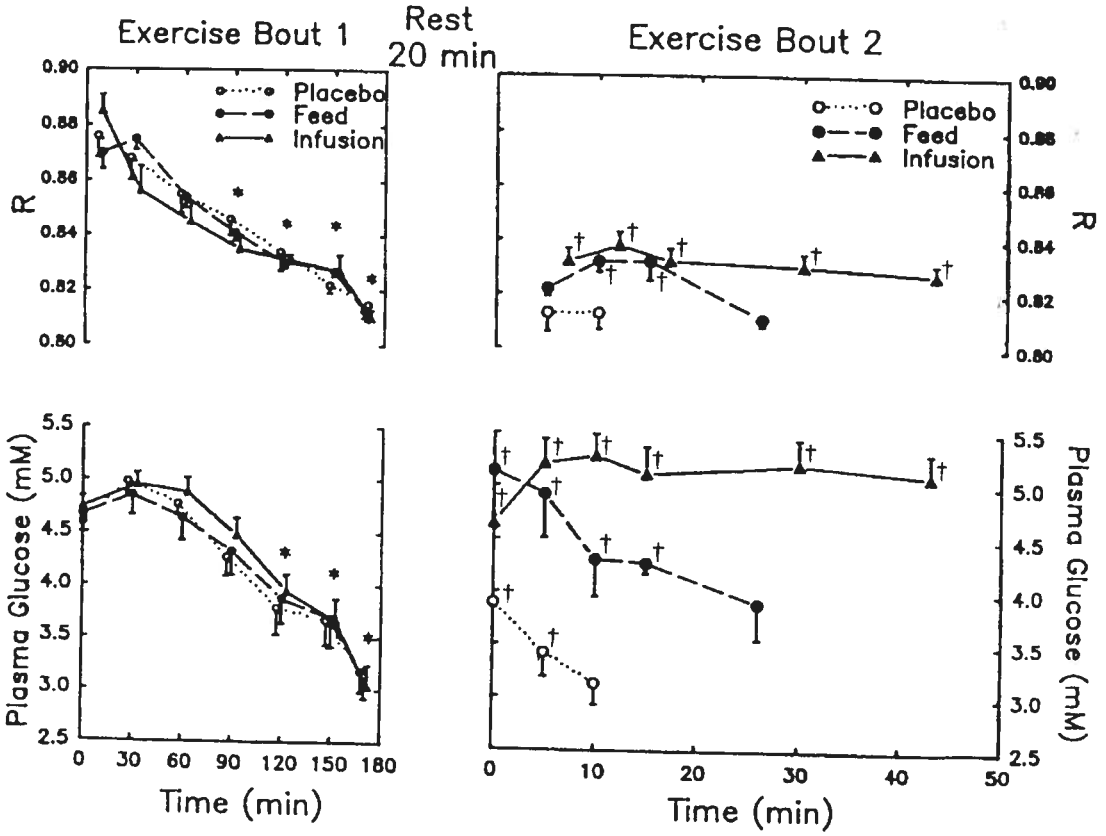

Fig. 3. Plasma glucose levels and R during three exercise trials (Coggan et al. 1987).

intravenously throughout Exercise Bout 2. The rate of glucose infusion was adjusted every 5 min during exercise to maintain plasma glucose concentration at ~5 mmol/l. This infusion of glucose allowed the subjects to complete an additional 43 ± 5 min of exercise, which was significantly different from the other trials (Figure 3). Finally, in a fourth trial, the subjects were provided with a single large CHO feeding (3 g maltodextrins/kg body weight) during Exercise Bout 1 after 135 min of exercise (Coggan et al. 1989). This large CHO load reversed the gradual decrease in plasma glucose (4 mmol/l), similar to glucose infusion or CHO ingestion at fatigue. Unlike the case when ingesting CHO at fatigue, however, ingesting CHO late in exercise helped maintain plasma glucose during additional exercise (5 mmol/l), thereby delaying fatigue by 36 ± 10 min, which was significantly different from control (Figure 4). The authors concluded that the better maintenance of blood glucose levels with CHO ingestion during prolonged exercise could be responsible for the increase in performance.

However, Felig et al. (1982) showed that hypoglycaemia fails to effect performance, and that its prevention does not consistently delay exhaustion. In that study, when water was ingested during exercise at 60 to 65 % VO_{2max}, blood glucose levels declined progressively and hypoglycaemia (blood glucose < 2.5 mmol/l) occurred in 7 of 19 healthy men. The hypoglycaemic subjects exercised for 15 to 70 min despite blood glucose levels of 1.4 to 2.7 mmol/l (Figure 5) and their exhaustion time (142 ± 15 min) was not significantly different from that in the 12 subjects whose blood glucose levels remained above 2.5 mmol/l (165 ± 11 min). In addition, results showed that perceived exertion during exercise was not

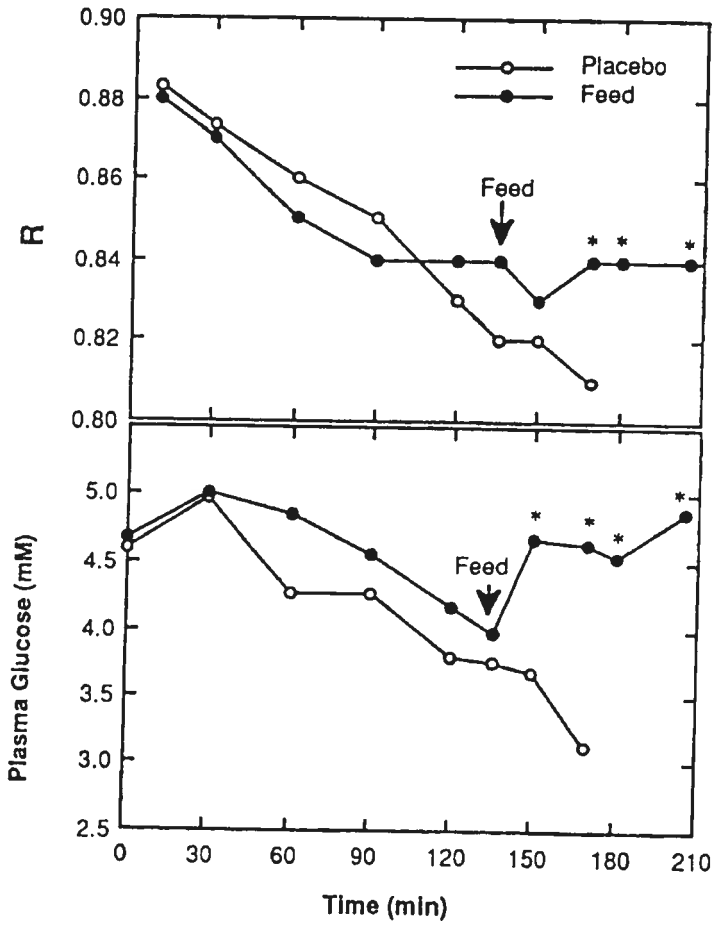

Fig. 4. Plasma glucose levels and R during prolonged exercise. Subjects were fed either a placebo or CHO after 135 min of exercise (Coggan et al. 1989).

higher in subjects who became hypoglycaemic. When subjects ingested 114 g of CHO during exercise, blood glucose levels at the end of exercise remained unchanged from values during the resting state (~4.5 mmol/l) and exhaustion time (171 ± 14 min) was not significantly different from that observed with water ingestion (164 ± 8 min). The authors concluded that glucose ingestion does not consistently delay exhaustion or alter the subjective sensation of exertion during exercise. These results suggest that hypoglycaemia may not necessarily be a mechanism of fatigue and that the better maintenance of blood glucose levels with the ingestion of glucose could not be a possible mechanism for improved performance.

4.0 Blood glucose oxidation and CHO administration during exercise: effect on performance

Coggan and Coyle hypothesized that it may not be the better maintenance of blood glucose *per se* but a better maintenance of blood glucose oxidation that increases performance during prolonged exercise. Therefore, in the 1980's a series of studies were conducted evaluating the importance of maintaining blood glucose oxidation in endurance exercise (Coyle et al. 1983; Coyle et al. 1986; Coggan et al. 1987). Coggan and Coyle hypothesized that CHO ingestion during prolonged exercise improves performance primarily by maintaining blood glucose oxidation at sufficiently high rates.

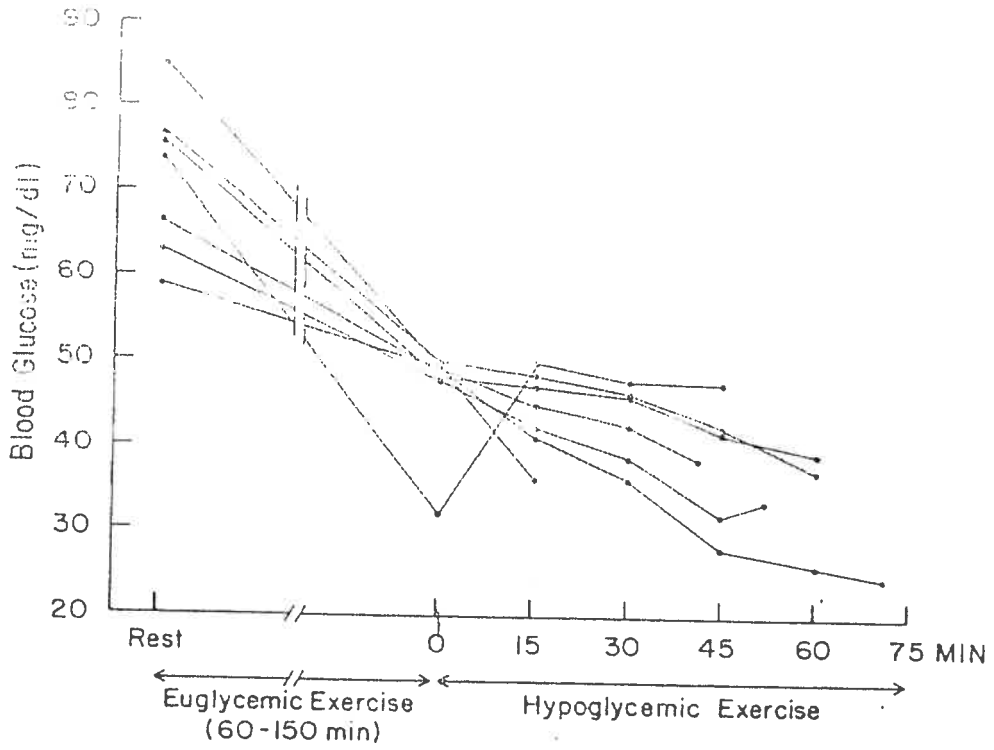

Fig. 5. Individual blood glucose values in the seven subjects whose blood glucose concentrations fell below 2.5 mmol/l water ingestion (Felig et al. 1982). "Euglycemic exercise" refers to the period of exercise during which blood levels remained above 2.8 mmol/l. "Hypoglycemic Exercise" refers to the duration of exercise after the blood glucose concentration fell below 2.8 mmol/l.

In these studies, CHO ingestion delayed fatigue by 30-60 min and this was associated with a better maintenance of a high rate of CHO oxidation at a time when muscle glycogen levels are low. These experiments consisted of performing bicycle exercise between 70-74 % VO_{2max} with and without CHO administration. For example, Coyle et al. (1986) showed that in the control group the rate of total CHO oxidation, at the start of exercise (2.0 g/min), was significantly reduced at fatigue (1.2 g/min). In contrast, when very large amount of CHO were ingested (432 g) over 4 hours the rate of total CHO oxidation was better maintained (2.0 g/min) (Figure 6). In these studies, the authors concluded that the lowering of blood glucose (2.5-3.0 mmol/l) during the latter stages of prolonged strenuous exercise plays a major role in the development of muscular fatigue by not allowing leg glucose uptake to increase sufficiently to offset reduced muscle glycogen availability. Moreover, it is not clear what induced fatigue in the CHO group, since plasma glucose and CHO oxidation remained unchanged throughout exercise (Figure 6).

In the study of Coyle et al. (1986), the authors drew a model (Figure 7) showing the percentage of energy and the absolute rate of CHO oxidation derived from various sources during prolonged cycling at 70-74 % VO_{2max} when fasted or when fed CHO throughout exercise. According to the model, the rate of muscle glycogen utilization during exercise is the same when fasted or when fed CHO. As the duration of exercise increases, progressively less energy is derived from muscle glycogen and progressively more is derived from blood glucose. During the first 2 hours of exercise, substrate utilization is generally similar when

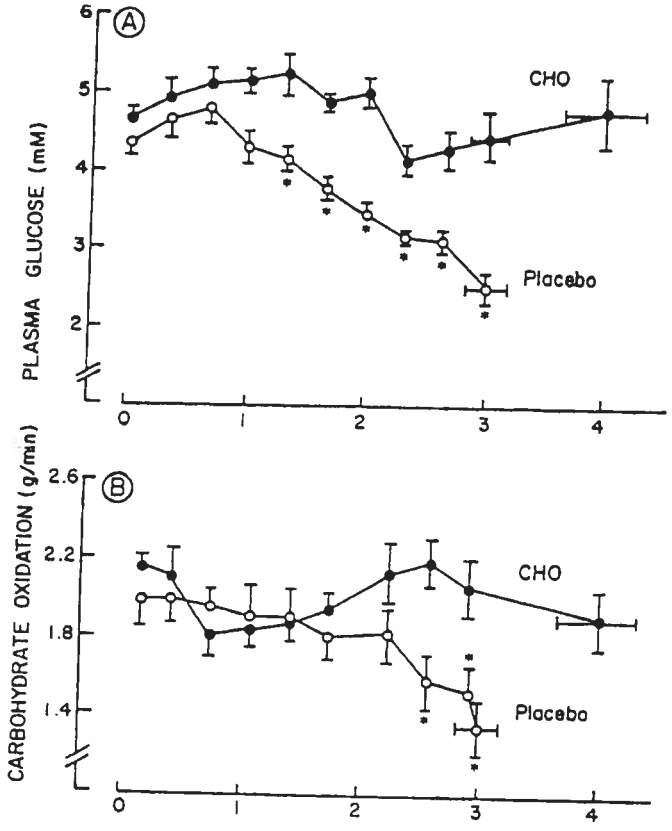

Fig. 6. Plasma glucose levels and total CHO oxidation during exercise when fed a placebo or CHO every 20-min (Coyle et al. 1986).

fasted or when fed CHO. However, according to the model, fatigue occurs after 3 hours of exercise when fasted owing to an insufficient rate of CHO oxidation as a result of a decrease in plasma glucose oxidation. CHO ingestion prevents this decrease in CHO oxidation by maintaining blood glucose concentration and availability and allowing blood glucose oxidation to increase to the point where it accounts for almost all of the CHO being oxidized. Therefore, over the course of 4 hours of exercise when fed CHO, muscle glycogen and blood glucose each contribute approximately one-half of the CHO energy and thus should be considered as equally important substrates.

This proposed model is widely accepted and is an interesting hypothesis. However it is oversimplified and a degree of caution must be taken with it. The actual amounts of exogenous blood glucose and muscle glycogen oxidation were not measured; thus estimated blood glucose oxidation when fed CHO does not distinguish between endogenous and exogenous sources of glucose. For example, during the first 2 hours of exercise, it is likely that ingested CHO partially replaces endogenous blood glucose. Furthermore, the percentage of energy derived from muscle glycogen was calculated with the assumption that 10 kg of muscle was active, thus the results are based on speculations and not direct measurements, which could make the data interpretation problematic. Furthermore, muscle glycogen stores between hours 3 & 4 are still being used in the model (figure 7), however in the actual study (Coyle et al. 1986) muscle glycogen stores are not being used between hours 3 & 4 (figure 9).

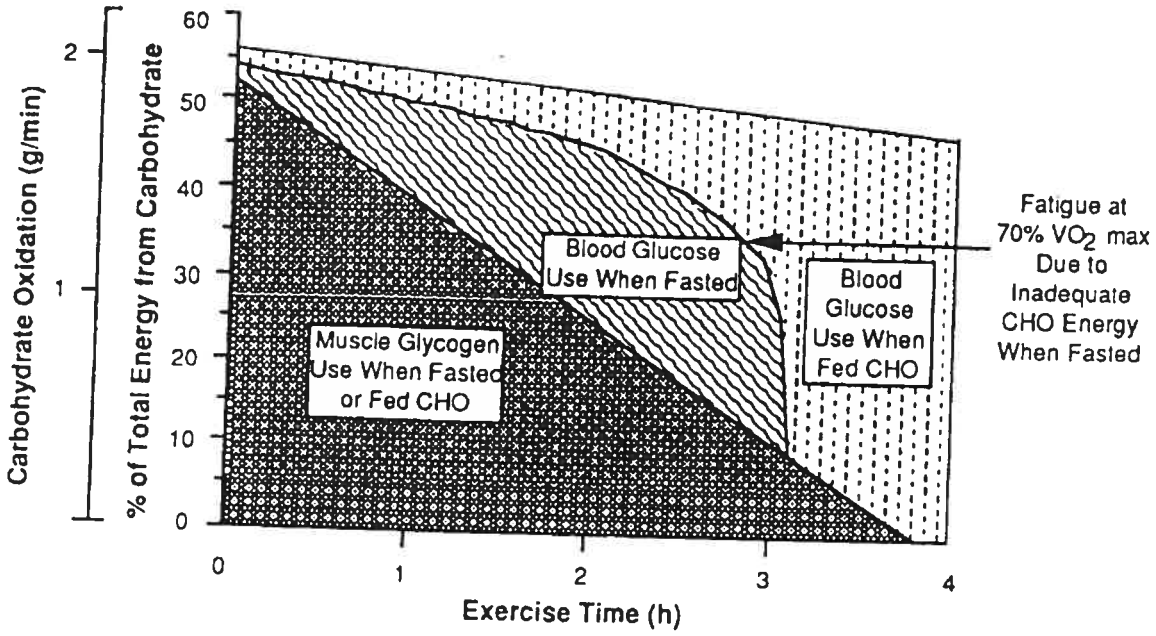

Fig. 7. Model showing various sources of energy during prolonged exercise. Note that blood glucose becomes predominant source of CHO energy during latter stages of exercise (Coyle et al. 1986).

5.0 Effects of CHO administration on muscle glycogen metabolism during exercise

Fatigue during prolonged exercise often coincides with low muscle glycogen content, and endurance performance could be improved by increasing initial muscle glycogen stores (see e.g., the review by Conlee, 1987). Therefore, it has been hypothesized that the administration of CHO during exercise slows the rate of muscle glycogenolysis. This possibility was first raised by the experiments of Bergstrom and Hultman (1967) who reported that the intravenous infusion of glucose at up to 3.5 g/min ([glucose] = 21 mmol/l) decreased net muscle glycogen degradation by ~20 % during bicycle ergometer at a workload of 950 kpm/min for 60 min. In addition, muscle glycogen sparing has also been observed in exercising rats when glucose was infused during prolonged running at a moderate intensity (Bagby et al. 1978). In that study, rats were infused intravenously with either saline or glucose (1.6 g kg^{-1} h^{-1}) ([glucose] = 7.8 mmol/l) during exercise on a treadmill at 21 m/min with a 10 % grade. Time to exhaustion was significantly increased in rats infused with glucose compared to control (225 vs. 164 min, respectively). These studies support the hypothesis that CHO ingestion during exercise improves performance by slowing the rate of muscle glycogen degradation. However, direct measurements of muscle glycogen utilization during exercise with and without CHO administration do not support this hypothesis. We have compiled 19 studies which show the rate of muscle glycogen utilisation with and without CHO ingestion during prolonged exercise (Figure 8). The means of muscle glycogen utilisation were not

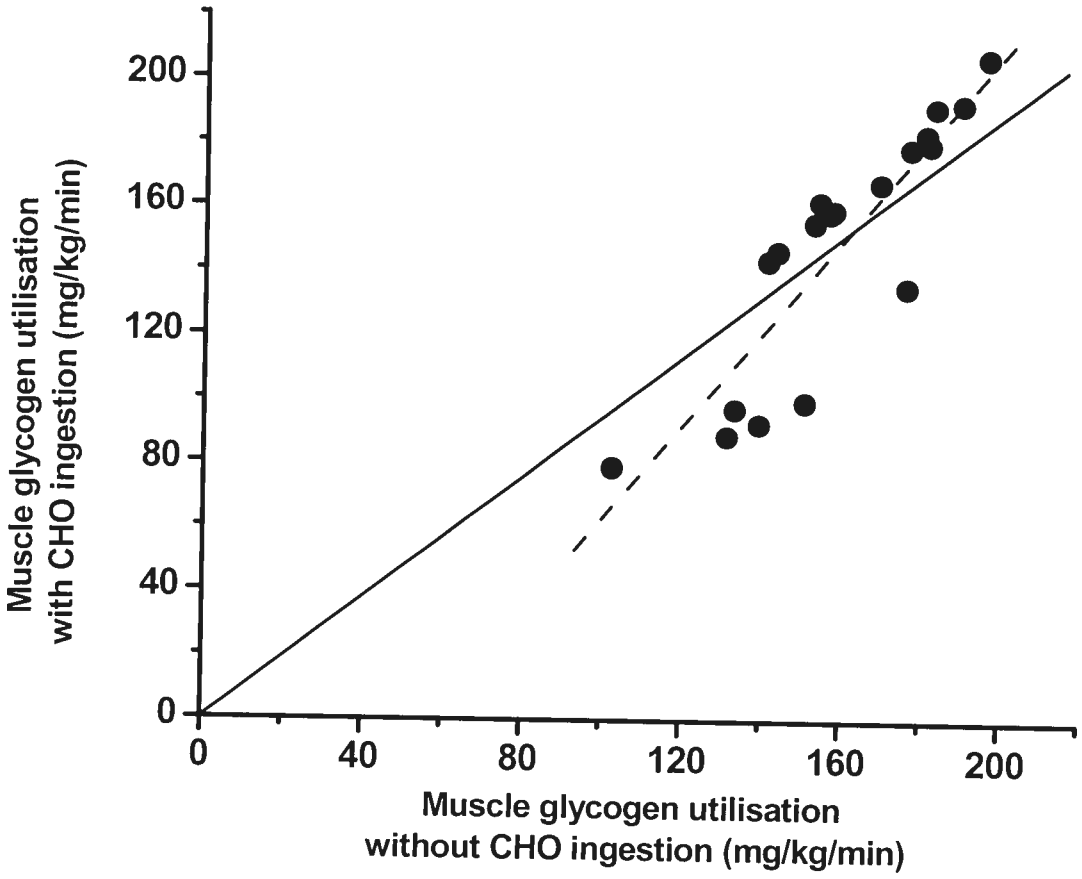

Fig. 8. Rate of muscle glycogen utilisation with CHO ingestion in function with the rate of utilisation in a control situation. Doted line represents the regression line ($r = 0.88$, $p < 0.01$) and solid line represents the identity line.

significantly different with (147.64 ± 39.3 mg/kg/min) or without (158.96 ± 23.9 mg/kg/min) CHO administration. In general, the literature indicates that CHO ingestion during continuous, moderate-intensity exercise does not reduce the utilization of muscle glycogen (Coyle et al. 1986; Coggan et al. 1987; Flynn et al. 1987; Hargreaves et al. 1987; Hargreaves et al. 1988; Mitchell et al. 1989; Coyle et al. 1991; Febbraio et al. 1996; Febbraio et al. 2000; Arkinstall et al. 2001; Chryssanthopoulos et al. 2002). Coyle et al. (1986) measured muscle glycogen concentration in the vastus lateralis before and after 105 min of cycling at 71% VO_{2max} with and without CHO ingestion throughout exercise. No significant differences in glycogen utilization were observed. The authors in that study obtained muscle biopsies from another group of cyclists at rest, after 120 min of exercise at 71% VO_{2max}, and at fatigue during both a placebo and a CHO ingestion trial. Fatigue was observed after 181 ± 11 min of exercise in the control group. In contrast, when subjects were fed CHO throughout exercise, fatigue was significantly delayed until min 241 ± 20. As shown in Figure 9, however, the decline in muscle glycogen concentration during exercise was similar both with and without CHO ingestion, with the additional 60 ± 16 min of exercise made possible by CHO ingestion accomplished without a further decrease in muscle glycogen. In a recent study, Chryssanthopoulos et al. (2002) showed that consuming a CHO meal 3 hours before treadmill running at 70 % VO_{2max} does not influence muscle glycogen use. Arkinstall et al. (2001) also reported that CHO ingested (63.6 g) did not attenuate muscle glycogen utilization during 60 min of continuous submaximal running or cycling. Moreover, studies in exercising

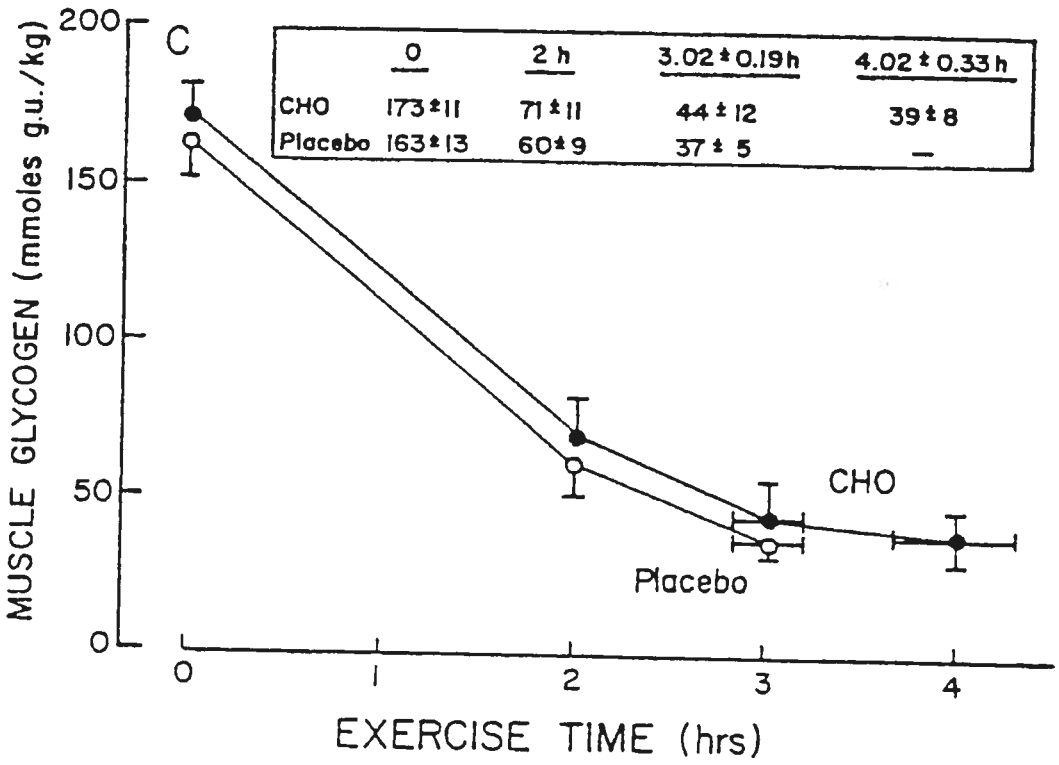

Fig. 9. Muscle glycogen utilization during exercise with and without the ingestion of CHO (Coyle et al. 1986).

rats have also observed no change in muscle glycogen utilization with CHO administration (Gorski et al. 1990; Slentz et al. 1990).

Nevertheless, some authors have reported reductions in muscle glycogen utilization ranging from 20 to 28 % with CHO administration during exercise (Bjorkman et al. 1984; Hargreaves et al. 1984; Tsintzas et al. 1995, 1996, 2001). For example, Hargreaves et al. (1984) observed that muscle glycogen utilization was significantly lower by ~26 % in subjects who ingested 172 g of sucrose during four hours of moderate prolonged exercise. Similar results were reported by Bjorkman et al. (1984) when subjects ingested 212 g of glucose during prolonged exercise. In another study, Tsintzas et al. (1995) investigated the effect of CHO ingestion on muscle glycogen utilization during running at 70 % VO_{2max}. In that study, pre-exercise glycogen levels (350 mmol/kg dm) and the duration of exercise (60 min) were kept the same. As a result of ingesting 50 g of CHO in a 5.5 % solution, a 28 % sparing of glycogen in the vastus lateralis muscle was observed. This glycogen sparing was accompanied by an increase in blood glucose levels (Figure 10). Glycogen determination in type I and type II muscle fibers revealed that the ingestion of the CHO solution resulted in a 42 % sparing of glycogen in type I fibers only, whereas in type II fibers, no significant differences were observed during the 60 min of exercise. In the study of Tsintzas et al. (1995), the amount of CHO ingested was small (only 50 g) compared to the studies of Hargreaves et al. (1984) (172 g) and Bjorkman et al. (1984) (212 g) which were much larger. The difference in muscle glycogen utilization between the control and CHO group in the study of Tsintzas et al. (1995) was large

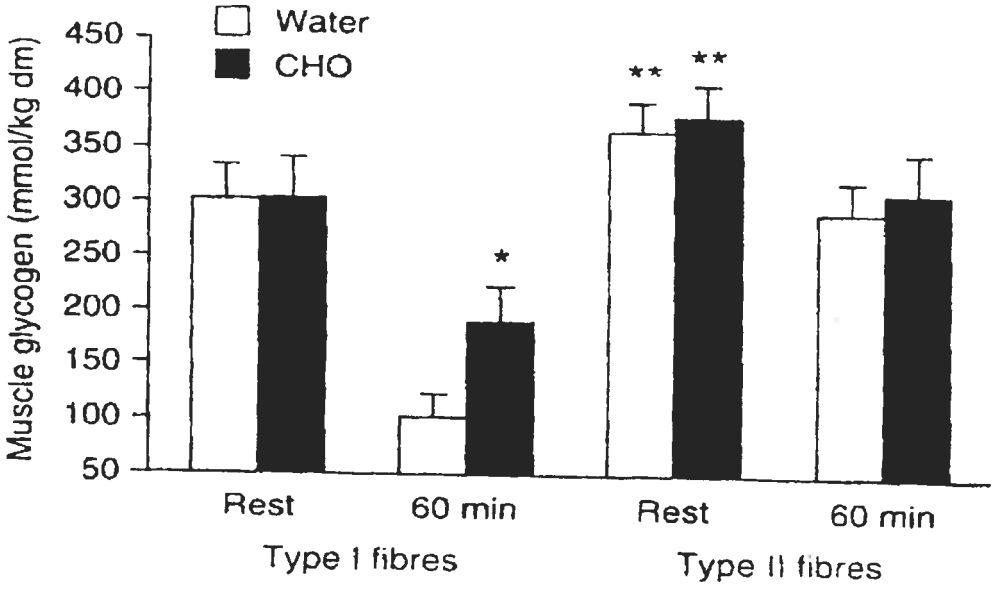

Fig. 10. Muscle glycogen levels during 60 min of exercise with and without the ingestion of CHO (Tsintzas et al. 1995).

(~100 mmol/kg dm) after 60 min of exercise at 70 % VO_{2max}. Therefore, the following calculations were performed to verify if 50 g of glucose could possibly spare 100 mmol/kg of muscle glycogen. In order to convert dry mass to wet weight 100 mmol/kg was divided by 3.3, thus 100 mmol/kg dm / 3.3 = 30.3 mmol/kg ww of muscle glycogen. One mmol of glucose = 0.180 g, thus 30.3 mmol/kg x 0.180 g = 5.45 g of glucose/kg. Ten kg of muscle was active, thus 5.45 g x 10 = 54.5 g of glucose is suppose to be used. Therefore, it seems unlikely that 50 g of ingested glucose could spare ~100 mmol/kg of muscle glycogen in 60 min of exercise, since muscle glycogen is predominantly used as a fuel source during prolonged aerobic exercise.

Finally, muscle glycogen sparing as well as the better maintenance of blood glucose levels and the increase in CHO oxidation during exercise with CHO ingestion are not **mechanisms** that could explain how the muscle can develop more force during exercise and thus increase performance. They are merely observations and the precise mechanism for the increase in performance is still unclear.

6.0 Effects of CHO administration on muscle metabolites during exercise

The underlying mechanism(s) of muscle fatigue remain(s) to be determined. Since substrate is needed to fuel the metabolism pathways involved in ATP generation, it is not unreasonable to suspect that substrate availability may be a fundamental cause of muscle fatigue. It has been suggested that metabolic disturbances at the cellular level could be implicated in the etiology of

muscle fatigue (Sahlin et al. 1998). This hypothesis is supported by the findings that muscle contraction and fatigue is associated with changes in metabolite concentration in the muscle such as ATP,IMP, PCr, and Pi (Sahlin et al. 1998). In addition, there is evidence to support that the beneficial effect of CHO administration on muscle performance during exercise could be associated with the changing of changes in metabolite concentrations in the muscle fiber (Lewis and Haller 1986; Spencer et al. 1991; Tsintzas et al. 1996; McConell et al. 1999; Snow et al. 2000; Tsintzas et al. 2001). Table 1 shows six studies that examined the effect of CHO ingestion on muscle metabolites during prolonged exercise. In all six studies, ATP levels were not significantly modified during exercise and no significant difference in ATP levels was observed between the control and CHO groups. In addition, all six studies showed significant reductions in PCr levels after exercise. Three studies reported that PCr levels were higher when CHO are ingested during exercise (Lewis and Haller 1986; Snow et al. 2000; Tsintzas et al. 2001). For example, Tsintzas et al. (2001) reported an increase in running performance with CHO ingestion and this was associated with a smaller decline of PCr concentration by 46 ± 17 % in type I fibers and by 36 ± 9 % in type II fibers (Figure 11). Only three (Spencer et al. 1991; Snow et al. 2000; McConell et al. 1999) of the six studies measured IMP levels, in which significant increases were observed during exercise. One study showed no significant difference between the control and CHO group (Snow et al. 2000), whereas two studies reported that IMP levels were lower when CHO are ingested during exercise (Spencer et al. 1991; McConell et al. 1999). For instance, McConell et al. (1999)

Table 1. Difference in ATP, PCr, IMP and Pi content during exercise with or without the ingestion of CHO

Study	ATP	PCr	IMP	Pi
Tsintzas et al. (1996)	No difference between groups	No difference between groups	-	-
Tsintzas et al. (2001)	No difference between groups	Significantly higher in CHO group	-	-
Snow et al. (2000)	No difference between groups	Significantly higher in CHO group only at min 30	No difference between groups	-
Spencer et al. (1991)	No difference between groups	No difference between groups	Significantly lower in CHO group	-
McConnel et al. (1999)	No difference between groups	No difference between groups	Significantly lower in CHO group	-
Lewis and Haller (1986)	No difference between groups	Significantly higher in CHO group	-	Significantly lower in CHO group

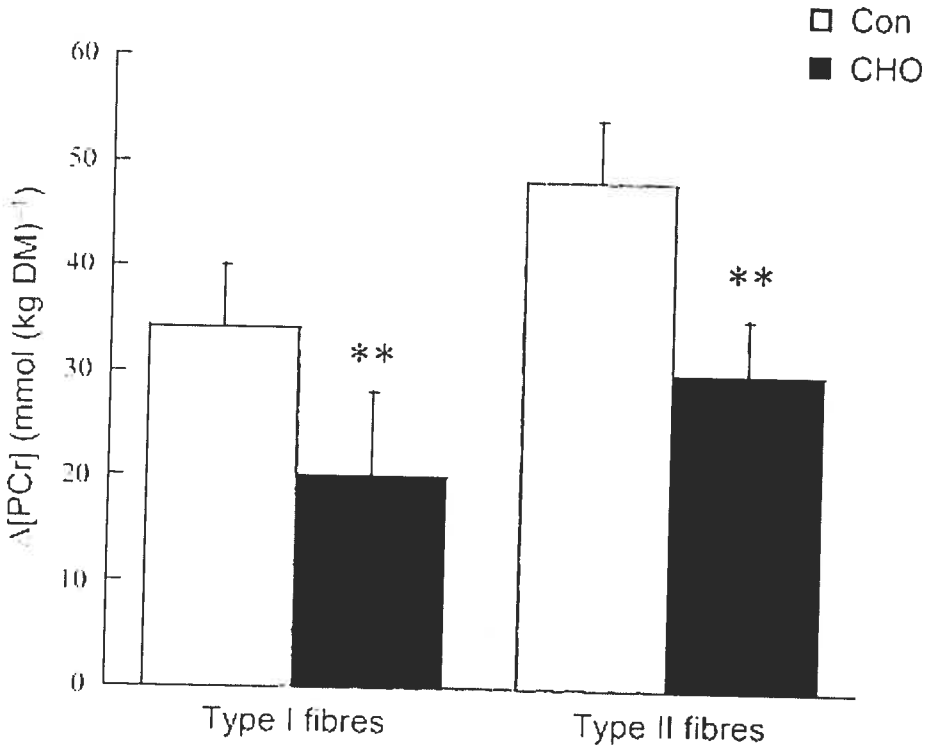

Fig. 11. Changes in type I and type II muscle fiber PCr concentrations during submaximal running with (CHO) and without (Con) carbohydrate supplementation (Tsintzas et al. 2001).

showed that muscle IMP concentration at the point of fatigue was lower when CHO was ingested, despite the subjects exercising 30 % longer during cycling at 69 ± 1 % of VO_{2max} (Figure 12). Finally, it appears that only one study examined the effect of CHO administration on inorganic phosphate levels during exercise, which was performed in subjects with McArdle disease (Lewis et al. 1986). In that study, lower levels of inorganic phosphate were observed when glucose was infused during exercise.

Moreover, it has also been suggested that the cause of muscle fatigue could be due to an insufficient energy supply mediated either through a limiting supply of substrate (acetyl-CoA) to the tricarboxylic acid cycle (TCA) or limitations in TCA activity due to reduced TCA intermediate concentration (Sahlin et al. 1990). Based on this hypothesis, Spencer et al. (1991) investigated the effect of CHO ingestion on TCA cycle intermediates during prolonged exercise at 70 % VO_{2max}. Results from that study show that performance was increased with CHO ingestion during prolonged exercise and this was associated with a higher content of TCA cycle intermediates.

Collectively, these authors (Spencer et al. 1991; McConell et al. 1999; Tsinzas et al. 2001) suggested that CHO ingestion during exercise could increase muscle performance, at least in part, by better maintaining oxidative ATP resynthesis. However, this hypothesis that may explain the increase in muscle performance observed with CHO administration during exercise is misleading. No significant differences in oxygen consumption were observed during exercise between the CHO and control group in these studies (Spencer et

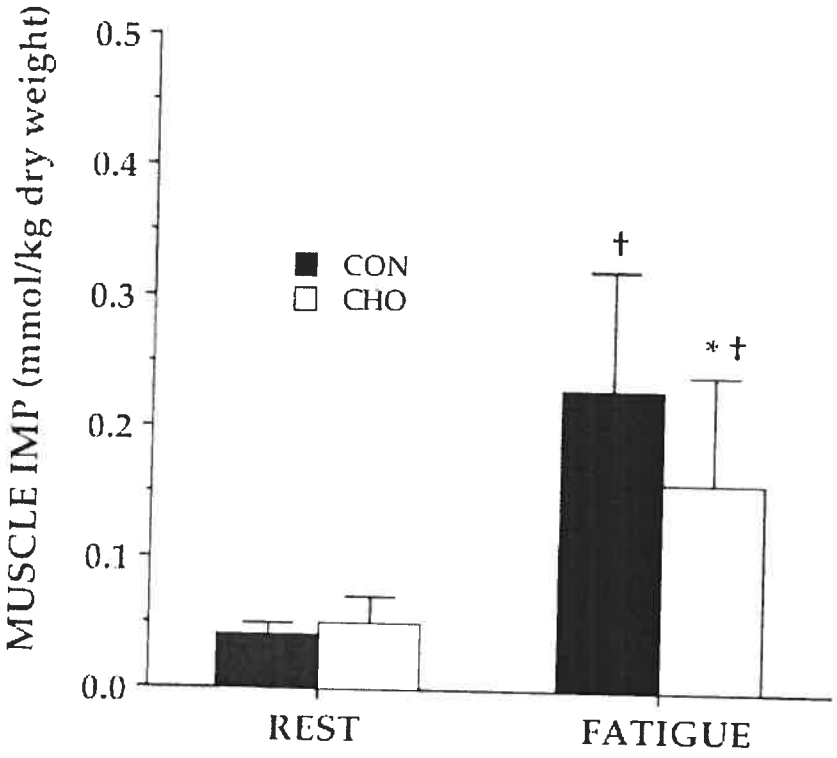

Fig. 12. Muscle IMP levels at rest and after exercise to fatigue with and without CHO ingestion (McConell et al. 1999).

al. 1991; McConell et al. 1999; Tsintzas et al. 2001). Therefore, the production of oxidative ATP during prolonged muscle contractions was the same with or without CHO ingestion. For example, in the study of McConell et al. (1999) no differences in oxygen uptake between the control and CHO group were observed at any time point during exercise.

7.0 The effect of CHO administration on muscle force production during prolonged electrical stimulation in the isolated muscle fiber

It is not known if CHO administration could increase performance through a central effect on the nervous system or through a direct improvement of peripheral neuromuscular functions or both. Therefore, this has led to the use of different isolated muscle fiber protocols, which excludes any possible central effect. Several investigators have examined the effect of lactate administration on muscle function and performance in various experimental models. In a recent study, Nielsen et al. (2001) showed that lactate may have a protective effect on force production. In that study, isolated rat soleus muscle was incubated at a concentration of extracellular K^+ of 11 mM, which reduced tetanic force by 75 %. Subsequent addition of 20 mM lactate led to an almost complete force recovery, which was also associated with an almost complete recovery of the electrical properties of the muscle (Figure 13). Similar results have been observed by Pedersen et al. (2003) on the soleus and EDL muscle incubated with 11 mM of K^+ and 10 mM of lactate. In contrast, Hogan et al. (1995) showed that lactate infusion, resulted in a significant decrease in force in isolated dog gastrocnemius muscle during isometric contractions at 2 Hz for 60 min. Erdogan et al. (2002)

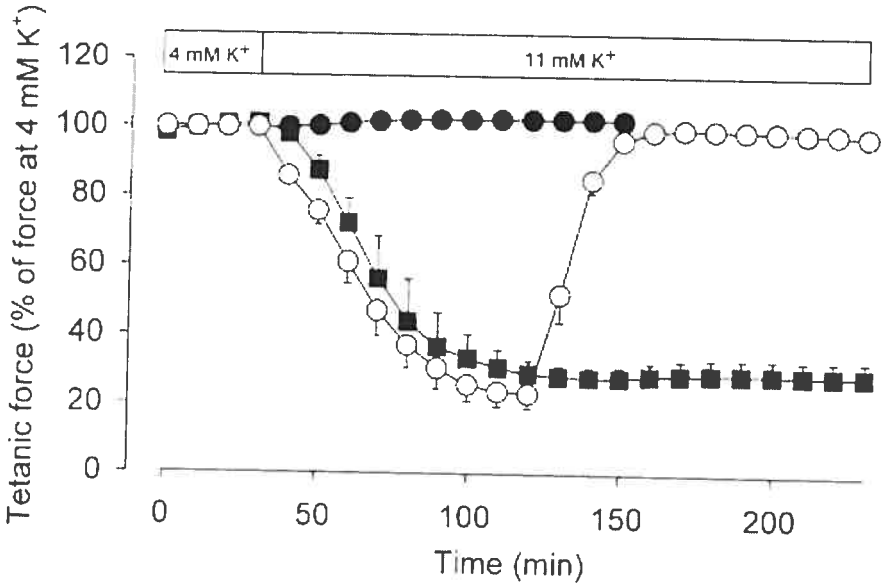

Fig. 13. Effect of 20 mM lactate on tetanic force in rat soleus muscles exposed to a [K+]o of 11 mM (Nielsen et al. 2001). $[K^+]_o$ was increased to 11 mM at time 30 min. ■, controls; ●, lactic acid added together with the increase in $[K^+]_o$; ○, lactic acid added after 90 min at 11 mM K^+.

observed that lactate administration reduced force *in vitro* in rat diaphragm muscle during isometric contractions at 0.5 Hz for 90 min. Furthermore, Chase and Kushmerick (1988) showed that 50 mM of lactate increased the maximum Ca^{2+} activated force by 5 % in permeabilized fibers and in the study by Posterino and Fryer (2000), 20 mM of lactate significantly increased submaximal or maximal force in mechanically skinned fibers.

Furthermore, it appears that only one study has examined the effect of glucose administration on muscle performance during electrical stimulation in an *in vitro* model (Phillips et al. 1993). In that study the authors examined different metabolic fuels (pyruvate, glucose, lactate) and no exogenous metabolic fuel on force production in isolated mouse soleus and extensor digitorum longus (EDL) muscles during isometric tetanic contractions at 50 Hz (soleus) and 150 Hz (EDL). Results show that pyruvate administration (20 mM) increased muscle performance in the soleus during isometric contractions, whereas glucose (11 mM) or lactate (20 mM) showed no significant effects. EDL muscles produced the same isometric force whether the metabolic fuel was glucose, pyruvate, lactate or if no exogenous metabolic fuel was supplied. The authors observed that the increase in muscle performance in the soleus with pyruvate administration was due to a lowering of inorganic phosphate by 17 %.

8.0 Introduction to manuscripts

This thesis is comprised of five related manuscripts contributing to the understanding of mechanisms by which CHO administration mediates improvements in muscle performance during prolonged contractions. Presently, no research has investigated possible improvements of peripheral neuromuscular function associated with elevated circulating glucose during prolonged indirect stimulation. We used an in situ nerve-muscle preparation in anesthetized rats for our experiments (Figure 14). In order to stimulate the muscle through the nerve a bipolar electrode was positioned in contact with the sciatic nerve, and the plantaris tendon was attached to a computer controlled servomotor for the measurement of muscle force. In order to see if glucose or lactate infusion had a direct effect on the neuromuscular junction, we stimulated the muscle directly by placing electrode wires directly through the plantaris muscle. The electrical properties of the muscle fiber membrane were measured using surface EMG, which was recorded with a ball electrode mounted on a spring and placed near the origin of the plantaris muscle. Changes in glycogen, ATP, PCr and lactate content as well as twitch force and half-relaxation time, maximum dynamic and isometric forces, resting membrane potential, pH, and M-wave characteristics were also measured, in order to identify some of the possible mechanisms by which glucose or lactate infusion could attenuate muscle fatigue in the present model.

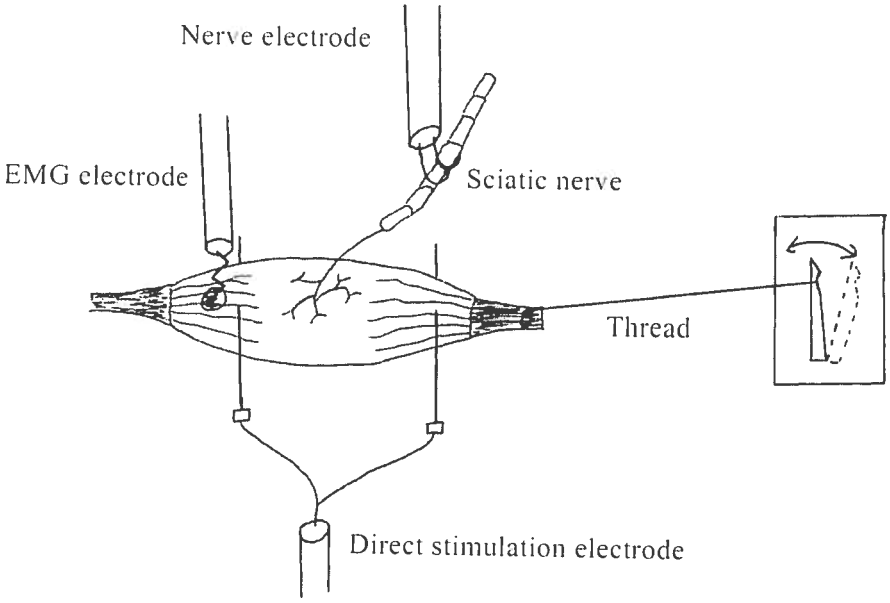

Fig. 14. Graphical representation of the model.

Chapter 2

MANUSCRIPT 1

Title: Glucose infusion attenuates muscle fatigue in rat plantaris muscle during prolonged indirect stimulation *in situ*

Authors: Antony D. Karelis, François Péronnet, and Phillip F. Gardiner

Journal : Experimental Physiology **87:** 585-592, 2002

Keywords: Muscle force, Peripheral Muscle Fatigue, M-wave

ABSTRACT

Carbohydrate ingestion increases endurance time to exhaustion during prolonged exercise as well as the ability to perform resistance exercise. The mechanism(s) underlying the increased performance due to glucose ingestion remain(s) unclear. The purpose of the present experiment was to verify the hypothesis that glucose infusion could attenuate peripheral muscle fatigue in the anaesthetized rat during prolonged indirect electrical stimulation *in situ*. For this purpose The plantaris muscle was electrically stimulated (50 Hz, for 200 ms, every 2.7 s, 5 V, pulse with: 0.05 ms) *in situ* through the sciatic nerve to perform concentric contractions for 60 min while infusing intravenously either only saline (7.25 mL kg^{-1} h^{-1}), or saline and glucose (1 g kg^{-1} h^{-1}: plasma glucose = 11 ± 1.1 vs. 4.9 ± 0.2 mM with infusion of saline) (8 rats/group). Glucose infusion attenuated the reduction in submaximal peak dynamic force (55 % decrease vs. 70 % in rats infused with saline only, $P<0.05$). In a third group of rats ($n = 8$), infusion of glucose beginning at min 30 partially restored submaximal peak dynamic force ($P<0.05$). Maximum dynamic and isometric forces at the end of the period of stimulation were also higher ($P<0.05$) in rats infused with glucose (4.0 ± 0.2 and 4.3 ± 0.2 N) than saline only (3.0 ± 0.2 and 3.5 ± 0.2 N). The beneficial effect of glucose infusion on peripheral muscle force during prolonged stimulation was not associated with a reduction in muscle glycogen utilisation, nor with a reduction of fatigue at the neuromuscular junction (as assessed through maximal direct muscle stimulation: 200 Hz, 200 ms, 150 V, pulse with: 0.05 ms). However, changes in M-wave peak-to-peak amplitude, duration and total area suggest that

glucose infusion and/or the associated increase in plasma insulin concentration could avoid the deterioration of electrical properties of the muscle fibre membrane.

INTRODUCTION

Carbohydrate (CHO) ingestion increases exercise time to exhaustion during prolonged exercise in man (Coggan & Coyle, 1987; Coggan & Coyle, 1991; Maughan, 1991; Coyle, 1997; Hargreaves, 1999). For example, Coyle *et al.* (1986) showed that ingestion of a glucose polymer (1.8 g/min) increased exercise time from 192 to 252 min during cycling at 70 % VO_{2max}. In the study by McConell *et al.* (1999) exercise time at 69 %VO_{2max} increased from 152 to 199 min with ingestion of 287 g of CHO. This phenomenon has also been described in animals (Dill *et al.* 1932; Bagby *et al.* 1978; Slentz *et al.* 1990). For instance, in the study by Slentz *et al.* (1990) running time to exhaustion, at 8 % grade and 30 m/min, increased from 180 to 276 min in rats with administration of 1.44 g of CHO. In addition, the ability to perform resistance exercise could also increase when CHO are ingested (Lambert *et al.* 1991; Haff *et al.* 2001). In the study by Haff *et al.* (2001), ingestion of 240 g of CHO before and during intermittent isokinetic leg exercise significantly increased the total amount of work performed from 38.1 to 41.1 kJ.

The effect of CHO ingestion on exercise time to exhaustion during prolonged exercise and on the ability to perform resistance exercise could be due to several factors including an increased contribution of plasma glucose oxidation to the energy yield (Coyle *et al.* 1986), muscle glycogen sparing (Tsintzas *et al.*

1996), maintenance of blood glucose concentration which could delay central fatigue (Davis *et al.* 1992) and has been shown to improve cognitive performance (Collardeau *et al.* 2001), an increase in the exercise-induced activation of muscle pyruvate dehydrogenase (Tsintzas *et al.* 2000), an attenuation of muscle and plasma ammonia accumulation (Snow *et al.* 2000), and an improved muscle energy balance (McConnell *et al.* 1999). However, the mechanism(s), which could mediate improvements in exercise performance, associated with CHO administration remain(s) unclear. In particular, it is not known if CHO administration attenuates fatigue through a central effect or through a direct improvement of peripheral neuromuscular function, or both. In fact, no data appear to be currently available on possible improvements of peripheral muscular function associated with glucose administration during prolonged submaximal stimulation.

Based on the results from Haff *et al.* (2001) it was hypothesized that glucose would attenuate peripheral muscle fatigue during prolonged muscle contraction. In order to test this hypothesis, peak submaximal dynamic force of the plantaris muscle was measured in anaesthetized rat during prolonged nerve electrical stimulation *in situ*, with and without glucose infusion. Changes in glycogen content, twitch force and half-relaxation time, maximum dynamic and isometric forces, and M-wave characteristics were also measured, in order to identify some of the possible mechanisms by which glucose infusion could attenuate peripheral muscle fatigue in this situation.

METHODS

Animal care and experimental groups

The experiments were conducted according to the directives of the Canadian Council on Animal Care. Female Sprague-Dawley rats (n = 24, 270-290 g) were obtained from Charles River Canada (St-Constant, Canada) and were housed in cages by pair in a room maintained at 20-23°C and 25 % relative humidity, and with a 12-h/12-h light cycle. They were fed pellet rat chow and tap water *ad libitum*. Three groups of eight animals were studied immediately before, during and after a one-hour period of electrical stimulation of the sciatic nerve with infusion of saline (7.25 mL kg^{-1} h^{-1}) with or without glucose (1.00 g kg^{-1} h^{-1}) through a catheter in the left jugular vein. The first group received only saline for 60 min. The second group received saline and glucose for 60 min. The third group received only saline for 30 min, and then saline and glucose for the subsequent 30 min.

Animal preparation

The animals were anesthetized with ketamine and xylazine I.P. (initial dose: 61.5 mg/kg ketamine and 7.7 mg/kg xylazine; 12.3 mg/kg ketamine and 1.5 mg/kg xylazine every 45 min thereafter). The experiment was conducted on the plantaris muscle, which contains a mixture of fiber types, and motor units (Gardiner & Olha, 1987; Delp & Duan, 1996), and is resistant to fatigue during submaximal prolonged stimulation such as that used in the present experiment. The plantaris muscle of the left leg was surgically isolated from the other ankle extensors. Care was taken not to disrupt its vasculature, innervation, or tendon and not to apply any tension on the muscle during isolation. The other ankle

extensors were denervated and tenotomized at the proximal end of their distal tendon to avoid separating the common tendon of the extensors and possibly damaging the plantaris tendon. The calcaneus was clipped, leaving a bone chip attached to the common tendon, and a silk ligature was firmly placed around the bone-tendon interface. Incisions were made on both sides of the lumbar spine in order to stabilize the animal with clamps. Care was taken not to damage the nerves leaving the spine. The animal was then placed on a stereotaxic table, ventral side on a warming pad. A head holder was used to support the head, and the pelvis and the foot were held immobile by clamps. The rectal temperature was monitored throughout the experiment using a rectal probe, and kept at 36°C. An oil (light mineral, Fisher Scientific, New Jersey, USA) bath was made around the exposed muscle and nerve and also maintained at 36°C. A bipolar stimulation electrode was positioned in contact with the sciatic nerve. The plantaris tendon was attached to a lever arm of a muscle puller servomotor (Cambridge LR 350, Aurora Scientific, Aurora, Canada) with the silk ligature (2-0 thread), which allowed for development of passive muscle tension and measurement of muscle force as previously described (Martineau & Gardiner, 2001). A ball electrode mounted on a spring was placed in contact with the origin side of the plantaris muscle for recording surface EMG, with the ground electrode placed through the gastrocnemius muscle.

Experimental protocol

Optimal length for maximal muscle twitch force was determined as follows: starting from a completely relaxed length, the muscle was slowly lengthened

while supramaximal (5 V) single square pulses of 0.05 ms in duration were delivered once every 3 seconds (Grass S88 stimulator). Several twitch force responses were recorded at the optimal length selected. Following this, the maximum dynamic force at a shortening velocity of 20 mm/s was recorded using one 200-ms train, at 200 Hz (pulse width: 0.05 ms) and 5 V. An evoked 200-Hz maximal contractile response to stimuli was determined in preliminary studies. The muscle was then allowed to rest for 5 min before beginning the 60-min period of stimulation, and the infusion of saline or glucose which were both started at the same time. The pattern of stretch, stimulation and contraction is shown in figure 1. The cycle lasted for 2.7 s. First the length of the muscle was increased by 2 mm for 100 ms, and maintained at this length for an additional 100 ms, without stimulation. The nerve was then stimulated for 200 ms at 50 Hz (pulse width: 0.05 ms) and the muscle was allowed to shorten by 4 mm at a velocity of 20 mm/s. The muscle was then allowed to passively return to its initial length over 200 ms, and to rest for 2.2 s before the beginning of the next cycle. Submaximal peak dynamic force and surface EMG were continuously monitored throughout the 60-min period of stimulation. At the end of the 60-min period of stimulation, twitch force, and the maximum dynamic force were recorded again. A bipolar electrode was, then, inserted in the belly of the muscle, and the muscle was alternatively stimulated indirectly (200 ms, pulse width: 0.05 ms, 200 Hz, 5 V) and directly (200 ms, pulse width: 0.05 ms, 200 Hz, 150 V) maximally while muscle length was kept constant. This measurement was only performed on the control group and the group who received glucose for 60 min. Muscle force and

surface EMG were monitored on an oscilloscope (Hewlett Packard 1741A) and recorded with a microcomputer. At the end of the experiment, the rat was killed by an overdose of ketamine/xylazine.

Measurements

Twitch force and half-relaxation time as well as maximum dynamic force were measured before and after the 60-min period of stimulation. Submaximal peak dynamic muscle force was measured throughout the 60-min period of stimulation. Maximum isometric force in response to indirect and direct muscle stimulation was measured after the 60-min period of stimulation, but not before in order to avoid muscle damage at the start of the experiment. Comparisons were made with maximum isometric force developed through indirect and direct stimulation, in a separate group of rats (n = 8) at the end of a 60-min period of saline infusion without stimulation. Finally, the first evoked M-wave from concentric contractions was analyzed after full-wave rectification, for the measurement of peak-to-peak amplitude, duration and total area throughout the 60-min period of stimulation. Custom designed software's performed all of the above measurements.

Blood samples (0.20 mL) were taken from an incision in the tail before beginning the stimulation and infusion, after 30 min of stimulation and at the end of the period of stimulation. Blood samples were centrifuged at 16,000 g for 4 min at 4°C and the plasma was stored at –80°C for subsequent analysis of glucose (HK 20 glucose kit technique Sigma diagnostics) and insulin concentrations (KTPS-11001, Immunocorps Sciences, Montreal, Canada). Both plantaris

muscles were surgically removed and plunged into liquid nitrogen immediately after the end of each experiment and stored at –80°C until analysis. Muscle glycogen levels were measured using the technique of Lo *et al.* (1970).

Statistical Analysis

The data are expressed as the mean ± SD. Data were analyzed using a two-way ANOVA. When significant differences were revealed, a Scheffe post hoc test was performed. The level of statistical significance was set at $P<0.05$.

RESULTS

Plasma glucose and insulin concentrations remained unchanged when only saline was infused, but significant increases were observed in response to glucose infusion (Table 1). The 60-min period of stimulation significantly decreased muscle glycogen concentrations in the three groups (Table 1), with no significant difference in the initial and final, respectively, values between the three groups.

When saline or saline + glucose was infused for 60 min, submaximal peak dynamic force decreased significantly over the first 5 min of stimulation and remained constant thereafter (Figure 2). However, the reduction was significantly smaller in rats infused with glucose (~55 % and ~70 %, respectively). In rats infused with saline for 30 min and with glucose for the subsequent 30 min, the initial reduction in submaximal peak dynamic force was similar to that observed in rats infused with saline only. However, submaximal peak dynamic force was

partially restored and was significantly different at min 60 when compared to the control group.

Twitch force significantly decreased after the 60-min period of stimulation with no significant difference between the three groups. Twitch half-relaxation time did not change significantly after the 60-min period of stimulation for the three groups, but was significantly lower in the group infused with glucose for 60 min when compared to the other groups (Table 2).

Figure 3 shows the maximum dynamic force before and after the 60-min period of stimulation. Maximum dynamic force significantly decreased in the control group, but remained unchanged in rats infused with glucose for 60 min. Infusion of glucose beginning at min 30 did not fully restore maximum dynamic force.

The maximum isometric force developed under indirect and direct stimulation at the end of the 60-min period of stimulation was ~19 % higher in rats infused with glucose for 60 min than in the control group. Direct stimulation increased muscle force by ~22 % in the control group and ~24 % in rats infused with glucose (Figure 4).

Figure 5 shows changes in M-wave peak-to-peak amplitude, duration and total area during the 60-min period of stimulation. When saline only was infused throughout the experiment a significant reduction in M-wave peak-to-peak amplitude and total area, and an increase in duration was observed. When glucose was infused throughout the experiment the reduction in M-wave peak-to-peak amplitude and the increase in duration were lower, and no change in total

area was observed. Infusion of glucose beginning at min 30 partially restored the values of peak-to-peak amplitude, duration and total area.

DISCUSSION

Results from this study indicate that the beneficial effect of glucose administration on endurance performance could be at least partly due to an improvement of peripheral neuromuscular function. Indeed, the *in situ* nerve-muscle preparation used in the present experiment in anaesthetized rat excludes any possible central effect of glucose. However, glucose infusion attenuated fatigue during prolonged electrical stimulation. The reduction in submaximal peak dynamic force over the first 5 min of stimulation was much smaller in rats infused with glucose than with saline only (~55 % and ~70 %, respectively). In addition, in rats infused with saline for 30 min, submaximal peak dynamic force was partially restored when glucose was administered for the subsequent 30 min. Thus, not only did glucose infusion from the beginning of muscle contraction attenuated muscle fatigue but also partly reversed muscle fatigue later in the period of stimulation.

Fatigue during prolonged exercise often coincides with low muscle glycogen content, and endurance performance could be improved by increasing initial muscle glycogen stores or decreasing muscle glycogen utilization (Conlee, 1987). Muscle glycogen sparing could, thus, be responsible for the increase in endurance performance associated with CHO administration (Tsintzas *et al.* 1996), and could also explain in part the attenuation of peripheral muscle fatigue

observed in the present experiment when glucose was infused. This hypothesis was not supported, however, by our findings. Indeed, a large and significant decrease in muscle glycogen content was observed at the end of the 60-min period of stimulation, but glucose infusion from the onset of stimulation or beginning at min 30, was not associated with any muscle glycogen sparing. This observation is in line with data from several studies showing an improvement in performance with no muscle glycogen sparing during prolonged exercise with CHO administration in man (Coyle *et al.* 1986; Coggan & Coyle, 1991; see Tsintzas & Williams, 1998 for review). Gorski *et al.* (1990) also showed that muscle glycogen utilization in rats was not significantly modified during treadmill running (45 min) or electrical stimulation (30 min) when glucose was administered (0.88 g given both intraperitoneally and by a stomach tube).

The neuromuscular junction (NMJ) is a possible site of peripheral fatigue during nerve stimulation (Sieck & Prakash, 1995). We, thus, hypothesized; that the beneficial effect observed with glucose infusion could be at least partly due to a better maintenance of the function of the NMJ. In order to test this hypothesis, maximal isometric force was compared when elicited through maximum nerve stimulation and through massive direct stimulation of the whole-muscle (thus by-passing the NMJ) (Sieck & Prakash, 1995) at the end of the experiment at a time when a significant reduction in submaximal peak dynamic force was observed. Maximal isometric force through direct as well as indirect stimulation was ~19 % higher in rats infused with glucose than in control rats, which confirmed the beneficial effect of glucose administration on peripheral muscle function. Also the

maximal isometric force elicited through direct massive stimulation of the muscle was ~22 % higher than that elicited through nerve stimulation in the control group, indicating that fatigue developed at the NMJ during the 60-min period of stimulation. However, the difference in maximal isometric force elicited through direct and indirect stimulation was similar in rats infused with glucose (~24 %) and in rats infused with saline only. Accordingly, the beneficial effect of glucose infusion on peripheral neuromuscular function does not appear to be due to an attenuation of fatigue at the NMJ.

Another mechanism for peripheral muscle fatigue is a possible impairment of action potential propagation along the sarcolemma, and/or a possible failure of generating action potentials in some fibers (Fitts, 1994). These phenomena translate, for example, in an increase in duration, and in reductions in amplitude and area of muscle compound action potential (M-wave) (Gardiner & Olha, 1987; Enoka *et al.* 1989; Edman & Lou, 1992; Fuglevand *et al.* 1993). In the present study, the marked reduction in submaximal peak dynamic force observed after 5 min of stimulation in rats infused with saline only, was indeed, associated with an increase in M-wave duration and a decrease in M-wave peak-to-peak amplitude and total area. The lower reduction in submaximal peak dynamic force observed at min 5, with glucose infusion was associated with a better maintenance of M-wave characteristics. Furthermore, when glucose was infused beginning at min 30 in the stimulation period, both submaximal peak dynamic force and M-wave characteristics were partially restored. These observations are in line with the hypothesis that peripheral muscle fatigue is at least partly due to impairments of

action potential generation and/or propagation and suggest that the beneficial effect of glucose infusion could at least partly be due to a better maintenance of the electrical properties of the muscle fiber membrane. This mechanism could also explain that maximum dynamic force measured at the end of the experiment, along with submaximal peak dynamic force measured during the period of stimulation, was higher in rats infused with glucose.

The electrical properties of the membrane largely depend on proper gradients of Na^+ and K^+, which are maintained by the activity of ATP dependent Na^+/K^+-pumps (Overgaard *et al.* 1999; Nielsen & Clausen, 2000). Glucose infusion and the associated increase in plasma glucose concentration could increase the activity of Na^+/K^+-ATPase and Na^+/K^+-pumps by providing glycolytic ATP which appears to preferentially fuel membrane ion pumps in skeletal (Allen *et al.* 1999; Okamoto *et al.* 2001) and cardiac muscle (Saks *et al.* 1994). In addition, in the present experiment, in response to glucose infusion, plasma insulin concentration markedly rose. Insulin plays a major role in K^+ and Na^+ homeostasis, favoring K^+ uptake and Na^+ efflux through the stimulation of the Na^+/K^+-pump (Sweeny & Klip, 1998; Sweeny & Klip, 2001). The importance of insulin stimulated Na^+/K^+-ATPase activity in restoring contractility to K^+ paralyzed rat muscle has been shown by Clausen *et al.* (1993). In that study the reduction in isometric twitch and tetanic force observed when the muscle was exposed to high K^+ concentrations (10-12.5 mM), was partially reversed by insulin which increased Na^+ efflux from the cell and the resting membrane potential. In the present experiment, this phenomenon, along with the increased glucose supply

to the membrane, could in part explain the better maintenance of the membrane electrical properties and the attenuation of peripheral muscle fatigue.

In conclusion, results from the present experiment indicate that peripheral muscle fatigue is attenuated during prolonged indirect stimulation by infusing glucose in rats. Changes in M-wave characteristics suggest that this could be due to a better maintenance of the membrane electrical properties. This in turn could be due to the effect of glucose *per se* on ATP production in the vicinity of Na^+/K^+, and/or to the effect of insulin on Na^+/K^+ -pump. However, it cannot be ruled out that other mechanisms could also be involved in the beneficial effect of glucose infusion on peripheral muscular function, such as changes in energy production and/or in metabolite concentrations in the muscle fiber (Spencer *et al.* 1991; Tsintzas *et al.* 1996; McConell *et al.* 1999; Tsintzas *et al.* 2001).

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Table 1. Plasma glucose and insulin concentrations, and muscle glycogen content.

		Control	Glucose 0-60 min	Glucose 30-60 min
Glucose (mM)	0 min	5.1 ± 0.3	5.0 ± 0.3	5.1 ± 0.2
	30 min	4.9 ± 0.2	11.1 ± 1.1*	4.9 ± 0.2
	60 min	4.2 ± 0.2	9.8 ± 0.7*	9.3 ± 0.5*
Insulin (pM)	0 min	300 ± 11	301 ± 11	305 ± 13
	30 min	283 ± 11	798 ± 20*	279 ± 12
	60 min	257 ± 12	777 ± 19*	682 ± 22*
Glycogen (μmol of glucose/g of wet muscle)	0 min	38.6 ± 2.3	41.3 ± 3.0	40.1 ± 2.6
	60 min	14.9 ± 2.9*	17.5 ± 2.2*	16.8 ± 2.1*

Values are means ± SD (n = 8). * Significantly different from values at 0 min ($P<0.05$).

Table 2. Initial and final twitch force and half-relaxation time.

		Control	Glucose 0-60 min	Glucose 30-60 min
Optimal isometric twitch force (N)	Initial	0.71 ± 0.06	0.74 ± 0.05	0.72 ± 0.04
	Final	0.36 ± 0.02*	0.38 ± 0.03*	0.37 ± 0.03*
Half-relaxation time times (ms)	Initial	11.7 ± 1.7	10.8 ± 1.1	10.5 ± 0.9
	Final	11.0 ± 0.9	9.4 ± 0.9†	11.2 ± 1.1

Values are means ± SD (n = 8). * Significantly different from initial values ($P<0.05$). † Significantly different from other groups ($P<0.05$).

Figure Legends

Fig. 1. Pattern of stretch, stimulation and contraction. On average the muscle length of the plantaris muscle of the rat is approximately 30 mm. The muscle was stretched by 2 mm for 100 ms, and maintained at this length for an additional 100 ms, without stimulation. The nerve was, then, stimulated for 200 ms at 50 Hz, 5 V and with a pulse width of 0.05 ms, and the muscle was allowed to shorten by 4 mm at a velocity of 20 mm/s. The muscle was then allowed to passively return to its initial length over 300 ms, and to rest for 2.0 sec before the beginning of the next cycle.

Fig. 2. Submaximal peak dynamic force. Submaximal peak dynamic force during the 60-min period of stimulation in control rats (■), and in rats infused with glucose from the beginning of stimulation (●) or from min 30 (▲). Values are means ± SD (n = 8).

* Significantly different from initial value ($P<0.05$). † Significantly different from the control group ($P<0.05$).

Fig. 3. Initial and final maximum dynamic force. Initial and final maximum dynamic force in control rats (hatched), and in rats infused with glucose from the beginning of stimulation (black) or from min 30 (white). Values are means ± SD (n = 8).

* Significantly different from initial values ($P<0.05$). † Significantly different from other groups ($P<0.05$).

Fig. 4. Maximum isometric force. Maximum isometric force under indirect (black) and direct (white) stimulation (200 Hz, 200 ms at 150 V and with a 0.05-ms pulse width), following the 60-min period of stimulation in the control rats and in rats infused with glucose for 60 min. Comparisons were made with observation made in a separate group of rats at the end of a 60-min period of saline infusion without stimulation. Values are means ± SD (n = 8). * Significantly different from the group without stimulation. † Significantly different from the control group ($P<0.05$). [2] Significantly different from indirect stimulation ($P<0.05$).

Fig. 5. M-wave characteristics. M-wave peak-to-peak amplitude, duration and total area during the 60-min period of stimulation in control rats (■), and in rats infused with glucose from the beginning of stimulation (●) or from min 30 (▲). Values are means ± SD (n = 8). † Significantly different from the control group ($P<0.05$). * Significantly different from the initial value ($P<0.05$).

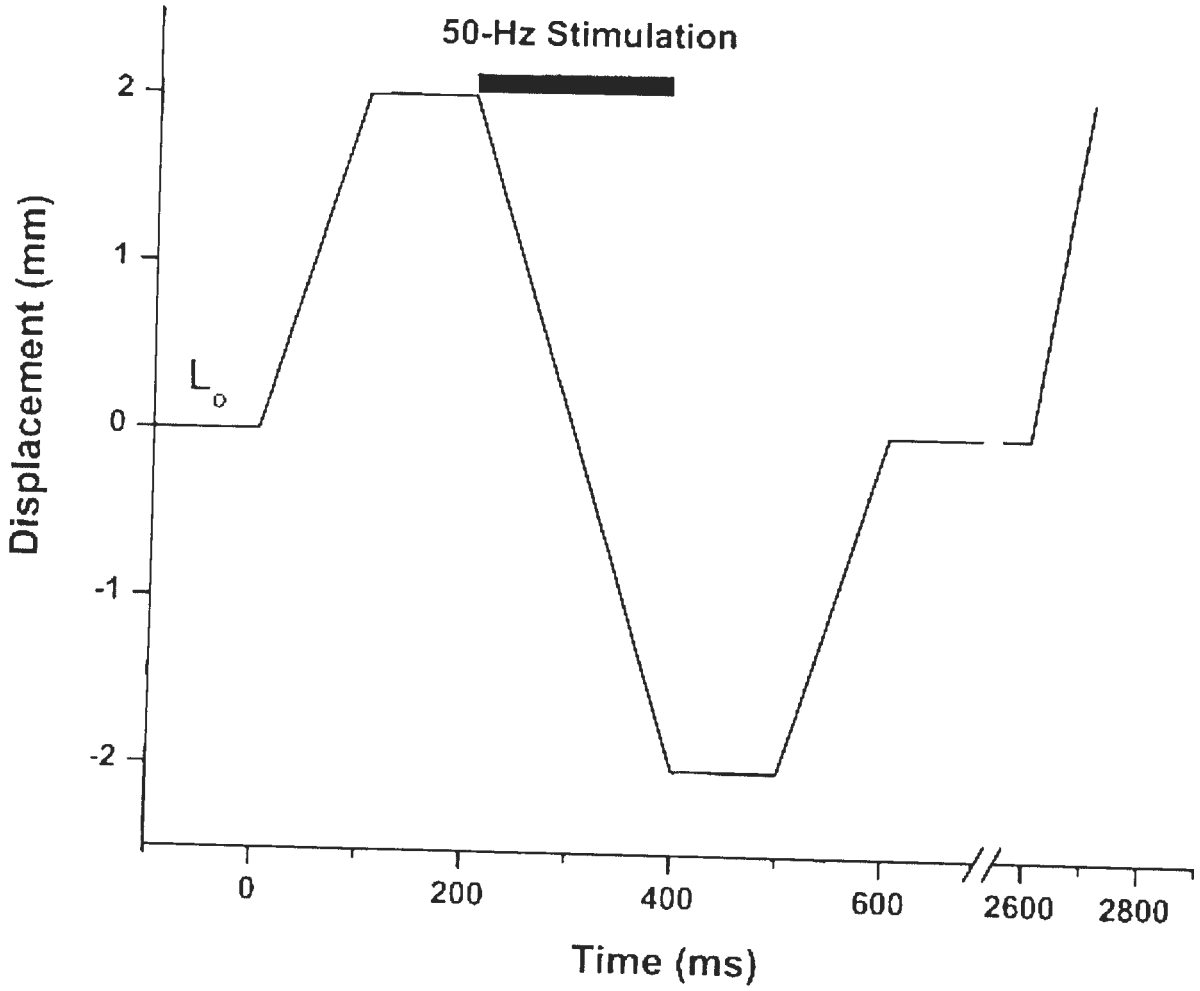
50-Hz Stimulation
Displacement (mm)
2
1
0
-1
-2
L_o
0
200
400
600
2600
2800
Time (ms)

Fig. 1

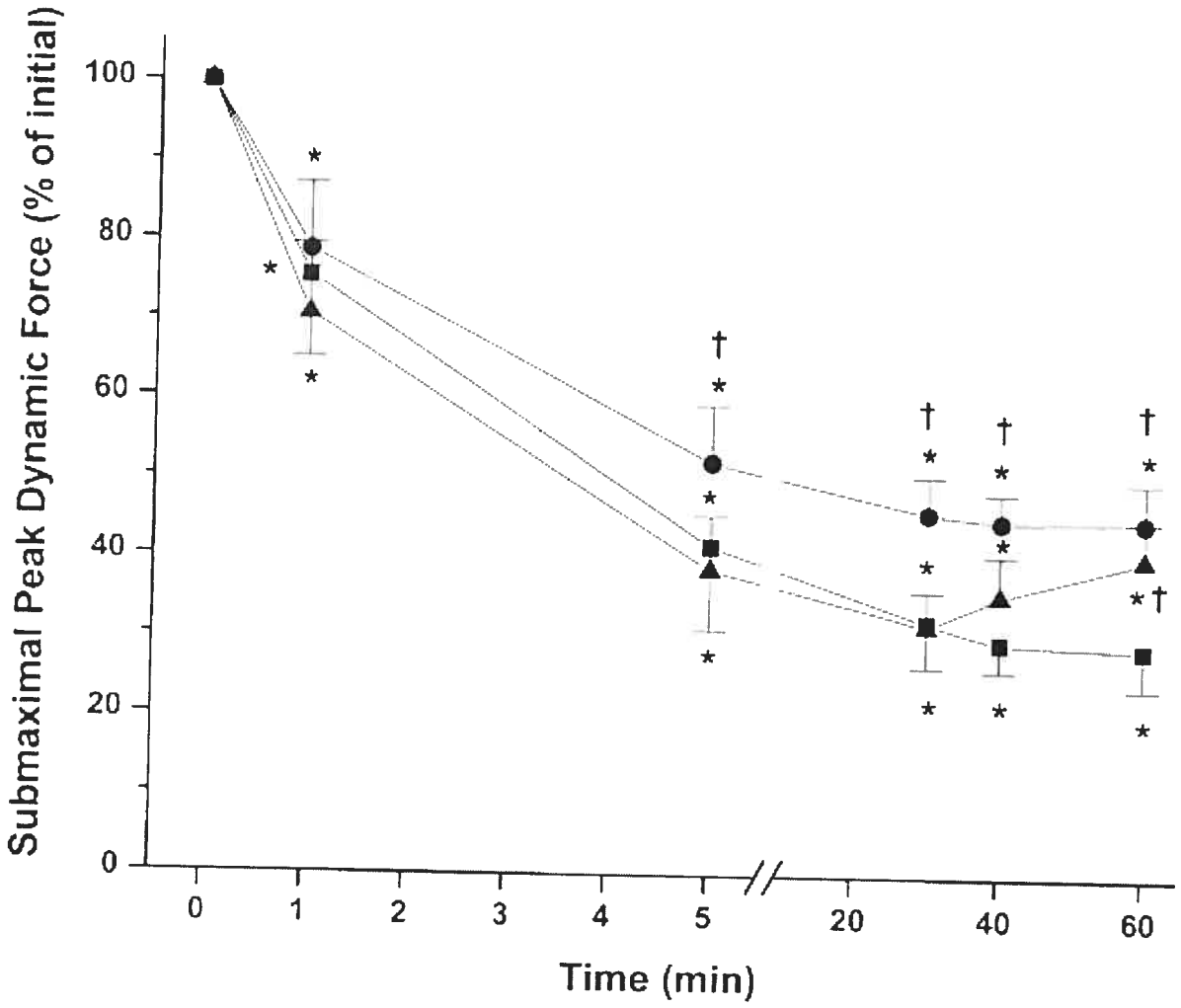
Submaximal Peak Dynamic Force (% of initial)
100
80
60
40
20
0
0
1
2
3
4
5
20
40
60
Time (min)

Fig. 2

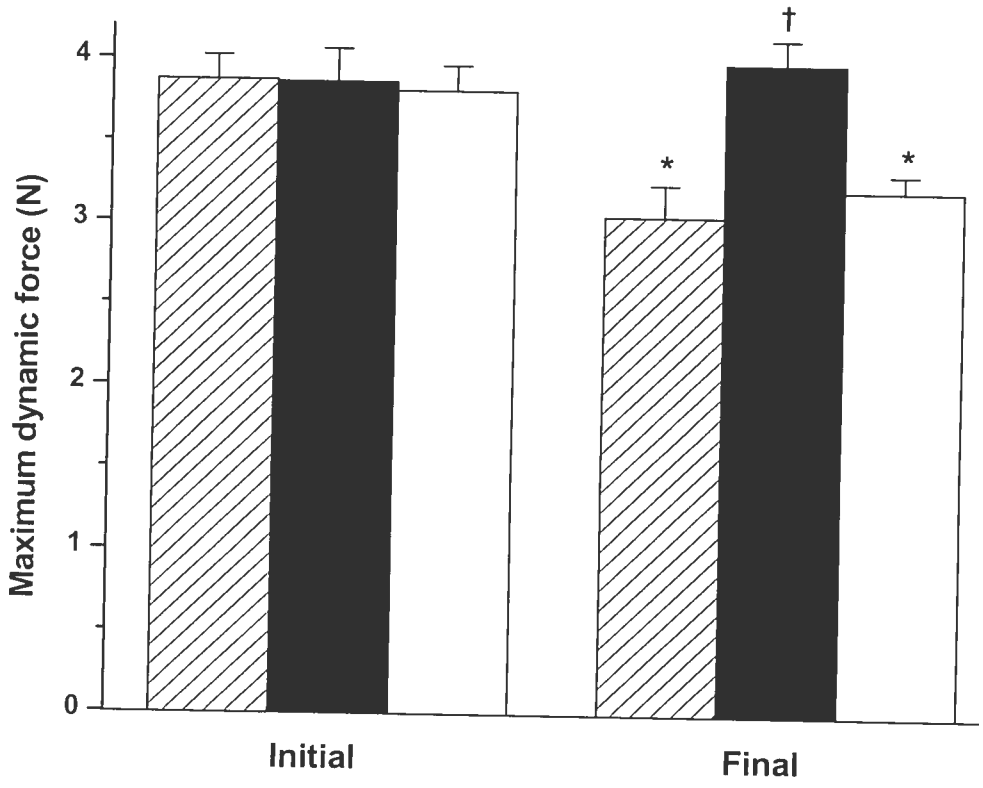

Fig. 3

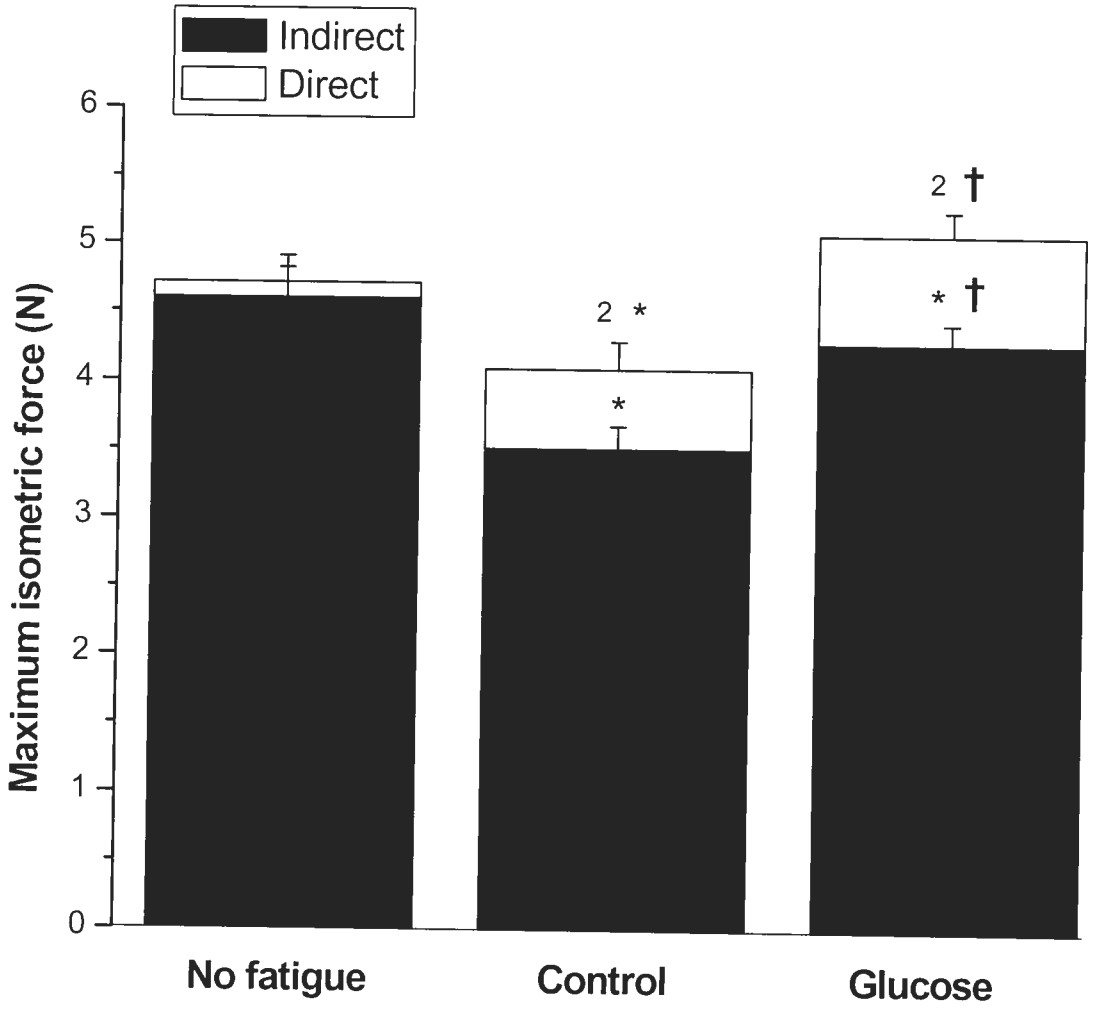
Indirect
Direct
Maximum isometric force (N)
0
1
2
3
4
5
6
2 *
*
2 †
* †
No fatigue
Control
Glucose

Fig. 4

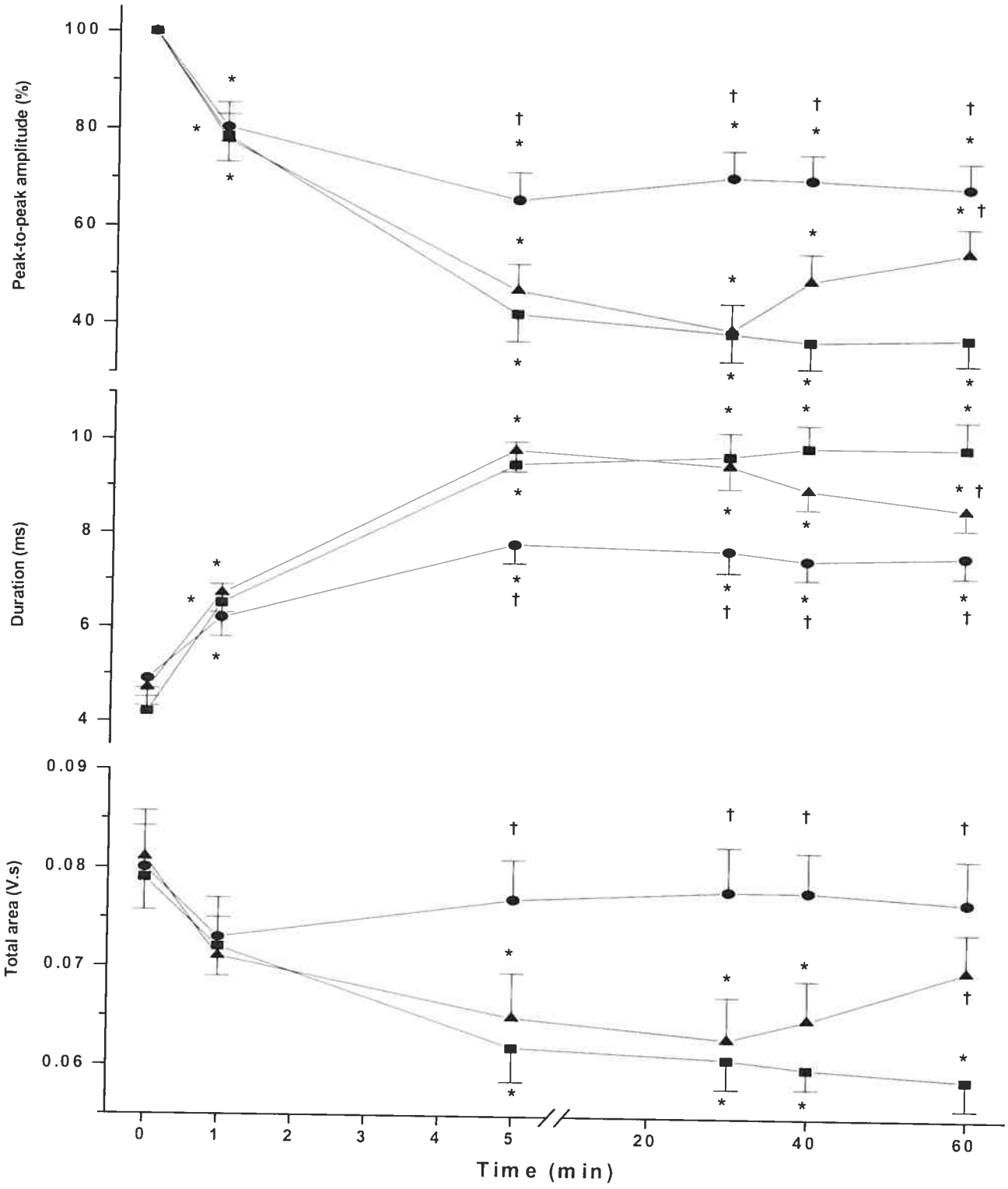
Peak-to-peak amplitude (%)
100
80
60
40
Duration (ms)
10
8
6
4
Total area (V.s)
0.09
0.08
0.07
0.06
0
1
2
3
4
5
20
40
60
Time (min)

Fig. 5

Chapter 3

MANUSCRIPT 2

Title: Insulin does not mediate the attenuation of muscle fatigue associated with glucose infusion in rat plantaris muscle stimulated *in situ*

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Journal: Journal of Applied Physiology 95: 330-335, 2003

Keywords: Muscle force, M-wave, Hyperinsulinemic-euglycemic clamp, Hyperinsulinemic-hyperglycemic clamp

ABSTRACT

Glucose infusion attenuates fatigue in rat plantaris muscle stimulated *in situ*, and this is associated with a better maintenance of electrical properties of the fiber membrane (Karelis et al. Exp Physiol 87:585, 2002). The purpose of the present study was to test the hypothesis that elevated plasma insulin concentration due to glucose infusion (~900 pmol/l), rather than high plasma glucose concentration (~10-11 mmol/l), could be responsible for this phenomenon, since insulin has been shown to stimulate the Na^+/K^+ pump. The plantaris muscle was indirectly stimulated (50 Hz, for 200 ms, 5 V, every 2.7 s) via the sciatic nerve to perform concentric contractions for 60 min, while insulin (8 mU kg^{-1} min^{-1}: plasma insulin ~900 pmol/l) and glucose was infused in order to maintain plasma glucose concentration between 4-6 (6.2 ± 0.4 mg kg^{-1} min^{-1}: hyperinsulinemic-euglycemic or HE) or 10-12 mmol/l (21.7 ± 1.1 mg kg^{-1} min^{-1} : hyperinsulinemic-hyperglycemic or (HH) (6 rats/group). The reduction in submaximal dynamic force was significantly ($P<0.05$) less with HH (-53 %) than with HE and saline only (-66 and -70 %, respectively). M-wave characteristics were also better maintained in the HH than in HE and control groups. These results demonstrate that the increase in insulin concentration is not responsible for the increase in muscle performance observed following the elevation of circulating glucose.

INTRODUCTION

We have recently shown that glucose infusion attenuates fatigue in rat plantaris muscle stimulated indirectly for 60 min *in situ* (15). This was associated with a better maintenance of the electrical properties of the membrane of the muscle fiber as shown by the characteristics of M-wave (peak-to-peak amplitude, duration and total area). The electrical properties of the membrane depend largely on gradients of Na^+ and K^+, which are maintained by the activity of ATP dependent Na^+/K^+-pump (20, 22). Glucose infusion and the associated increase in plasma glucose concentration could have favored the maintenance of these gradients by providing glycolytic ATP, which could preferentially fuel the Na^+/K^+-pump (1, 21). However, in our experiment, glucose infusion and the resultant high plasma glucose concentration (~11 mmol/l) also resulted in a marked increase in plasma insulin concentration (~900 pmol/l). In addition to increasing glucose uptake, insulin directly favors K^+ uptake and Na^+ efflux through the stimulation of the Na^+/K^+-pump (24, 25). This effect could, at least in part, explain the maintenance of electrical properties of the membrane, and the attenuation of muscle fatigue when glucose was infused. The effect of insulin on Na^+/K^+ homeostasis, and on the electrical properties of the muscle fiber membrane has been shown, for example, by Clausen *et al.* (3). In that study, the reduction in isometric twitch and tetanic force observed when the muscle was exposed to high K^+ concentrations (10-12.5 mmol/l), was partially reversed by insulin, and this was associated with an efflux of Na^+ from the cell, and an increase in the resting membrane potential.

The purpose of the present study was to test the hypothesis that elevated plasma insulin concentration due to glucose infusion rather than high plasma glucose concentration *per se* could attenuate muscle fatigue during prolonged indirect electrical stimulation *in situ*. For this purpose, submaximal dynamic force of the plantaris muscle along with changes in M-wave characteristics was measured in anaesthetized rats during prolonged nerve electrical stimulation *in situ*, in a control situation, and when plasma insulin concentration was raised markedly (~900 pmol/l) by infusion of insulin, with glucose maintained between 4-6 mmol/l (hyperinsulinemic-euglycemic clamp) or between 10-12 mmol/l (hyperinsulinemic-hyperglycemic clamp). Maximum dynamic and isometric forces, twitch force and half-relaxation time were also measured before and after the stimulation period.

METHODS

Animal care and anesthesia of animals

Adult female Sprague-Dawley rats weighing approximately 250 g were obtained from Charles River (St. Constant, PQ, Canada). The animals were housed by pairs in grid cages in a room maintained at 20-23°C and 25 % relative humidity, with a 12:12-h light-dark cycle. The animals were provided with commercially available laboratory rat chow and water *ad libitum* from the time of reception until the day of the experiment. The care and treatments of animals were conducted according to the directives of the Canadian Council on Animal Care. The animals were anesthetized by intraperitoneal injections of an initial

dose of ketamine and xylazine (61.5 mg/kg ketamine and 7.7 mg/kg xylazine). Two supplemental doses were given in the middle of preparation (~min 45), and immediately after the beginning of stimulation (12.3 mg/kg ketamine and 1.5 mg/kg xylazine, i.p.) in order to maintain deep anesthesia.

Animal preparation

The experiments were conducted on the plantaris muscle, which contains a mixture of fiber types (7), and motor units (10), and is resistant to fatigue during submaximal prolonged stimulation. As previously described (15) the plantaris muscle and sciatic nerve were surgically isolated, and the plantaris tendon was attached to the lever arm of a muscle puller servomotor (Cambridge LR 305B, Aurora Scientific, Aurora, ON, Canada). The rectal temperature was monitored throughout the experiment, and kept at 36°C using a heating pad.

The animals were divided randomly into three groups of six, and studied before, during and after a 60-min period of electrical stimulation of the sciatic nerve with infusion of either saline (10 mL kg^{-1} h^{-1}) or insulin (8 mU kg^{-1} min^{-1}; administered with saline: 10 mL kg^{-1} h^{-1}; (Pump: Havard Apparatus, St-Laurent, PQ, Canada) (Human insulin, Sigma Chemical, Oakville, ON, Canada). Based on a series of preliminary experiments, infusion of insulin was started 30 min before the beginning of the stimulation in order to attain a plateau at ~900 pmol/l of plasma, which was maintained throughout the experiment (Figure 1). In order to limit blood loss, plasma insulin concentration at the beginning and in the middle of the stimulation period (t = 0, and t = 30 min) was measured in additional groups of rats. Plasma glucose was monitored at 5-min intervals and was

maintained either between ~4.0 and ~6.0 mmol/l (hyperinsulinemic-euglycemic clamp) or between ~10 and ~12 mmol/l (hyperinsulinemic-hyperglycemic clamp) by infusing glucose as needed. The total amounts of glucose infused were 6.2 ± 0.4 and 21.7 ± 1.1 mg kg^{-1} min^{-1}, for the hyperinsulinemic-euglycemic and hyperinsulinemic-hyperglycemic clamps, respectively. The insulin and glucose were infused through a catheter in the left jugular vein. In the control group the infusion of saline alone was started 30 min before the beginning of stimulation.

Experimental protocol

Following determination of optimal muscle length, as previously described (15), twitch forces were recorded using supramaximal (5 V) single square pulses of 0.05 ms in duration delivered once every 3 seconds (Grass S88 stimulator; Quincy, MA, USA). The muscle was, then, subjected to a single maximum dynamic contraction (200-ms train, at 200 Hz and 5 V), and was allowed to rest for 5 min before beginning the 60-min period of stimulation.

Figure 2 shows the pattern of stretch, stimulation and contraction used, which was identical to that in the previous experiment (15). Submaximal dynamic force and surface EMG from a ball electrode mounted on a spring, were continuously monitored on an oscilloscope (Hewlett Packard 1741A Mississauga, ON, Canada) and recorded throughout the 60-min period of stimulation. At the end of the 60-min period of stimulation, twitch force, and maximum dynamic force were recorded again. A bipolar electrode was, then, inserted in the belly of the muscle, and the muscle was alternatively stimulated indirectly (200 ms, 200 Hz, 5 V) and directly (200 ms, 200 Hz, 150 V) maximally while muscle length was kept

constant. Following the experiment, animals were killed by an overdose of ketamine/xylazine.

Measurements

Twitch force and half-relaxation time together with maximum dynamic force were measured before and after the 60-min period of stimulation. Submaximal dynamic muscle force was measured throughout the 60-min period of stimulation. Maximum isometric forces in response to indirect and direct muscle stimulation were measured after the 60-min period of stimulation. Comparisons were made with maximum isometric force developed through indirect and direct stimulation, in a separate group of rats (n = 6) at the end of a 60-min period of saline infusion without stimulation. Finally, the first evoked M-wave during each concentric contraction was analyzed after full-wave rectification, for the measurement of peak-to-peak amplitude, duration and total area, throughout the 60-min period of stimulation. Custom designed software was used to perform all of the above measurements.

Plasma glucose concentrations were determined in blood droplets sampled by incision of the tail (Glucometer Elite, Toronto, ON, Canada). For the measurement of plasma insulin concentration (automated radioimmunoassay: Medicorp, Montreal, Canada), ~ 1ml blood samples were withdrawn from a catheter placed in the right femoral vein, centrifuged at 16,000 g for 4 min at 4°C, and the plasma was stored at -80°C for subsequent analysis. Both plantaris muscles were excised and frozen into liquid nitrogen immediately after the end of

each experiment, and stored at −80°C until analysis. Muscle glycogen levels were measured using the technique of Lo *et al.* (17).

Statistical Analysis

Data are expressed as the mean ± SD. Comparisons were made using a two-way ANOVA. When significant differences were revealed, a Scheffé post hoc test was performed. The level of statistical significance was set at $P<0.05$.

RESULTS

In the control group, with infusion of saline only, submaximal dynamic force significantly decreased over the first 5 min of stimulation and remained constant thereafter (Figure 3). When compared to the initial value, over the last 55 min of stimulation the average reduction of force developed was 70.4 ± 3.8 %. Maximum dynamic force (Figure 4), isometric force (Figure 5), and twitch force (Table 1), were also significantly reduced following the period of stimulation. The pattern of changes in submaximal dynamic force was similar in the animals infused with insulin (Figure 3). In the euglycemic group, the average reduction in submaximal force over the last 55 min of stimulation (66.3 ± 3.2 %), as well as the reduction in maximum dynamic (0.85 ± 0.04 N), twitch (0.42 ± 0.03 N), and maximal isometric force developed in response to indirect stimulation (1.5 ± 0.08 N), were not significantly different from those observed in the control group. In contrast, in animals infused with insulin, but with a high plasma glucose concentration, the reduction in submaximal dynamic force (53.1 ± 2.9 %) (Figure 3), maximal dynamic force (0.21 ± 0.01 N) (Figure 4), and maximal isometric

force developed in response to indirect stimulation (0.78 ± 0.04 N) (Figure 5) were lower. The reduction in twitch force was similar to those observed in the control group and in the euglycemic group (Table 1), and no significant change was observed for half relaxation time in any of the three groups, following the stimulation period (Table 1). No significant difference was observed between the maximum isometric force developed in response to indirect and direct stimulation, in the group of rats which were not submitted to the fatigue protocol (Figure 5). In contrast, the maximum isometric force developed in response to direct stimulation following the end of the experiment was significantly higher than that developed in response to indirect stimulation. However, the differences were not significantly different in rats infused with saline (19.2 ± 0.9 %) and with insulin (21.1 ± 1.0 and 18.4 ± 0.8 % in the euglycemic and hyperglycemic group, respectively).

Figure 6 shows changes in M-wave peak-to-peak amplitude, duration and total area during the 60-min period of stimulation. The reduction in peak-to-peak amplitude (~60 - 57 %), and in total area (from ~0.08 to ~0.059 – 0.062 V.s), and the increase in duration (from ~5.0 to ~10.1 – 9.6 ms), were similar in rats infused with saline, and in rats infused with insulin but maintained euglycemic. The reduction in peak-to peak amplitude (~35 %) and the increase in duration (from ~5.0 to ~7.6 ms) were significantly lower in rats infused with insulin but with a very high plasma glucose concentration, while M-wave total area was not significantly modified.

The 60-min period of stimulation significantly decreased muscle glycogen content in the three groups (Table 1), with no significant difference in the initial and final values, respectively, among the three groups.

DISCUSSION

Ingestion of carbohydrates during prolonged exercise has been shown to increase exercise time to exhaustion in man (4, 5, 6, 12, 19) and animals (2, 8, 23), and could also increase the ability to perform resistance exercise (11, 16). In a recent experiment, we have shown that the beneficial effect of infused glucose on exercise performance could be in part due to a direct effect on the neuromuscular function (15). In this study, the plantaris muscle was stimulated indirectly in anesthetized rats over a 60-min period while either saline or glucose was infused. When glucose was infused (17 $mg.kg^{-1}.min^{-1}$), plasma glucose concentration was maintained between 10 and 11 mmol/l (vs ~5 mmol/l in rats infused with saline), and this was associated with a significant attenuation of muscle fatigue, as shown by the lower reduction in submaximal dynamic force during the period of stimulation (~55 % decrease vs ~70 % in control rats), and a lower reduction in maximum dynamic and isometric force after the period of stimulation. In addition, in rats infused with saline for 30 min, submaximal dynamic force was partially restored when glucose was administered for the subsequent 30 min. Thus, not only did glucose infusion from the beginning of muscle contraction attenuate muscle fatigue but also partly reversed muscle fatigue later in the period of stimulation. Comparison between maximal isometric

force developed with indirect and massive direct muscle stimulation, showed that the attenuation of fatigue was not due to a better maintenance of the neuromuscular junction. In contrast, M-wave characteristics indicated that glucose infusion helped alleviate deterioration of the electrical properties of the muscle fiber membrane. This phenomenon could be due to the effect of a high plasma glucose concentration per se, which could increase the supply of glycolytic ATP to the Na^+/K^+-pump (1, 21). However, the high plasma insulin concentration observed, in this situation, in response to glucose infusion, and high plasma glucose concentration, could also be involved in the better maintenance of the electrical properties of the muscle fiber membrane. Indeed, as shown by Hundal *et al.* (13) and Marette *et al.* (18), insulin increases the activity of Na^+/K^+-ATPase in the membrane of the muscle fiber. Data from Clausen *et al.* (3) indicate that insulin could have a beneficial effect on muscle force. In that experiment, when extracellular potassium concentration was increased from the control value of 4 mmol/l to 12.5 mmol/l, the tetanic force of the soleus muscle was reduced by 96 %. Insulin administration (100 mU/ml) produced a 38 % recovery of force within 20 min, and this was associated with a 21 % increase in efflux of Na^+ from the cell, and a 14 % increase in K^+ uptake. The purpose of the present experiment was, thus, to test the hypothesis that the beneficial effect of glucose infusion on muscle fatigue observed in our previous experiment, could be due to the associated high plasma insulin concentration, rather than the high plasma glucose concentration and delivery to the muscle, *per se*. It should be recognized, however, that carbohydrate ingestion during

prolonged exercise in man is not associated with high plasma insulin concentrations (9, 14). As a consequence, although the high plasma insulin concentration could explain our previous findings, it is unlikely to be responsible for the improvement in performance in endurance events when carbohydrates are ingested.

The above hypothesis was not confirmed by the results obtained. When saline was infused, with plasma glucose and plasma insulin concentrations averaging ~5 mmol/l and ~265 pmol/l throughout the experiment, submaximal dynamic force, as well as maximal dynamic and isometric forces were significantly reduced following the 60-min stimulation period. Similar reductions were observed, when plasma insulin concentration was increased to ~900 pmol/l but with plasma glucose concentration maintained between 4-6 mmol/l by infusing only a small amount of glucose (~93 mg over 60 min) (hyperinsulinemic-euglycemic clamp). In contrast, when plasma glucose concentration was raised between 10-12 mmol/l by infusing a larger amount of glucose (~325 mg over 60 min), while maintaining plasma insulin concentration at ~900 pmol/l (hyperinsulinemic-hyperglycemic clamp), the reduction in submaximal dynamic force, as well as maximal dynamic and isometric forces were much lower. These observations indicate that increased plasma glucose concentration and/or glucose delivery to the muscle, and not the associated increase in plasma insulin concentration, was responsible for the attenuation of muscle fatigue when glucose was administered.

The mechanisms by which increased plasma glucose concentration and/or glucose delivery to the muscle attenuates muscle fatigue remain to be determined. However, as observed in the previous experiment (15), this was not related to difference in muscle glycogen utilization, which was similar in the three groups, nor to a better maintenance of the properties of the neuromuscular junction, as shown by differences between direct and indirect stimulation of the muscle at the end of the experiment. In contrast, as also observed in the previous experiment (15), the reduction in muscle force when saline was infused, as well as with the hyperinsulinemic-euglycemic clamp, was associated with a concomitant reduction in M-wave peak-to-peak amplitude, and total area, and an increase in M-wave duration. The larger submaximal and maximal dynamic forces, and maximal isometric forces observed with the hyperinsulinemic-hyperglycemic clamp were associated with a better maintenance of M-wave characteristics. These observations confirm that muscle fatigue was at least partly due to impairments of action potential generation and/or propagation along the muscle fiber membrane, and that high plasma glucose concentration and/or delivery to the muscle helps alleviate these impairments. This phenomenon could be due in part to the fact that infused glucose could provide glycolytic ATP, which appears to preferentially fuel the Na^+/K^+-pump, as shown by data from Okamoto *et al.* (21). An alternate or complementary explanation is that glycolytic ATP from infused glucose could also preferentially fuel the Ca^{2+} pump in the sarcoplasmic reticulum, as shown by Xu *et al.* (27), and could, thus, help attenuate muscle

fatigue related to change in Ca^{2+} handling, which also develops under prolonged stimulation in situ (26).

ACKOWLEDGEMENTS

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Table 1. Initial and final muscle glycogen content, twitch force and half-relaxation time in the control, hyperinsulinemic-euglycemic (HE) and hyperinsulinemic-hyperglycemic (HH) groups.

		Control	HE	HH
Glycogen	Initial	39.4 ± 2.4	40.1 ± 3.1	41.8 ± 2.8
μmol of glucose/g w.w.	Final	16.2 ± 2.5*	17.5 ± 2.3*	18.1 ± 2.6*
Twitch Force (N)	Initial	0.74 ± 0.06	0.78 ± 0.04	0.77 ± 0.07
	Final	0.39 ± 0.03*	0.36 ± 0.03*	0.40 ± 0.04*
Half-relaxation time (ms)	Initial	11.2 ± 1.6	10.9 ± 1.3	11.6 ± 1.9
	Final	10.5 ± 0.8	10.4 ± 1.0	9.9 ± 1.1

Values are means ± SD (n = 6). * Significantly different from initial value ($P<0.05$).

FIGURE LEGENDS

Fig. 1. Plasma glucose and insulin concentrations in the control (!), in the hyperinsulinemic-euglycemic (,), and in the hyperinsulinemic-hyperglycemic (7) groups. (means ± SD, n = 6; * significantly different from initial value, P<0.05).

Fig. 2. Pattern of stretch, stimulation and contraction (on average, L_0 was approximately 30 mm).

Fig. 3. Submaximal dynamic force during the 60-min period of stimulation in the control (!),in the hyperinsulinemic-euglycemic (,), and in the hyperinsulinemic-hyperglycemic (7) groups (means ± SD, n = 6; * significantly different from initial value;

† Significantly different from the other groups, P<0.05).

Fig. 4. Initial and final maximum dynamic force. Initial and final maximum dynamic force in the control (black), and in the hyperinsulinemic-euglycemic (white) or in the hyperinsulinemic-hyperglycemic (shaded) groups. Values are means ± SD (n = 6).

* Significantly different from initial values (P<0.05). † Significantly different from other groups (P<0.05).

Fig. 5. Maximum isometric force. Maximum isometric force under indirect (black) (200 Hz, 200 ms at 5 V), and direct (shaded) stimulation (200 Hz, 200 ms

at 150 V), following the 60-min period of stimulation in the control, hyperinsulinemic-euglycemic (HE) and hyperinsulinemic-hyperglycemic (HH) groups. Comparisons were made with observation made in a separate group of rats at the end of a 60-min period of saline infusion without stimulation. Values are means ± SD (n = 6). * Significantly different from the group without stimulation (P<0.05). † Significantly different from indirect stimulation (P<0.05). ‡ Significantly different from the control group (P<0.05).

Fig. 6. M-wave characteristics. M-wave peak-to-peak amplitude, duration and total area during the 60-min period of stimulation in the control (!), and in the hyperinsulinemic-euglycemic (,) or in the hyperinsulinemic-hyperglycemic (7) groups. Values are means ± SD (n = 6). * Significantly different from the initial value (P<0.05). † Significantly different from the other groups (P<0.05).

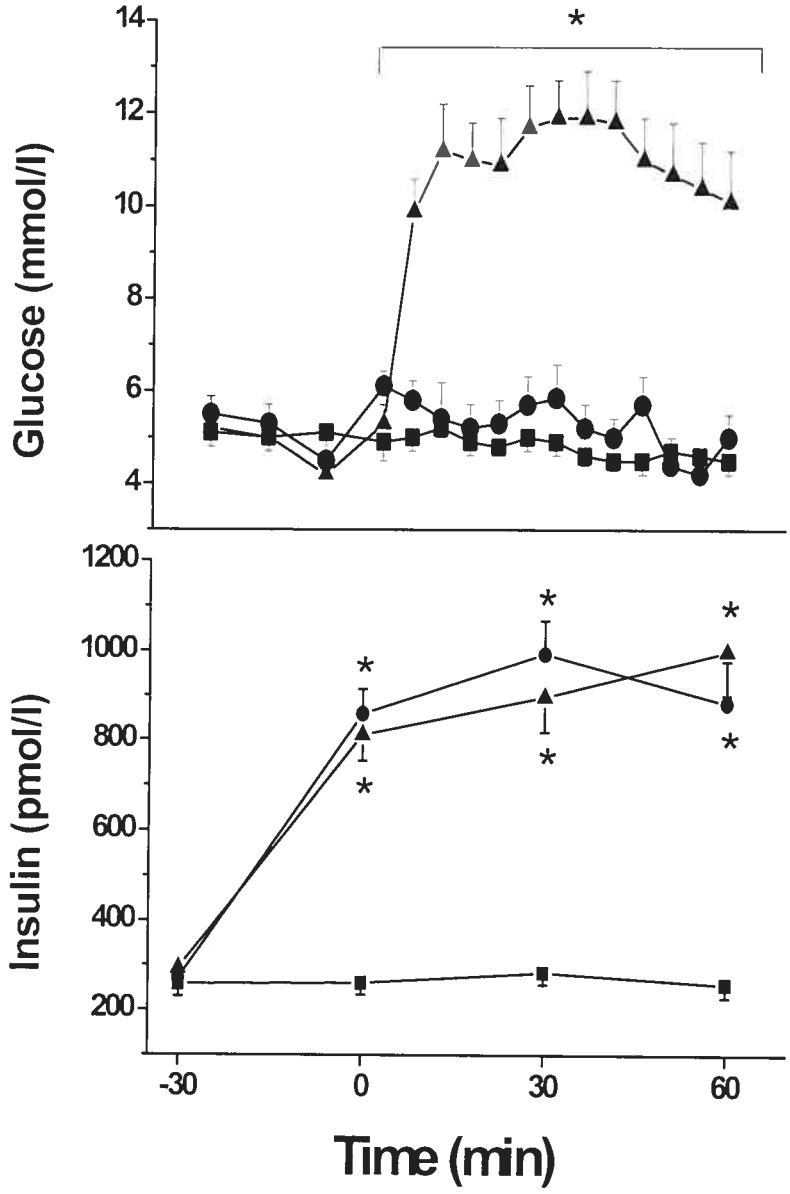
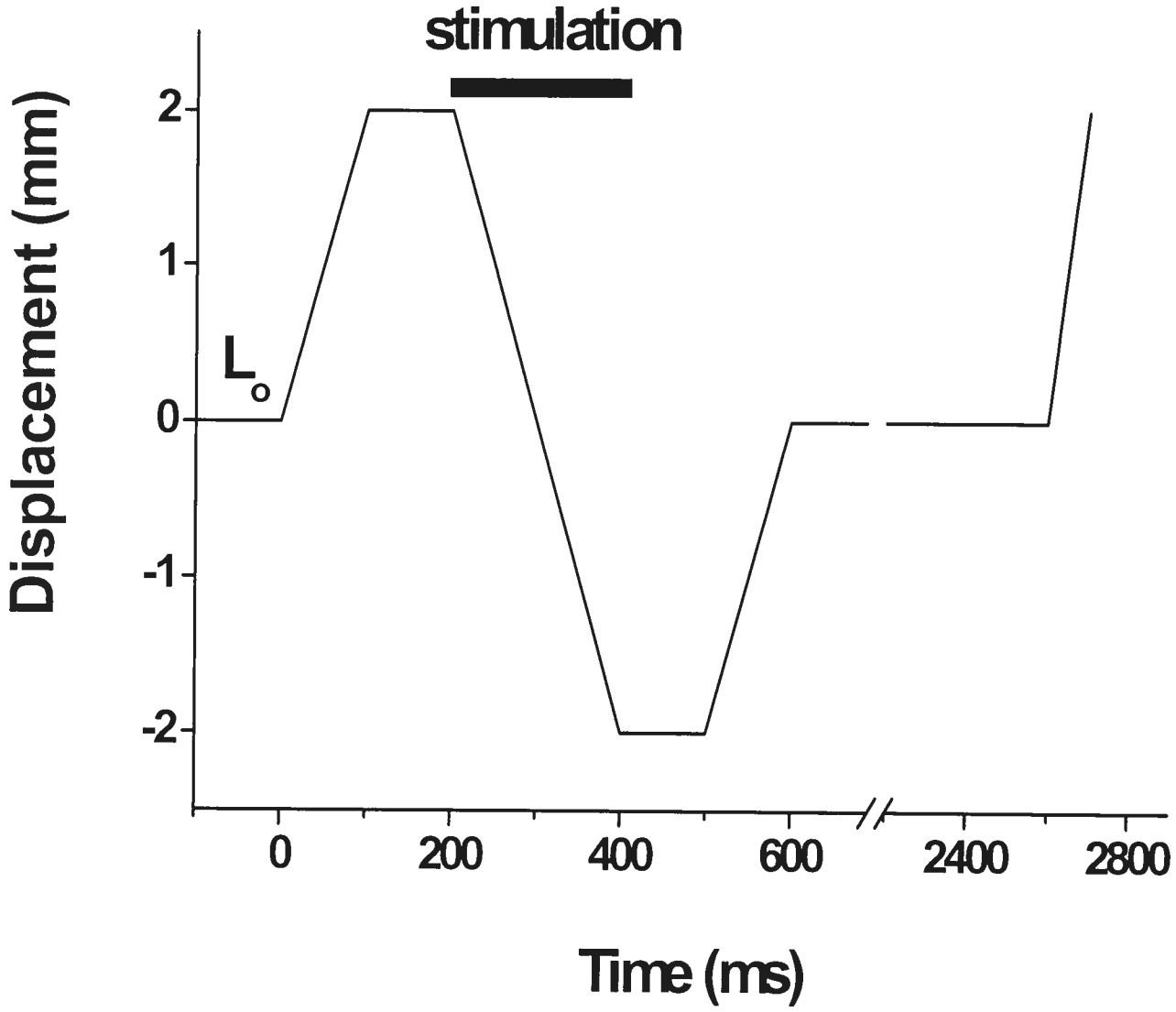

Glucose (mmol/l)
14
12
10
8
6
4
*
Insulin (pmol/l)
1200
1000
800
600
400
200
*
*
*
*
*
*
-30
0
30
60
Time (min)

Fig.1

Fig. 2


stimulation
Displacement (mm)
2
1
0
-1
-2
L_o
0
200
400
600
2400
2800
Time (ms)

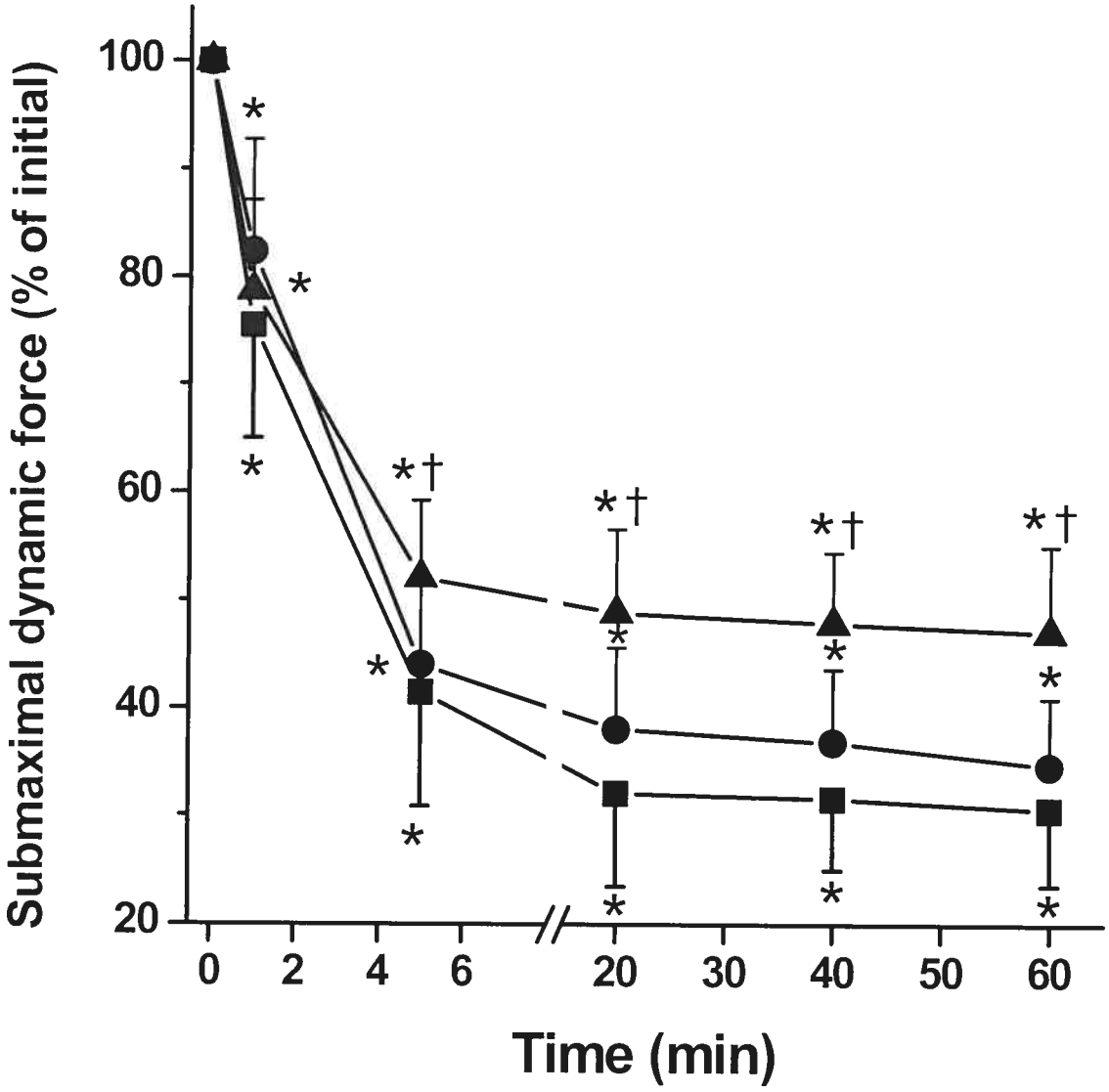

Submaximal dynamic force (% of initial)
100
80
60
40
20
0
2
4
6
20
30
40
50
60
Time (min)
*
†

Fig. 3

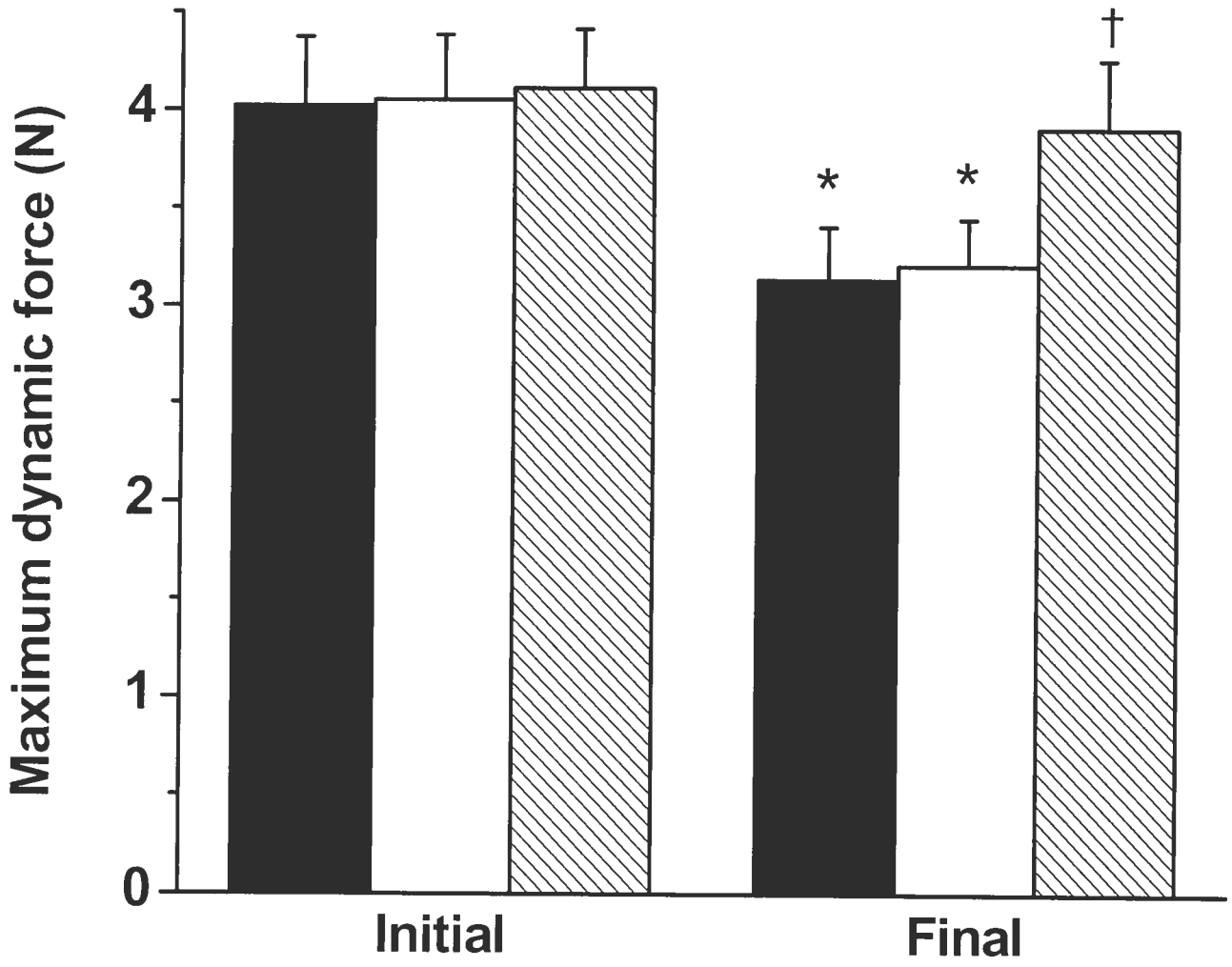


Fig. 4

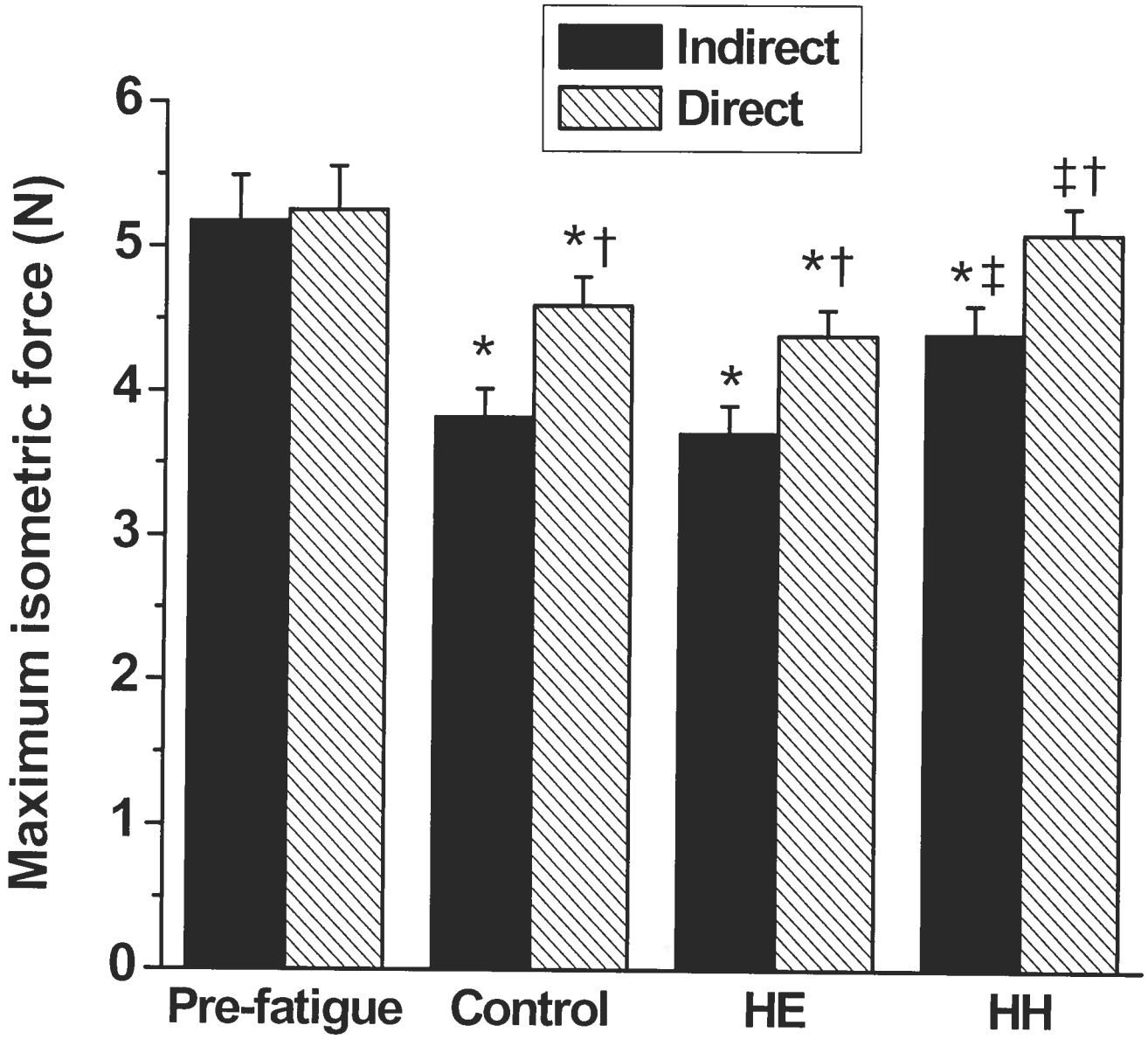

Indirect
Direct
Maximum isometric force (N)
6
5
4
3
2
1
0
*
*†
*
*†
*‡
‡†
Pre-fatigue
Control
HE
HH

Fig. 5

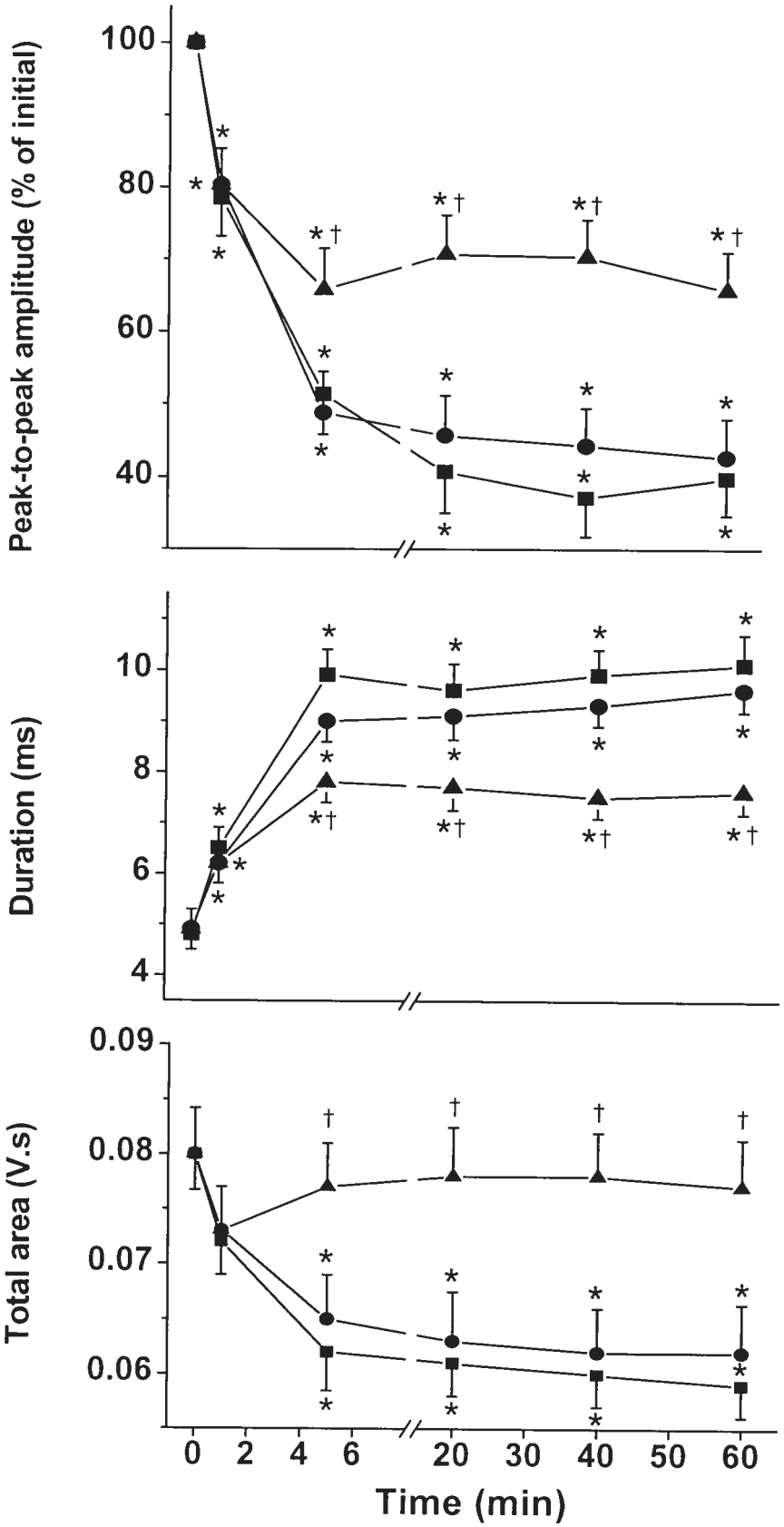

Peak-to-peak amplitude (% of initial)
100
80
60
40
Duration (ms)
10
8
6
4
Total area (V.s)
0.09
0.08
0.07
0.06
0
2
4
6
20
30
40
50
60
Time (min)

Fig. 6

Chapter 4

MANUSCRIPT 3

Title: Effect of lactate infusion on M-wave characteristics and force in the rat plantaris muscle during repeated stimulation *in situ*

Authors: Antony D. Karelis, Mariannick Marcil, François Péronnet, and Phillip F. Gardiner

Journal: Journal of Applied Physiology 96:2133-8, 2004

Keywords: Muscle Fatigue, Muscle force, M-wave, Performance

ABSTRACT

It is unclear whether accumulation of lactate in skeletal muscle during exercise contributes to muscle fatigue. The purpose of the present study was to examine the effect of lactate infusion on muscle fatigue during prolonged indirect stimulation *in situ*. For this purpose the plantaris muscle was electrically stimulated (50 Hz, for 200 ms, every 2.7 s, 5 V) *in situ* through the sciatic nerve to perform concentric contractions for 60 min while infusing intravenously either saline or lactate (8 rats/group). Lactate infusion ([lactate] ~12 mM) attenuated the reduction in submaximal dynamic force (-49 % vs. –68 % in rats infused with saline, $P < 0.05$). Maximum dynamic and isometric forces at the end of the period of stimulation were also higher ($P<0.05$) in rats infused with lactate (3.8 ± 0.3 and 4.4 ± 0.3 N) compared to saline (3.1 ± 0.2 and 3.6 ± 0.2 N). The beneficial effect of lactate infusion on muscle force during prolonged stimulation was associated with a better maintenance of M-wave characteristics compared to control. In contrast, lactate infusion was not associated with any reduction in muscle glycogen utilization or with any reduction of fatigue at the neuromuscular junction (as assessed through maximal direct muscle stimulation: 200 Hz, 200 ms, 150 V).

INTRODUCTION

It has been repeatedly suggested that lactic acid accumulation in the muscle and in the extracellular fluid and/or a decrease in pH in response to exercise could be in part responsible for the development of muscle fatigue (see (3, 13) for review). However, results from several studies which have examined the effect of lactate administration on muscle function in various experimental models do not consistently support this hypothesis (1, 2, 4, 5, 8, 11, 12, 18, 22, 23, 25-28, 30). Favero *et al.* (12) showed a large reduction in Ca^{2+} release from isolated sarcoplasmic reticulum vesicles incubated with lactate (37 % decrease at 20 mM lactate), but in mechanically skinned fibers this effect appears much smaller (less than 10 % decrease at 30 mM lactate) (8) or even reversed (220 % increase at 20 mM lactate) (2). Andrews *et al.* (2) also reported that lactate slightly reduced Ca^{2+} uptake by the sarcoplasmic reticulum in intact isolated muscle fibers (13 % decrease over 15 s but no effect over 1 min with 20 mM lactate). Consistent data indicate no inhibition of the excitation-contraction coupling by lactate in skinned (1, 4, 27, 28) and in intact muscle fibers (22, 26, 30). As for the electrical properties of the sarcolemma, Erdogan *et al.* (11) showed that lactate (20 mM) does not modify resting membrane potential or the amplitude of the action potential in muscle strips of the rat diaphragm stimulated indirectly *in vitro*. Nielsen *et al.* (23) and Pedersen *et al.* (25), actually showed that in intact muscle stimulated directly or indirectly *in vitro*, lactate (20 and 10 mM, respectively) could partly restore M-wave area previously deteriorated by increasing $[K^+]$ in the bath. Finally, the effect of lactate on force development

appears variable. Phillips *et al.* (26), in the mouse soleus and EDL muscles and Coast *et al.* (5) in rat diaphragm muscle strip *in vitro*, did not observe any change in isometric force when lactate concentration was increased to 20 or 10 mM, respectively. In contrast, Erdogan *et al.* (11) in rat diaphragm muscle strips stimulated indirectly *in vitro*, and Hogan *et al.* (18), in dog gastrocnemius muscle stimulated indirectly *in situ*, showed that lactate (20 and 14 mM, respectively) significantly reduced isometric muscle force. However, Nielsen *et al.* (23) in the rat soleus muscle, and Pedersen *et al.* (25), in both the rat EDL and soleus muscles, stimulated directly or indirectly *in vitro*, showed that the reduction in isometric force due to increasing $[K^+]$ in the bath, was totally (23) or partially (25) reversed when lactate concentration was increased to 20 and 10 mmM, respectively.

Muscle fatigue has been associated with impairments of action potential generation and/or propagation along the muscle fiber membrane (6, 13, 14, 24). Therefore, the purpose of the present study was to further investigate the effect of lactate infusion on the electrical properties of the muscle fiber membrane and the development of muscle fatigue. Based on the studies from Nielsen et al. (23) and Pedersen et al. (25), we hypothesized that the electrical properties of the muscle fiber membrane and the development of muscle fatigue would be better maintained with lactate infusion during prolonged indirect stimulation *in situ*. Submaximal dynamic force of the plantaris muscle along with changes in M-wave characteristics were measured in anaesthetized rat during prolonged nerve electrical stimulation *in situ*, in a control situation, and with lactate infusion.

METHODS

Animal care and anesthesia

Adult female Sprague-Dawley rats weighing approximately 250 g were obtained from Charles River (St. Constant, PQ, Canada). The animals were housed by pair in grid cages in a room maintained at 20-23 °C and 25 % relative humidity, with a 12:12-h light-dark cycle. The animals were provided with commercially available laboratory rat chow and water *ad libitum* from the time of reception until the day of the experiment. The care and treatments of animals were conducted according to the directives of the Canadian Council on Animal Care. The animals were anesthetized by intraperitoneal injections of an initial dose of ketamine and xylazine (61.5 mg/kg ketamine and 7.7 mg/kg xylazine). Two supplemental doses were given in the middle of preparation (~min 45), and immediately before the beginning of stimulation (12.3 mg/kg ketamine and 1.5 mg/kg xylazine, i.p.) in order to maintain deep anesthesia.

Animal preparation

As previously described (19), the plantaris muscle of the left leg was surgically isolated from the other ankle extensors which were denervated and tenotomized at the proximal end of their distal tendon to avoid separating the common tendon of the extensors. The plantaris muscle was chosen because it contains a mixture of motor units (16) and fiber types (7), and is resistant to fatigue during submaximal prolonged stimulation (16, 19, 20). The calcaneus was clipped, leaving a bone chip attached to the common tendon, and a silk ligature (2-0 thread) was firmly placed around the bone-tendon interface. The animal was

placed in a prone position on a stereotaxic table, and stabilized by clamps fixed in the head, vertebral column, left knee, and left foot. The hindlimb skin flaps were used to prepare a pool that was filled with mineral oil kept at 36-37 °C by recirculation through a thermoregulated bath. The rectal temperature was monitored throughout the experiment, and kept at 36 °C using a heating pad. For the nerve-muscle evoked contractions, a bipolar stimulation electrode was positioned in contact with the sciatic nerve, and the plantaris tendon was attached to a lever arm of a muscle puller servomotor (Cambridge LR 305B, Aurora Scientific, Aurora, ON, Canada) with a silk ligature. Surface EMG was recorded using a ball electrode mounted on a spring, which was placed in contact with the origin side of the plantaris muscle, with the ground electrode placed through the gastrocnemius muscle.

The animals were divided randomly into two groups of eight animals and studied immediately before, during and after a 60-min period of electrical stimulation of the sciatic nerve with a continuous infusion through a catheter in the left jugular vein of either saline alone (NaCl 0.9 %) (7.25 ml kg^{-1} h^{-1}), or sodium L-(+)-lactate (sodium 2-hydroxypropionate) (Sigma Chemicals) (0.96 g kg^{-1} h^{-1}, administered with saline 7.25 ml kg^{-1} h^{-1}). Four additional groups of eight rats each were used to measure maximal isometric force developed through indirect and direct stimulation in the unfatigued muscle, initial plasma insulin concentration and pH, as well as final plasma insulin concentration and pH following infusion of saline or lactate (see below).

Experimental protocol

Optimal length for muscle twitch force was determined starting from a completely relaxed length. The muscle was slowly lengthened while supramaximal (5 V) single square pulses of 0.05 ms in duration were delivered once every 3 seconds (Grass S88 stimulator; Quincy, MA, USA). When optimal muscle length was achieved, several twitch force responses were recorded. Following this, the muscle was subjected to a single maximum dynamic contraction (200-ms train, at 200 Hz and 5 V) which was recorded. The muscle was, then, held at this length and allowed to rest for 5 min before beginning the 60-min period of stimulation.

The infusion of lactate and the stimulation were both initiated at min 0. The inset in figure 2 shows the pattern of stretch (2 mm for 100 ms), stimulation (200 ms at 50 Hz) and contraction (4 mm at a velocity of 20 mm/s), relaxation (300 ms) and rest (2.1 s), which was repeated every 2.7 s for 60 min. Submaximal dynamic force and surface EMG were continuously monitored on an oscilloscope (Hewlett Packard 1741A Mississauga, ON, Canada) and were recorded with a microcomputer throughout the 60-min period of stimulation. At the end of the 60-min period of stimulation, twitch force and the maximum dynamic force were recorded again. A bipolar electrode was, then, immediately inserted in the belly of the muscle, and the muscle was alternatively stimulated indirectly (200 ms, 200 Hz, 5 V, pulse duration: 0.05 ms) and directly (200 ms, 200 Hz, 150 V, pulse duration: 0.05 ms) maximally while muscle length was kept constant. We have previously shown (19, 20) that these parameters used for direct muscle

stimulation in this preparation result in the same force with and without neuromuscular block using curare. Following the experiment, animals were killed by an overdose of ketamine/xylazine.

Measurements

Twitch force and half-relaxation time together with maximum dynamic force were measured before and after the 60-min period of stimulation. Submaximal dynamic muscle force was measured throughout the 60-min period of stimulation. Maximum isometric force in response to indirect and direct muscle stimulation was measured after the 60-min period of stimulation, but not before in order to avoid muscle damage at the start of the experiment. Comparisons were made with maximum isometric force developed through indirect and direct stimulation, in a separate group of rats (n = 8) at the end of a 60-min period of saline infusion without stimulation. Finally, the first evoked M-wave from each 200-ms train of stimulation was analyzed for the measurement of peak-to-peak amplitude, duration (before full-wave rectification) and total area (after full-wave rectification), throughout the 60-min period of stimulation. Custom designed software was used to perform all of the above measurements and computations.

Plasma glucose (Glucometer Elite, Toronto, ON, Canada) and lactate (lactate analyzer model YSI 2300, Yellow SpringsInstruments, Yellow Springs, OH) concentrations were measured at regular intervals (see figure 2 & 3) in 0.2-ml blood samples taken from incisions on the tail before beginning the stimulation and infusion, during the stimulation, and at the end of the period of stimulation. Plasma insulin concentration (automated radioimmunoassay: Medicorp,

Montreal, Canada) and pH (Corning pH meter 120, Essex, UK) were also measured at the beginning and the end of the stimulation period, in blood withdrawn from a catheter placed in the right femoral artery. Because of the large amounts of blood needed for the determination of pH (~8 ml) and plasma insulin concentration (~1 ml), these variables were measured in additional groups of rats infused with saline, or lactate as described above. Both plantaris muscles were excised and frozen into liquid nitrogen immediately after the end of each experiment, and stored at –80 °C until analysis. Muscle glycogen levels were measured using the technique of Lo *et al.* (21).

Statistical Analysis

Data are expressed as the mean ± SD. Comparisons were made using a two-way ANOVA. When significant differences were revealed, a Scheffé post hoc test was performed. The level of statistical significance was set at $P < 0.05$.

RESULTS

Plasma glucose (Figure 1) and insulin (Table 1) concentrations remained unchanged when saline or lactate was infused. However plasma lactate concentrations, which remained unchanged when saline was infused, significantly increased in response to lactate infusion (Figure 1). The 60-min period of stimulation significantly decreased muscle glycogen concentrations with no significant difference in the initial and final values, respectively between the two groups (Table 1). Blood pH did not significantly change in response to

electrical stimulation of the plantaris muscle with infusion of saline, or lactate (Table 1).

Submaximal dynamic force significantly decreased over the first 5 min of stimulation in the two groups and remained stable thereafter (Figure 2). However, the reduction was significantly smaller in rats infused with lactate compared to control at min 60 (-49 % vs. –68 %, respectively).

Twitch force significantly decreased after the 60-min period of stimulation with no significant difference between the two groups, while twitch half-relaxation time was not significantly modified (Table 1).

Figure 3 shows the maximum dynamic force before and after the 60-min period of stimulation. Maximum dynamic force significantly decreased in the control group, but remained unchanged in rats infused with lactate for 60 min.

The maximum isometric force developed under indirect stimulation at the end of the 60-min period of stimulation was significantly higher in rats infused with lactate for 60 min than in the control group (Figure 4). Direct stimulation increased muscle force ~20 % in both groups.

Figure 5 shows changes in M-wave peak-to-peak amplitude, duration and total area during the 60-min period of stimulation. When saline was infused throughout the experiment, a significant reduction in M-wave peak-to-peak amplitude and total area as well as an increase in duration was observed. When lactate was infused throughout the experiment, the reduction in M-wave peak-to-peak amplitude and the increase in duration were significantly lower, and no change in total area was observed.

DISCUSSION

Results from the present study show that muscle force significantly decreased by 71 % of initial value in the control group during the 60-min period of stimulation. This marked reduction in submaximal dynamic force was associated with an increase in M-wave duration, and a decrease in M-wave peak-to-peak amplitude and total area. These observations confirm that muscle fatigue, in this model, is at least partly due to impairments of action potential generation and/or propagation along the muscle fiber membrane (9, 10, 15, 16).

We have recently shown that glucose infusion helped maintain the electrical properties of the muscle fiber membrane, and attenuated fatigue in the rat plantaris muscle stimulated indirectly for 60-min *in situ* (19). This was also shown in the present study with lactate infusion. Indeed, when compared to the control situation, when lactate concentration was increased to ~12 mM, the characteristics of M-wave (peak-to-peak amplitude, duration and total area) were better maintained, the reduction in submaximal dynamic force was much smaller over the first 5 min of stimulation (-45 %, and –58 % of the initial value, respectively), and throughout the period of stimulation (-49 %, and –68 % of the initial value, respectively, at min 60). The decrease in force in the control condition differed among the measurements taken: force decrease was 50 % for twitches, 70 % for submaximal dynamic contractions, 20 % for maximal dynamic contractions, and 15 % for maximal isometric contractions. The latter two measures are most likely closest to reflecting the maximal contractile capacity of the muscle, and thus contractile capability decreased by 15-20 %. Submaximal

dynamic contractions and twitches were evoked at submaximal stimulation frequencies and were, thus, subject to fatigue effects on factors, which determine rate of force development and relaxation. We included these measurements in an attempt to include all contractile "types" that one would expect during voluntary contractions. Lactate infusion attenuated all decreases, except the decrease in twitch response. Since twitch characteristics are determined by sarcoplasmic reticulum function to a greater extent than are the other measures, it may indicate sarcoplasmic function is not implicated in the attenuating effects of lactate infusion on fatigue.

The observation that lactate alleviates the deterioration of the electrical properties of the muscle fiber membrane and attenuates muscle fatigue during prolonged electrical stimulation is in line with results from Nielsen *et al.* (23) indicating that lactate has a protective effect on muscle force production and M-wave area. In that study, tetanic force was reduced by 75 % when isolated rat soleus muscles were incubated at a concentration of extracellular K^+ of 11 mM. Increasing [lactate] to 20 mM completely restored tetanic force. Furthermore, M-wave area was reduced when the muscle was exposed to high extracellullar K^+, but almost completely recovered with administration of 20 mM lactate. In a subsequent study, Pedersen *et al.* (25) also showed that 10 mM lactate partially restored muscle force in EDL and soleus muscle incubated in 11 mM K^+. In contrast, Hogan *et al.* (18) and Erdogan *et al.* (11) showed that lactate administration in a cross-over design resulted in a reduction in muscle performance. In these studies, isometric force was significantly reduced when

plasma lactate concentration was increased to 14 mM (18) or 20 mM (11) in the dog gastrocnemius muscle stimulated submaximally *in situ* through the sciatic nerve at 2Hz, and in rat diaphragm muscle strip stimulated supramaximally *in vitro* through the phrenic nerve, respectively. Results from the studies by Hogan *et al.* (18) and Erdogan *et al.* (11) on one hand, as well as Nielsen *et al.* (23) and Pedersen *et al.* (25) on the other hand, are difficult to compare, since fatigue was induced by different methods: prolonged electrical stimulation (11, 18) vs. large increase in [K+] (23, 25). As for the differences between the present study and those of Hogan *et al.* (18) and Erdogan *et al.* (11), they could be due to difference in the muscle studied (diaphragm (11) and gastrocnemius (18) vs. plantaris in the present experiment), difference in the plasma lactate concentration achieved (20 mM (11) vs. ~12 mM in the present experiment), and/or difference in the pattern of stimulation and the fatigue induced (low frequency fatigue (11, 18) vs. high frequency fatigue in the present experiment).

Taken together, data in the literature as well as data from the present experiment, do not consistently support the hypothesis that increase in lactate concentration is associated with muscle fatigue. On the contrary, as shown by Nielsen *et al.* (23), and Pedersen *et al.* (25), as well as in the present experiment, lactate could attenuate muscle fatigue. However, the mechanism(s) underlying this phenomenon remain(s) to be determined. In the studies by Nielsen *et al.* (23) and Pedersen *et al.* (25), both direct and indirect muscle stimulation were performed, but no comparison was made between the forces evoked in these two modes of stimulation. In the present experiment, maximal isometric force

was compared at the end of the 60-min period of stimulation when the motor nerve or the muscle was stimulated. In rats infused with saline for 60 min but without stimulation of the nerve muscle preparation, the maximal isometric force produced was similar with direct and indirect stimulation. The maximal isometric force evoked by the indirect stimulation was significantly lower by 26 % following the 60-min period of stimulation when saline was infused, but only 15 % lower when lactate was infused. However, direct stimulation of the muscle significantly increased the maximal isometric force developed by ~20 %, with no significant difference in the two groups. Accordingly, the protective effect of lactate infusion on force production during prolonged stimulation does not appear to be due to an attenuation of fatigue at the neuromuscular junction. In addition, as observed in our previous experiments with glucose infusion (19, 20), lactate infusion did not modify muscle glycogen utilization, which was similar in the two groups.

As discussed by Nielsen *et al.* (23), the beneficial effect of lactate on muscle performance could be due to the associated reduction in pH. In that study, lactate significantly decreased intracellular pH from 7.28 to 6.89. The authors concluded that acidification could counteract the depressing effects of elevated extracellular [K^+] on muscle excitability and force, and suggested that this could be due to a reduced inactivation of Na^+ channels. Intracellular pH was not measured in the present study, however as observed in other studies (11, 18), extracellular pH showed no changes with lactate infusion. Lactate could also modify Ca^{2+} handling by the sarcoplasmic reticulum. Nielsen *et al.* (23) did not report any effect of lactate administration on Ca^{2+} influx and total Ca^{2+} content of

the muscles. However, Posterino & Fryer (28) observed a small increase in the rate of Ca^{2+} release from the sarcoplasmic reticulum of EDL fibers in presence of lactate, while Posterino *et al.* (27) showed that the rate of relaxation of the tetanic response was faster in the presence of lactate. It could also be suggested that changes in the osmolarity and/or $[Na^{+}]$ of the fluid surrounding the muscle fibers, due to sodium lactate infusion, could help maintain the electrical properties of the membrane and muscle performance. The infused lactate could also be a fuel for aerobic metabolism in the contracting muscle. Finally, it has been shown that an antioxidant supplementation in animals improved muscle performance (29), and Groussard *et al.* (17) showed that lactate could act as an antioxidant, able to scavenge both OH^{-} and O_2^{-}.

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Table 1. Initial and final plasma pH, and insulin, muscle glycogen content, twitch force and half-relaxation time in rats infused with saline (control) and in rats infused with lactate.

		Control	Lactate
pH	Initial	7.39 ± 0.01	-
	Final	7.37 ± 0.01	7.42 ± 0.02
Insulin (pM)	Initial	284 ± 12	-
	Final	259 ± 11	270 ± 11
Glycogen	Initial (#)	39.4 ± 2.4	41.6 ± 2.7
(μmol of glucose/g ww)	Final	16.2 ± 2.5*	18.1 ± 2.3*
Twitch Force (N)	Initial	0.75 ± 0.06	0.78 ± 0.05
	Final	0.39 ± 0.03*	0.40 ± 0.04*
Half-relaxation time (ms)	Initial	11.2 ± 1.6	11.8 ± 1.7
	Final	10.5 ± 0.8	11.0 ± 1.0

Values are means ± SD (n = 8). * Significantly different from initial value ($P < 0.05$).

Measured in the contra-lateral muscle.

FIGURE LEGENDS

Fig. 1. Plasma lactate and glucose concentrations during the 60-min period of stimulation in rats infused with saline (control: !), and in rats infused with lactate (,). Values are means ± SD (n = 8). * Significantly different from initial value ($P<0.05$).

Fig. 2. Submaximal dynamic force during the 60-min period of stimulation in rats infused with saline (control: !), and in rats infused with lactate (,). Values are means ± SD (n = 8). Significant decreases in muscle force from initial were observed after min 1 ($P < 0.05$). † Significantly different from the control group ($P < 0.05$). Inset figure is the pattern of stretch, stimulation, and contraction. Optimal muscle length (L_0) was approximately 30 mm.

Fig. 3. Initial and final maximum dynamic force in rats infused with saline (control) (black), and in rats infused with lactate (white). Values are means ± SD (n = 8).

* Significantly different from initial values ($P < 0.05$). † Significantly different from control group ($P < 0.05$).

Fig. 4. Maximum isometric force under indirect (black) (200 Hz, 200 ms at 5 V), and direct (white) stimulation (200 Hz, 200 ms at 150 V), following the 60-min period of stimulation in rats infused with saline (control) and in rats infused with lactate for 60 min. Comparisons were made with observation made in a separate

group of rats at the end of a 60-min period of saline infusion without stimulation. Values are means ± SD (n = 8).

* Significantly different from the group without stimulation ($P < 0.05$). † Significantly different from indirect stimulation ($P < 0.05$). ‡ Significantly different from control group ($P < 0.05$).

Fig. 5. M-wave peak-to-peak amplitude, duration and total area during the 60-min period of stimulation in rats infused with saline (control: !) and in rats infused with lactate (,). Values are means ± SD (n = 8). * Significantly different from initial value in both groups ($P < 0.05$). † Significantly different from the control group ($P < 0.05$).

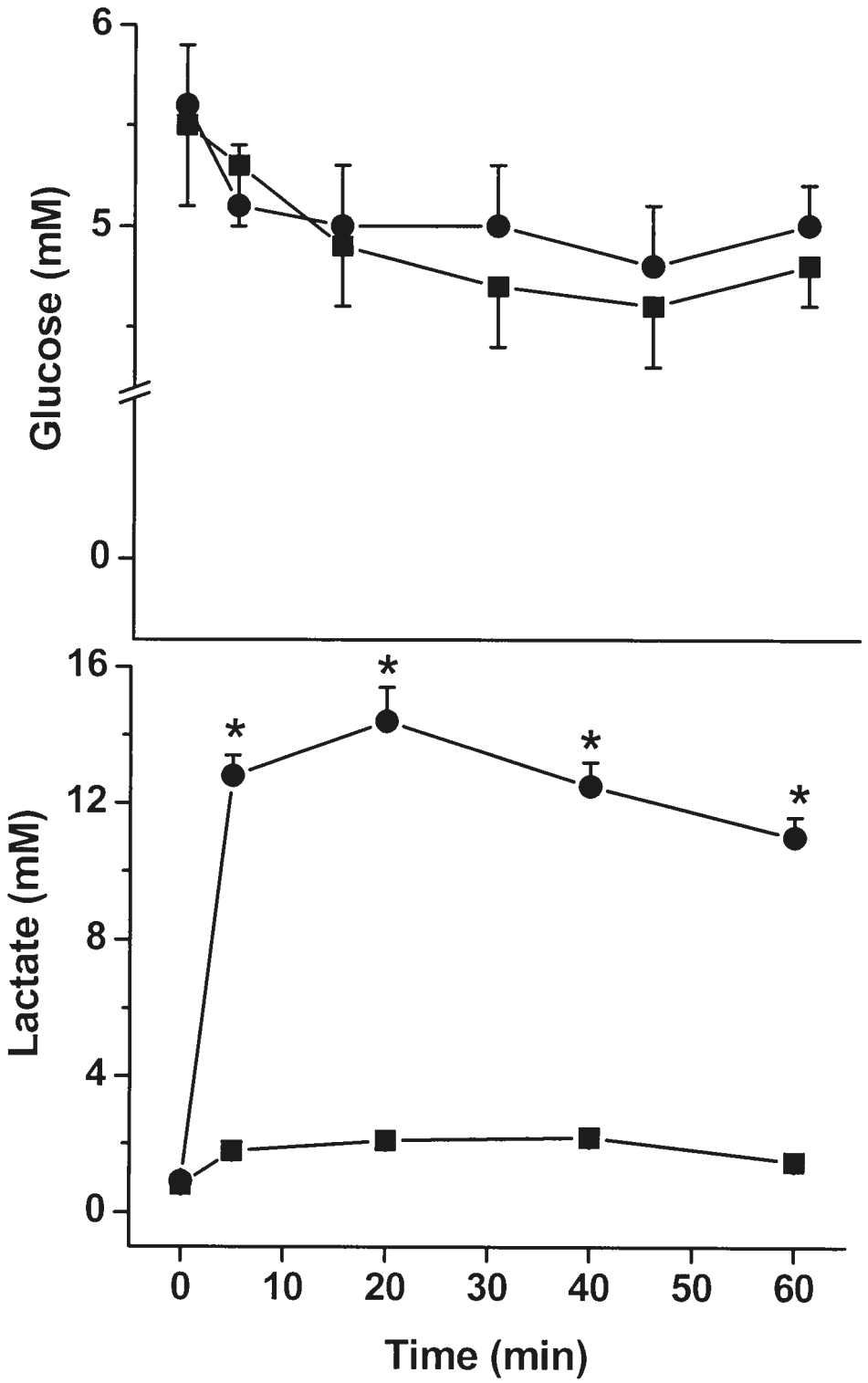

Fig. 1

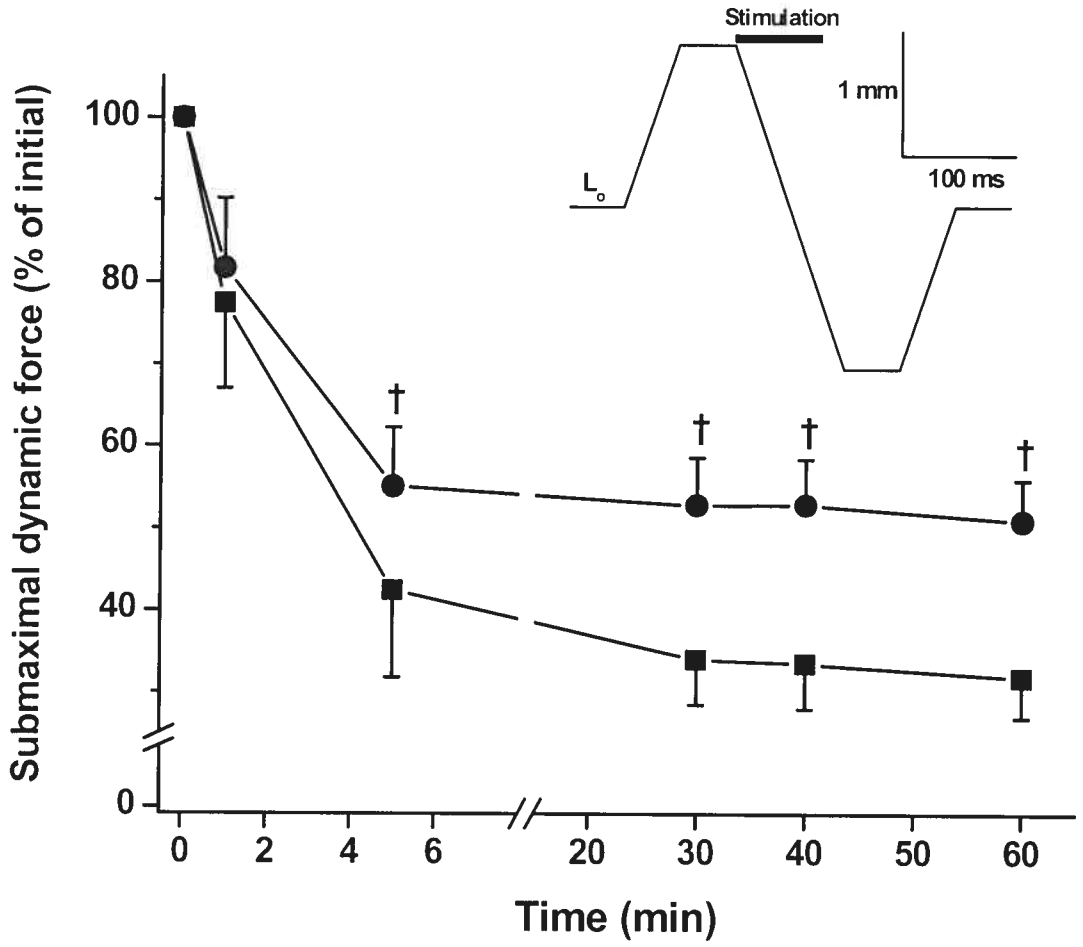
Stimulation
1 mm
100 ms
L_o
Submaximal dynamic force (% of initial)
100
80
60
40
0
0
2
4
6
20
30
40
50
60
Time (min)
†
†
†
†

Fig. 2

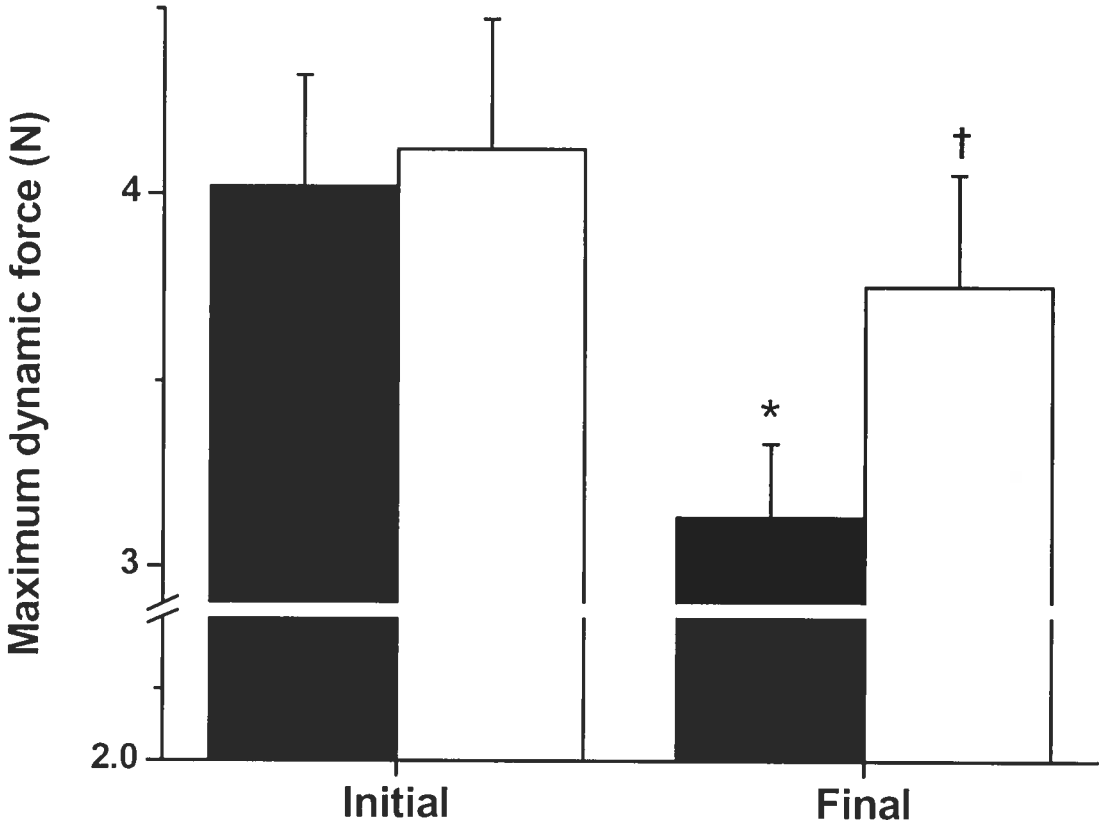

Fig. 3

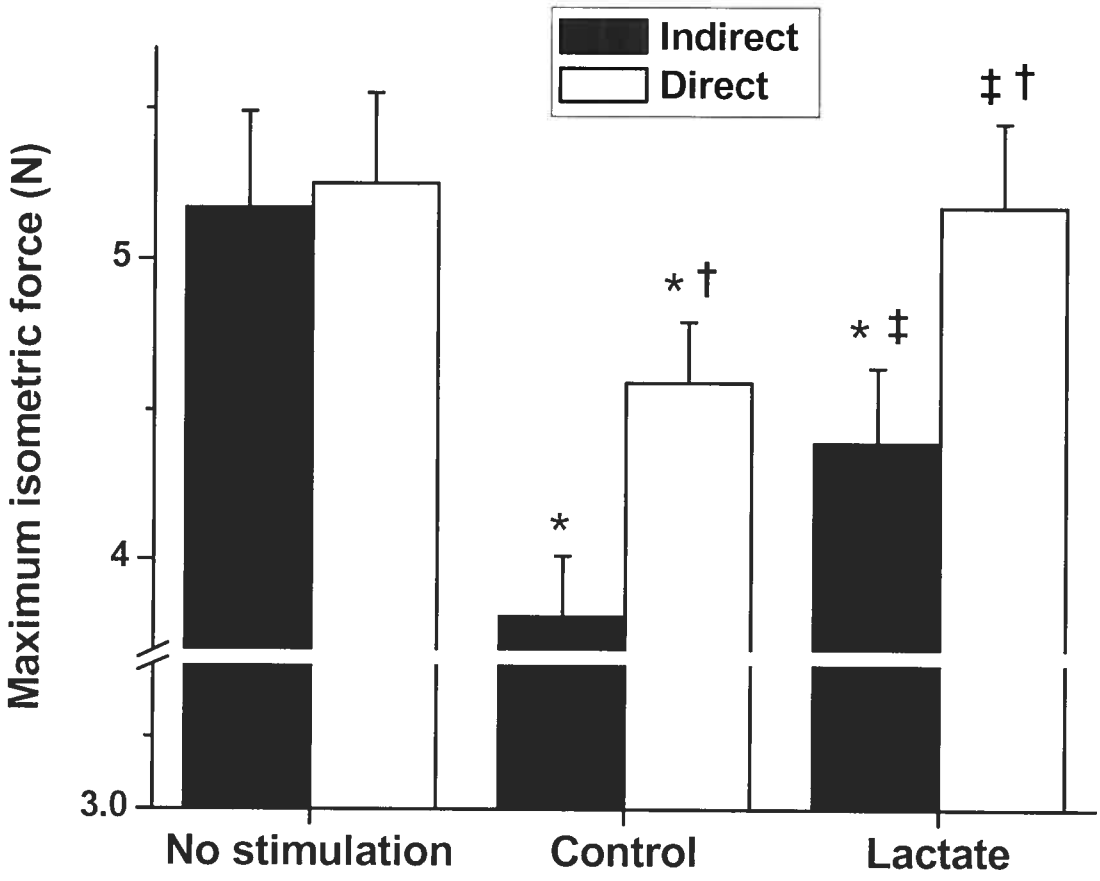
Indirect
Direct
Maximum isometric force (N)
5
4
3.0
* †
* ‡
‡ †
*
No stimulation
Control
Lactate

Fig. 4

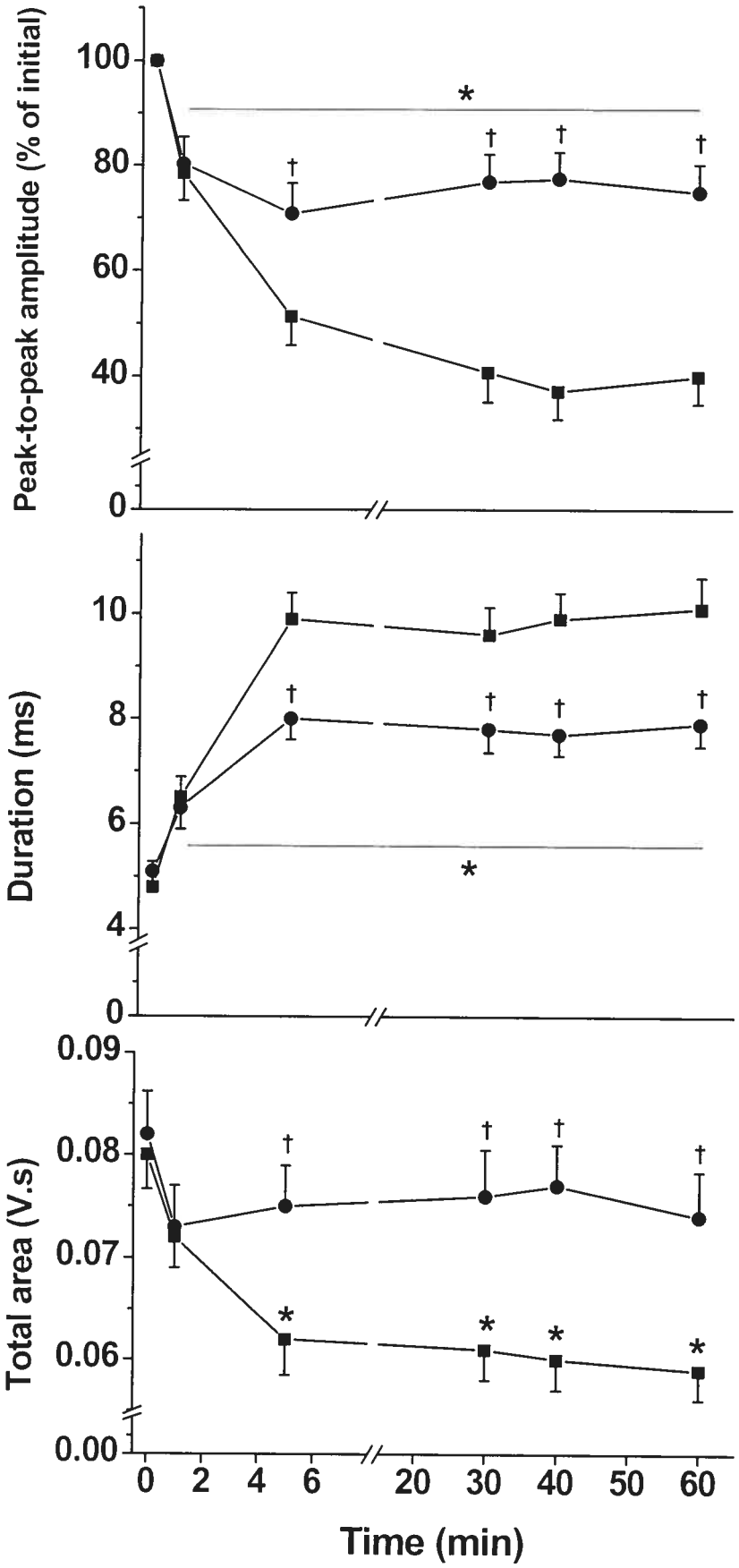
Peak-to-peak amplitude (% of initial)
100
80
60
40
0
*
†
Duration (ms)
10
8
6
4
0
Total area (V.s)
0.09
0.08
0.07
0.06
0.00
0
2
4
6
20
30
40
50
60
Time (min)

Fig. 5

Chapter 5

MANUSCRIPT 4

Title: Resting membrane potential of rat plantaris muscle fibers after prolonged indirect stimulation *in situ*: effect of glucose infusion

Authors: Antony D. Karelis, François Péronnet, and Phillip F. Gardiner

Journal: Canadian Journal of Applied Physiology, 2004 (in press)

Keywords: Resting Membrane Potential, Muscle Fatigue, Muscle force, M-Wave

Abstract

The resting membrane potential (RMP) measured at min 1 in the recovery period following indirect stimulation of the rat plantaris muscle for 60 min *in situ* was significantly decreased in control rats, but was back to baseline values within 2 min. When glucose was infused ([glucose] ~10 mM) no change in RMP was observed and muscle fatigue and the reduction in M-wave peak-to-peak amplitude were both attenuated. However, muscle force and the electrical properties of the membrane were deteriorated both in rats infused with glucose, and in control rats at min 2 during the recovery period, at a time when RMP was not modified. These observations suggest that the maintenance of RMP, when glucose was infused, or its quick restoration at the end of the stimulation period in the control group, does not alleviate the reduction in muscle membrane excitability and in muscle contractility.

Introduction

We have recently shown that glucose infusion attenuates fatigue in rat plantaris muscle stimulated indirectly for 60-min *in situ* (Karelis et al. 2002), and this was associated with a better maintenance of the electrical properties of the muscle fiber membrane, as shown by the characteristics of M-wave (peak-to-peak amplitude, duration and total area). This suggests that glucose infusion may help alleviate the deterioration of action potential generation and/or propagation associated with fatigue, which depend on several factors including the resting membrane potential (RMP) (Clausen et al. 1998; Nielsen and Clausen 2000). Several studies have shown that the absolute value of RMP significantly decreases when muscle fibers are subjected to prolonged tetanic contractions (Lannergren and Westerblad 1986; Renaud and Kong 1991; Balog et al. 1994; Cairns et al. 1995), and this could impair the excitability of the muscle membrane, and could be involved in the development of fatigue (Cairns et al. 1995; Fitts and Balog 1996; Cairns et al. 1997; Sejersted and Sjogaard 2000).

Based on changes in M-wave characteristics observed following prolonged *in situ* stimulation of the rat plantaris muscle in our previous experiment (Karelis et al. 2002), we hypothesized that glucose infusion would attenuate the decrease in RMP observed immediately after prolonged muscle contractions (Lannergren and Westerblad 1986; Renaud and Kong 1991; Balog et al. 1994). In order to test this hypothesis the plantaris muscle was electrically stimulated *in situ* through the sciatic nerve to perform concentric contractions for 60 min while infusing intravenously either saline or glucose, in the anaesthetized

rat. RMP was measured in individual fibers over a 4-min period beginning immediately at the end of the period of stimulation, along with the recovery of submaximal dynamic force, and M-wave peak-to-peak amplitude.

Material and Methods

Experimental groups

The experiments were conducted according to the directives of the Canadian Council on Animal Care. Female Sprague-Dawley rats (n = 24, ~250 g) were obtained from Charles River Canada (St-Constant, Canada) and were housed in cages by pair in a room maintained at 20-23°C and 25 % relative humidity, and with a 12-h/12-h light cycle. They were fed pellet rat chow and tap water *ad libitum*. Two groups of 12 animals were studied before and immediately after a one-hour period of electrical stimulation of the sciatic nerve with infusion of saline (7.25 mL kg^{-1} h^{-1}) with or without glucose (1.00 g kg^{-1} h^{-1}) through a catheter in the left jugular vein. The first group, which served as control, received only saline for 60 min. The second group received saline and glucose for 60 min (experimental group). Since RMP measurements during muscle contraction are problematic, in both the control and experimental groups, submaximal dynamic force and M-wave peak-to-peak amplitude were measured in a sub-group of six rats, while RMP was measured in the other sub-group (n = 6), during the 4-min recovery period following stimulation.

Experimental protocol

As previously described (Karelis et al. 2002) the plantaris muscle and sciatic nerve were surgically isolated, and the plantaris tendon was attached to

the lever arm of a muscle puller servomotor (Cambridge LR 305B, Aurora Scientific, Aurora, ON, Canada). Following determination of optimal muscle length, as previously described (Karelis et al. 2002), twitch forces were recorded using supramaximal (5 V) single square pulses of 0.05 ms in duration delivered once every 3 seconds (Grass S88 stimulator; Quincy, MA, USA). The pattern of stretch, stimulation and contraction used was identical to that in the previous experiment (Karelis et al. 2002). The muscle was then electrically stimulated for 60 min, while infusing either saline or glucose. Immediately after the period of stimulation the muscle was studied over a 4-min recovery period. Following the experiment, the animals were killed by an overdose of ketamine/xylazine.

Measurements

Submaximal dynamic force and surface EMG were monitored on an oscilloscope (Hewlett Packard 1741A Mississauga, ON, Canada) and recorded with a microcomputer before the 60-min period of stimulation, and every minute over the first 4 min of recovery. The first evoked M-wave from each 200-ms train of stimulation was analyzed after full-wave rectification, for the measurement of peak-to-peak amplitude. Custom designed software was used to perform all of the above measurements.

RMP was measured (Triplett, Model 4000) in several individual fibers, and noted in the laboratory notebook, as previously described (Desaulniers et al. 2001), using a micropositioner (Burleigh Instruments, Burleigh Park, NY, USA) and glass microelectrodes with tip diameters of approximately 1 μm containing 3

M of KCl (resistance <10 MΩ). Measurements were categorized into bins of 0-1 min, 1-2 min, 2-3 min and 3-4 min following the 60-min period of stimulation.

Statistical Analysis

Data are expressed as the mean ± SD. Comparisons were made using a two-way ANOVA. When significant differences were revealed, a Scheffé post hoc test was performed. The level of statistical significance was set at $P<0.05$.

Results

The pre-stimulation RMP were not significantly different in the control and experimental group: -79.4 ± 3.3 mV and -80.3 ± 3.8 mV, respectively (Figure 1). Immediately after the 60-min period of stimulation, RMP was significantly lower in the control group between min 0 and 1 (-71.2 ± 2.7 mV) but returned to baseline pre-stimulation values between min 1 and 2 (-76.9 ± 3.7 mV) and was not significantly modified thereafter. When glucose was infused, RMP recorded between min 0 and 4 in the recovery period, was not significantly different from the initial value, and was significantly higher than in the control group between min 0 and 1 (-76.8 ± 2.6 mV).

Glucose infusion attenuated the reduction in submaximal dynamic force (-56 % vs. -71 % in control rats, respectively), and M-wave peak-to-peak amplitude (-38 % vs. -55 % in control rats, respectively) observed during the 60-min period of stimulation. These values partially recovered over the 4 min following cessation of stimulation, and the recovery was faster in the experimental than in control group (Figure 2).

Discussion

It has been shown that deterioration in action potential generation and/or propagation or in muscle excitability could be associated with a reduction in M-wave peak-to-peak amplitude (Harrison and Flatman 1999), which, in turn could be related to a decrease in RMP (Fitts and Balog 1996; Sejersted and Sjogaard 2000). Results from the present study show that muscle force and M-wave peak-to-peak amplitude significantly decreased by 71 and 55 % of the corresponding initial value, respectively, in the control group during the 60-min period of stimulation. These observations confirm that, in the model used in the present experiment, muscle fatigue could be at least in part due to impairments of action potential generation and/or propagation along the muscle fiber membrane (Gardiner and Olha 1987; Enoka et al. 1989; Edman and Lou 1992; Fuglevand et al. 1993; Fitts 1994). In addition, RMP was significantly decreased by ~8 mV in the control group immediately after the 60-min period of stimulation, which is in line with results observed in other studies in intact muscles (Renaud and Kong 1991; Balog et al. 1994; Balog and Fitts 1996). This phenomenon could be due to an increase in intracellular [Na^+] and extracellular [K^+] observed with fatigue suggesting that the activity of the muscle membrane Na^+/K^+ pump could be involved (Overgaard et al. 1999; Nielsen and Clausen 2000; Clausen 2003).

Results from the present study confirm that glucose infusion attenuates fatigue in the rat plantaris muscle during prolonged indirect electrical stimulation *in situ*. This is shown by the reduction in submaximal dynamic force, which was significantly less in rats infused with glucose than with saline (-56 % and -71 % of

the initial value, respectively, at the end of the 60-min period of stimulation). M-wave peak-to-peak amplitude was also better maintained in rats infused with glucose, when compared to the rats in the control group (-38 % vs. -55 % of initial value, respectively, at the end of the 60-min period of stimulation). This suggests that glucose infusion could at least partly help alleviate the impairments of action potential generation and/or propagation along the muscle fiber membrane, and, thus, protect against muscle fatigue. In addition, when glucose was infused, RMP was not significantly reduced at the end of the stimulation period (-3.5 ± 0.3 mV, NS vs. -8.2 ± 0.9 mV, $P < 0.05$ when saline was infused). However, in spite of this lack of change in RMP at min 1 in the recovery period, when glucose was infused, muscle force and M-wave peak-to-peak amplitude were significantly decreased. This observation suggests that the impairment in the electrical properties of the muscle fiber membrane and the reduction in muscle force following prolonged stimulation are not entirely due to a decrease in RMP. This is further confirmed by the observation that in the control group, although RMP returned to the initial value after 1 min of recovery, both M-wave peak-to-peak amplitude and muscle force had not fully recovered after 4 min of recovery. The observation of a deterioration of M-wave peak-to-peak amplitude without any changes in RMP is in line with the idea that during prolonged stimulation of the muscle fiber membrane, RMP is preserved in spite repetitive influxes of Na^+. However, as summarized by Clausen (2003), this is achieved at the expense of a K^+ efflux, which exceeds the capacity of the Na^+/K^+-pump. As a

consequence, membrane excitability and muscle contractility are altered due to the progressive rise in extracellular K^+ and intracellular Na^+ accumulation.

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Figure Legends

Fig. 1. Resting membrane potential recorded before (Pre) and after (Recovery) the 60-min period of stimulation in control rats (!), and in rats infused with glucose (,). Values are means ± SD (n = 6 muscles/ n = 3 fibers/muscle). * Significantly different from the initial value (P<0.05). † Significantly different from the control group (P<0.05).

Fig. 2. M-wave peak-to-peak amplitude, and submaximal dynamic force during the 4-min recovery following the 60-min period of stimulation in control rats (!), and in rats infused with glucose (,). . Values are means ± SD (n = 6). * Significantly different from the initial value (P<0.05). † Significantly different from the control group (P<0.05).

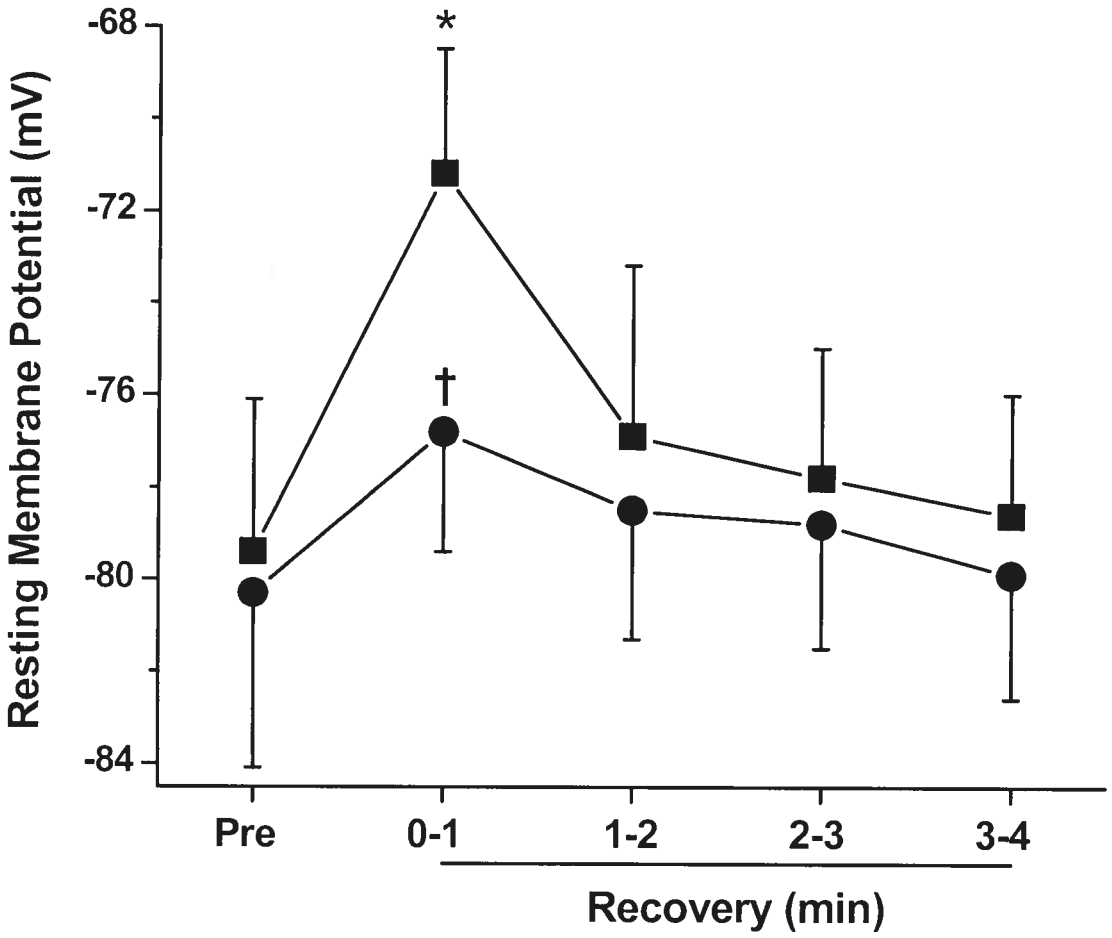
-68
-72
-76
-80
-84
Resting Membrane Potential (mV)
*
†
Pre
0-1
1-2
2-3
3-4
Recovery (min)

Fig. 1

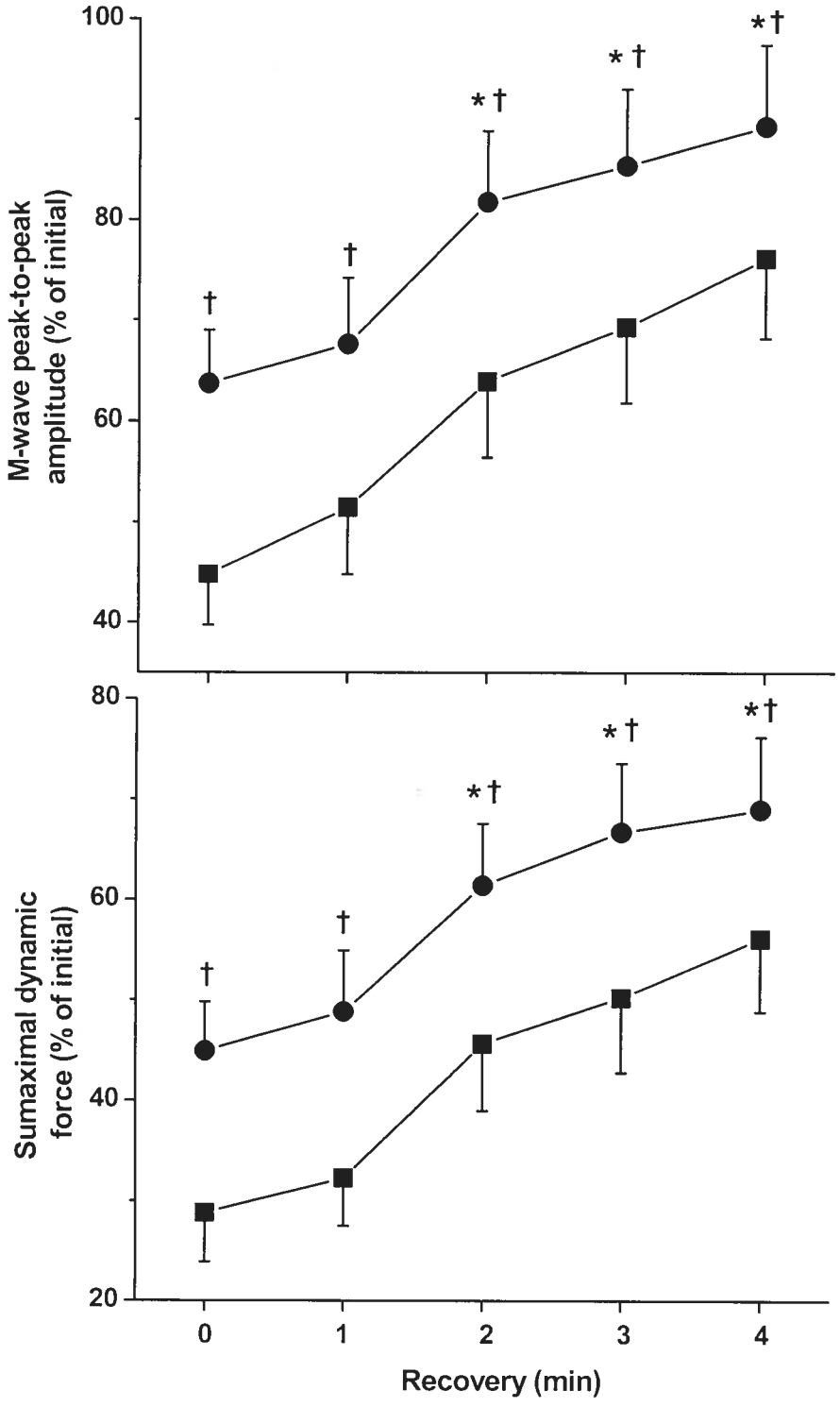
M-wave peak-to-peak amplitude (% of initial)
100
80
60
40
*†
*†
*†
†
†
†
Sumaximal dynamic force (% of initial)
80
60
40
20
*†
*†
*†
†
†
0
1
2
3
4
Recovery (min)

Fig. 2

Chapter 6

MANUSCRIPT 5

Title: Glucose infusion and changes in ATP, PCr and lactate concentration in rat plantaris muscle during prolonged indirect stimulation *in situ*

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Journal: Metabolism

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Keywords: Muscle Fatigue, Muscle force, M-wave, Phosphate potential

ABSTRACT

The attenuation of muscle fatigue (rat plantaris muscle stimulated *in situ* for 60 min) with glucose infusion, was associated with a lower reduction in PCr concentration (from 23.1 ± 1.6 to 14.2 ± 1.1 μmol/g vs. 22.7 ± 1.4 to 9.9 ± 0.9 μmol/g in the control group) but no change in ATP or lactate concentration vs. control. These observations are in line with those reported during prolonged submaximal whole-body exercise in man with administration of carbohydrate. Thus, the mechanism(s) underlying the increase in performance in these two models could be at least partly similar.

INTRODUCTION

It is well established that carbohydrate (CHO) ingestion during prolonged exercise increases endurance time to exhaustion in man (Coggan and Coyle 1991; Hargreaves 1999) and in animals (Bagby et al., 1978; Slentz et al., 1990). In addition, the ability to perform resistance exercise could also increase when CHO are ingested (Haff et al., 2003). We have shown that glucose infusion attenuated fatigue in rat plantaris muscle stimulated indirectly for 60 min *in situ* (Karelis et al., 2002, 2003). However, the underlying mechanism(s) by which CHO ingestion or infusion increases muscle performance remain(s) to be determined.

Fatigue is associated with changes in metabolite concentration in the muscle such as ATP, IMP, PCr, inorganic phosphate and lactate (Sahlin et al., 1998). There is evidence to support that the beneficial effect of CHO administration on muscle performance could be due to changes in metabolite concentrations in the muscle fiber (Spencer et al., 1991; Tsintzas et al., 1996; McConell et al., 1999; Snow et al., 2000; Tsintzas et al., 2001). For example, CHO ingestion increased muscle performance during submaximal exercise and this was associated with a lower accumulation of muscle IMP levels (Spencer et al., 1991; McConell et al., 1999). In addition, other studies have indicated that the reduction in PCr levels were smaller with the ingestion of CHO during submaximal exercise and this was also associated with an increase in muscle performance (Tsintzas et al., 1996; Snow et al., 2000; Tsintzas et al., 2001). Lewis and Haller (1986) also showed that glucose infusion in subjects with

McArdle disease during exercise reduced the decline in PCr levels as well as the increase in inorganic phosphate levels.

The purpose of the present study was to verify the hypothesis that, in this model, improvement in muscle performance could be associated with similar changes in the response of ATP, PCr and lactate concentrations to those, which have been observed with CHO ingestion in humans.

METHODS

The experiments were conducted according to the directives of the Canadian Council on Animal Care. Female Sprague-Dawley rats (n = 24, ~250 g) were obtained from Charles River Canada (St-Constant, Canada) and were housed in cages by pair in a room maintained at 20-23°C and 25 % relative humidity, and with a 12-h/12-h light cycle. They were fed pellet rat chow and tap water *ad libitum.* Two groups of six animals were studied before, during and immediately after a 60-min period of electrical stimulation of the sciatic nerve with infusion of saline (7.25 mL kg^{-1} h^{-1}) with or without glucose (1.00 g kg^{-1} h^{-1}) through a catheter in the left jugular vein. The first group, which served as control, received only saline for 60 min. The second group received saline and glucose for 60 min (experimental group). Two separate groups of six rats receiving saline or glucose, were used in order to measure muscle ATP, PCr and lactate concentrations at min 5.

As previously described (Karelis et al., 2002) the plantaris muscle and sciatic nerve were surgically isolated, and the plantaris tendon was attached to

the lever arm of a muscle puller servomotor (Cambridge LR 305B, Aurora Scientific, Aurora, ON, Canada).

Following determination of optimal muscle length, as previously described (Karelis et al., 2002), twitch forces were recorded using supramaximal (5 V) single square pulses of 0.05 ms in duration delivered once every 3 seconds (Grass S88 stimulator; Quincy, MA, USA). The pattern of stretch, stimulation and contraction used was identical to that in the previous experiment (Karelis et al., 2002). The muscle was then electrically stimulated for 60 min, while infusing either saline or glucose. Following the experiment (5 or 60 min), both plantaris muscles were freeze clamped, excised and plunged into liquid nitrogen immediately and stored at -80^{o} C until analysis and the animals were killed by an overdose of ketamine/xylazine.

Submaximal dynamic force was continuously monitored on an oscilloscope (Hewlett Packard 1741A Mississauga, ON, Canada) and recorded with a microcomputer during the 60-min period of stimulation. Custom designed software was used to perform the above measurement. Muscle ATP, PCr and lactate concentrations were measured by enzymatic fluorimetric using the technique by Lowry and Passoneau (1972).

Statistical Analysis

Data are expressed as the mean $\pm$ SD. Comparisons were made using a two-way ANOVA. When significant differences were revealed, a Scheffé post hoc test was performed. The level of statistical significance was set at $P<0.05$.

RESULTS

When saline or glucose was infused for 60 min, submaximal dynamic force significantly decreased over the first 5 min of stimulation and remained stable thereafter (Figure 1). However, the reduction was significantly smaller in rats infused with glucose ([glucose] ~10 mM) compared to control at min 60 (-57 % vs. -73%, respectively).

Muscle ATP concentration remained unchanged from the initial value at min 5 and min 60 in both groups during the 60-min period of stimulation (Figure 2). In contrast, muscle PCr concentration significantly decreased at min 5 and min 60 in both groups during the 60-min period of stimulation. However, the decrease in PCr concentration was significantly smaller in rats infused with glucose compared to control. Muscle lactate concentration significantly increased at min 5 and min 60 in both groups, however no significant difference was observed between the two groups.

DISCUSSION

Results from the present study confirm that glucose infusion attenuates fatigue in the rat plantaris muscle during prolonged indirect electrical stimulation *in situ*. This is shown by the reduction in submaximal dynamic force, which was significantly less in rats infused with glucose than with saline (-57 % and -73 % of the initial value, respectively, at the end of the 60-min period of stimulation).

It was hypothesized that glucose infusion could modify changes in muscle ATP, PCr or lactate concentrations during prolonged contractions. Muscle ATP

levels were not significantly modified during the 60-min period of stimulation in both groups, which is in line with findings reported in studies conducted in man during submaximal whole-body exercise with CHO ingestion (McConell et al., 1999; Snow et al., 2000; Tsintzas et al., 2001). Moreover, as shown in some studies in man (McConell et al., 1999; Snow et al., 2000) as well as in the present experiment, muscle lactate levels showed no significant differences between both groups during the 60-min period of stimulation. However, this finding does not appear to be consistent. For instance, Spencer et al., (1991) and Tsintzas et al., (2001) reported that muscle lactate levels either significantly increased or decreased with CHO ingestion during submaximal exercise, respectively. Finally, in the present experiment, glucose infusion resulted in a smaller reduction of PCr levels in the plantaris muscle at min 5 and 60 during the 60-min period of stimulation (16.9 ± 1.5 & 14.2 ± 1.1 μmol/g vs. 13.1 ± 1.5 & 9.9 ± 0.9 μmol/g in rats infused with saline, respectively). This observation is in line with results observed in other studies (Snow et al., 2000; Tsintzas et al., 1996, 2001). For example, Tsintzas et al., (2001) reported that CHO ingestion during exhaustive running, in man, attenuated the decline of PCr concentration by 46 ± 17 % in type I fibers and by 36 ± 9 % in type II fibers. Snow et al., (2000) also observed a smaller reduction in muscle PCr concentration at min 30 only (79.2 ± 3.0 mmol/kg in CHO group vs. 67.5 ± 3.4 mmol/kg in control group) when subjects ingested CHO during submaximal exercise for two hours. In another study, Tsintzas et al., (1996) showed that CHO ingestion increased exercise time from 102 min in the control group to 132 min in the CHO group. At the point of

fatigue, they observed that muscle ATP and PCr were lower than the resting value in the control group but not in the group receiving CHO. Collectively, the smaller reduction of PCr level in the absence of a decrease in ATP level, as observed in this study with glucose infusion, could reflect a better maintenance of phosphate potential. In support of this hypothesis, Spencer et al., (1991) and McConell et al., (1999) showed that muscle IMP concentration was lower during submaximal exercise with CHO ingestion, suggesting a reduction in ADP accumulation and conversion in AMP.

In conclusion, results from the present study indicate that the attenuation of fatigue in the rat plantaris muscle during prolonged indirect stimulation *in situ* with glucose infusion was associated with a higher concentration of PCr as already shown in man. This suggests that the mechanism(s) responsible for the increase in performance in the rat isolated muscle when glucose is infused, could be at least partly similar to those responsible for the increase in endurance observed during submaximal whole-body exercise when CHO is administered in man.

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FIGURE LEGEND

Fig. 1. Submaximal dynamic force during the 60-min period of stimulation in control rats (!), and in rats infused with glucose (,). Values are means ± SD (n = 6). * Significantly different from the initial value in both groups ($P < 0.05$). † Significantly different from the control group ($P < 0.05$).

Fig. 2. Muscle ATP, PCr and lactate concentration during the 60-min period of stimulation in control rats (black) and in rats infused with glucose (white). Values are means ± SD (n = 6). * Significantly different from the initial value in both groups ($P < 0.05$). † Significantly different from the control group ($P < 0.05$).

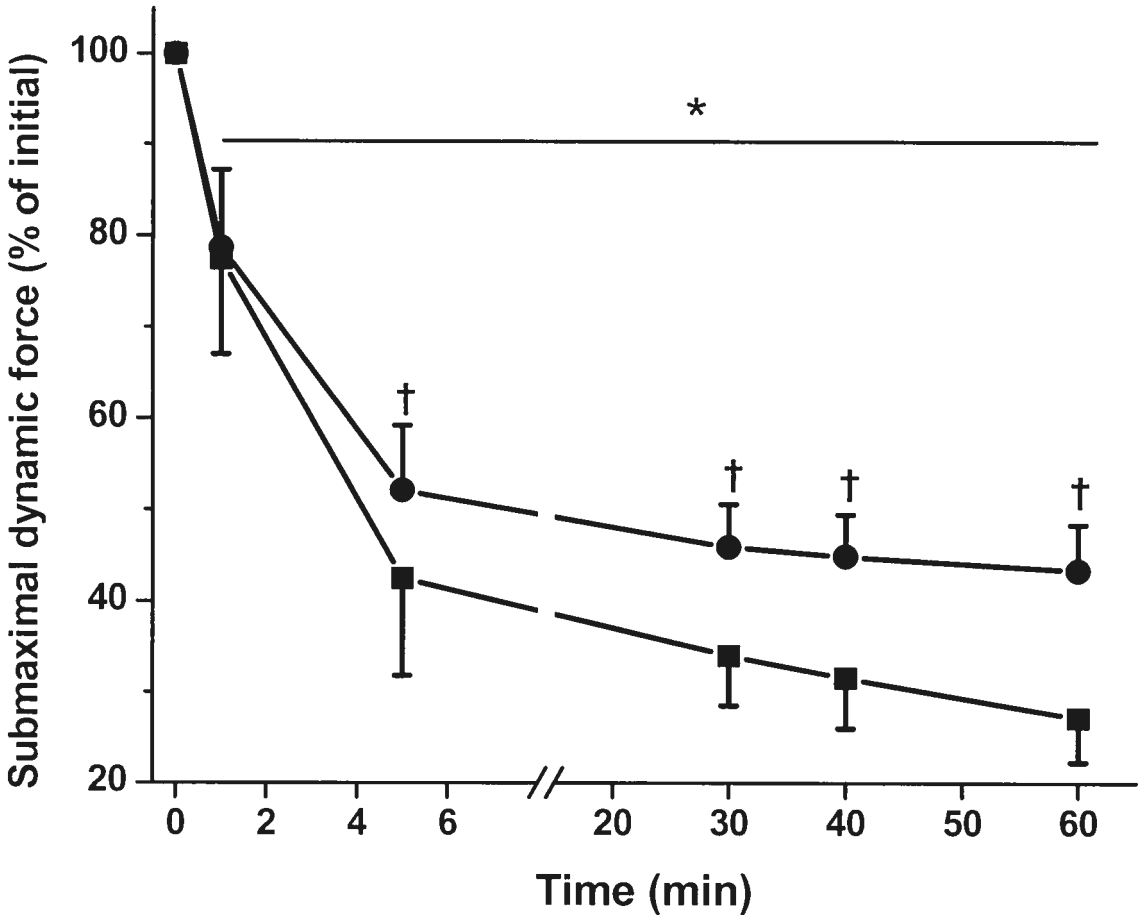
Submaximal dynamic force (% of initial)
100
80
60
40
20
*
†
†
†
†
0
2
4
6
20
30
40
50
60
Time (min)

Fig. 1

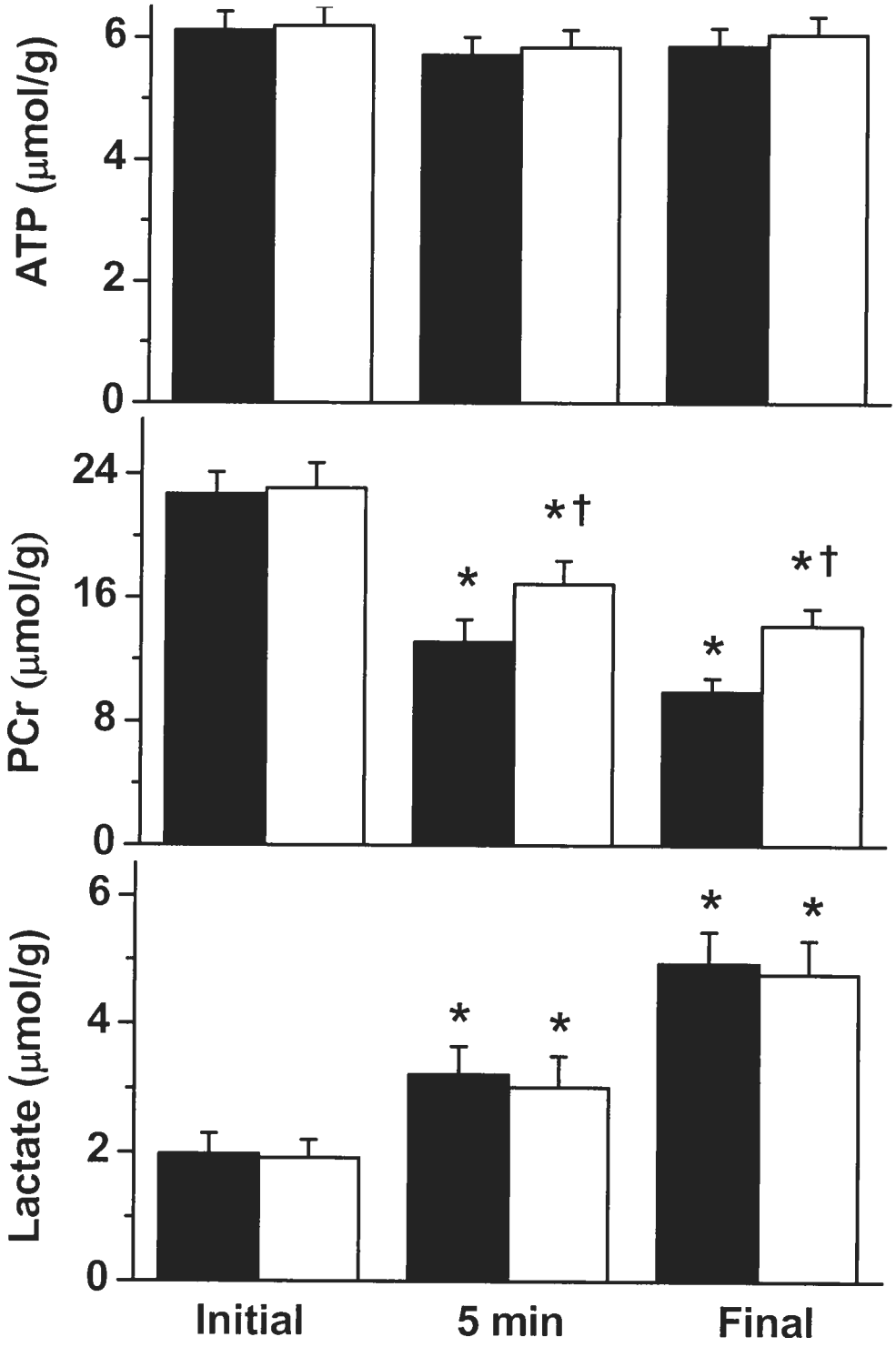
ATP (µmol/g)
6
4
2
0
PCr (µmol/g)
24
16
8
0
*
*†
*
*†
Lactate (µmol/g)
6
4
2
0
*
*
*
*
Initial
5 min
Final

Fig. 2

Chapter 7

DISCUSSION AND CONCLUSION

The five studies presented in this thesis share the common goal of furthering our understanding of the mechanisms through which CHO administration increases muscle performance during prolonged contractions. Results from these studies show that the beneficial effect of elevated circulating glucose on muscle performance could be due to an improvement of peripheral muscular function. Indeed, the *in situ* nerve-muscle preparation used in the present experiments in anaesthetized rats excludes any possible central effect of glucose. Our first study, showed that elevated blood glucose levels achieved by the infusion of glucose attenuated muscle fatigue during prolonged indirect stimulation. In that study, when saline or glucose was infused for 60 min, submaximal dynamic force significantly decreased over the first 5 min of stimulation and remained constant thereafter. However, the reduction was significantly smaller in rats infused with glucose (~55 % vs. ~70 % in rats infused with saline). In rats infused with saline for 30 min and with glucose for the subsequent 30 min, the initial reduction in submaximal dynamic force was similar to that observed in rats infused with saline only. However, submaximal dynamic force was partially restored and was significantly different at min 60 when compared to the control group.

Fatigue during prolonged exercise has been suggested to be associated with glycogen depletion in working muscles and increasing initial muscle glycogen stores has been shown to increase performance (Conlee 1987). As discussed in the review of the literature, it was hypothesized that the increase in performance with CHO administration during exercise could be due to a muscle

glycogen sparing. However, this hypothesis was not supported by our findings in study 1. Indeed, a large and significant decrease in muscle glycogen content was observed at the end of the 60-min period of stimulation, but glucose infusion from the onset of stimulation or beginning at min 30, was not associated with any muscle glycogen sparing.

The neuromuscular junction has been suggested as a possible site of muscle fatigue during nerve stimulation (Fitts 1994). Thus, we investigated if glucose infusion during prolonged stimulation could have an effect on the neuromuscular junction. We showed that the beneficial effect of glucose infusion on peripheral neuromuscular function in study 1 does not appear to be due to an attenuation of fatigue at the neuromuscular junction as shown by maximum nerve stimulation and through massive direct stimulation of the whole-muscle (thus by-passing the neuromuscular junction). Results show that the difference in maximal isometric force elicited through direct and indirect stimulation was similar in rats infused with glucose (~24 %) and in rats infused with saline only (~22 %).

Changes in M-wave characteristics suggest that the increase in performance could be due to a better maintenance of the electrical properties of the muscle fiber membrane. As shown in the results of study 1, when saline only was infused throughout the experiment a significant reduction in M-wave peak-to-peak amplitude and total area, and an increase in duration was observed. When glucose was infused throughout the experiment the reduction in M-wave peak-to-peak amplitude and the increase in duration was lower, and no change in total area was observed. Infusion of glucose beginning at min 30 partially restored the

values of peak-to-peak amplitude, duration and total area. As discussed in the manuscripts, a possible mechanism for explaining the increase in muscle performance with glucose infusion during prolonged stimulation may be ATP compartmentation. Glycolytic ATP appears to be the preferred fuel for membrane ion pumps, such as the Na^+/K^+-ATPase (Okamoto et al. 2001). By increasing the quantity of glucose at the muscle membrane this will in turn bring an increase supply of glycolytic ATP and a better function of electrical properties at the muscle membrane. Furthermore, insulin plays a major role in K^+ and Na^+ homeostasis, favoring K^+ uptake and Na^+ efflux through the stimulation of the Na^+/K^+-pump in the membrane of the muscle fiber (Sweeney et al. 1998). In study 1, plasma insulin concentration markedly rose in response to glucose infusion. This phenomenon could in part explain the better maintenance of the membrane electrical properties and the attenuation of muscle fatigue. In support of this hypothesis, Clausen et al. (1993) showed that insulin administration during prolonged stimulation increased muscle performance. In that study, extracellullaire K^+ concentration was increased from 4 to 12.5 mM, which decreased muscle force by 96% from initial. When insulin was infused, force increased by 38 % after 20 min and this was associated with a 21 % increase in efflux of Na^+ from the cell, and a 14 % increase in K^+ uptake.

Thus, in our second study we hypothesized that insulin is responsible for the increase in muscle performance observed in our previous study. To test this hypothesis plasma insulin concentration was raised markedly (~900 pmol/l) by infusion of insulin, with glucose maintained between 4-6 mmol/l

(hyperinsulinemic-euglycemic clamp) or between 10-12 mmol/l (hyperinsulinemic-hyperglycemic clamp). The above hypothesis was not confirmed by our findings. Submaximal dynamic force was significantly reduced following the 60-min stimulation period when plasma insulin concentration was increased to ~900 pmol/l but with plasma glucose concentration maintained between 4-6 mmol/l (hyperinsulinemic-euglycemic clamp). In contrast, when plasma glucose concentration was raised between 10-12 mmol/l while maintaining plasma insulin concentration at ~900 pmol/l (hyperinsulinemic-hyperglycemic clamp), the reduction in submaximal dynamic force was much lower. These observations indicate that increased plasma glucose concentration and/or glucose delivery to the muscle, and not the associated increase in plasma insulin concentration, was responsible for the attenuation of muscle fatigue when glucose was administered.

The deterioration of action potential generation and/or propagation could be associated with changes in RMP, which has been suggested to be implicated in the development of muscle fatigue (Clausen 2003). Based on changes in M-wave characteristics observed during prolonged stimulation in study 1 and 2, we investigated if changes in RMP with glucose infusion could explain the better maintenance of the electrical properties of the fiber membrane and increase performance. In study 4, we showed that RMP may not be implicated in the improvement of muscle performance during prolonged stimulation with glucose infusion and that RMP appears not to be involved in the development of muscle fatigue. In that study, RMP was significantly decreased by ~8 mV in the control

group immediately after the 60-min period of stimulation. In addition, when glucose was infused, RMP was not significantly reduced at the end of the stimulation period. However, in spite of this lack of change in RMP at min 1 in the recovery period, when glucose was infused, muscle force and M-wave peak-to-peak amplitude were significantly decreased. This observation suggests that the impairment in the electrical properties of the muscle fiber membrane and the reduction in muscle force following prolonged stimulation are not entirely due to a decrease in RMP. This is further confirmed by the observation that in the control group, although RMP returned to the initial value after 1 min of recovery, both M-wave peak-to-peak amplitude and muscle force had not fully recovered after 4 min of recovery.

We also examined other mechanisms that could also be involved in the beneficial effect of glucose infusion on muscular function. There is evidence to suggest that changes in metabolite concentrations with CHO administration during exercise may be associated with an increase in muscle performance (Lewis and Haller 1986; Spencer et al. 1991; Tsintzas et al. 1996; McConell et al. 1999; Snow et al. 2000; Tsintzas et al. 2001). Therefore, in our fifth study we examined several muscle metabolites during prolonged stimulation with glucose infusion. Results show that glucose infusion attenuated the decline in PCr degradation during prolonged indirect stimulation, thus suggesting a better maintenance of the potential phosphate. Moreover, this study also suggests that the mechanism(s) responsible for the increase in performance in the rat isolated muscle when glucose is infused could be at least partly similar to those

responsible for the increase in endurance observed during submaximal whole-body exercise when CHO is administered in man.

In our third study, we showed that the infusion of lactate also increased muscle performance during prolonged indirect stimulation and that this was also associated with a better maintenance of the electrical properties of the muscle fiber membrane. In addition, as observed in study 1, lactate infusion was also not associated with any reduction in muscle glycogen utilization or with any reduction of fatigue at the neuromuscular junction (as assessed through maximal direct muscle stimulation. These results could suggest that the beneficial effect of glucose infusion observed in study 1 was not due to fuelling of the Na^+/K^+-pump with glycolytic ATP. However, an alternate explanation for these results is that the attenuation of muscle fatigue was due to glycolytic ATP supply to the membrane pumps when glucose was infused, but to a different mechanism when lactate was infused.

Taken together, it appears that during prolonged stimulation when the ability of metabolism to match energy demand is exceeded, adjustments seem to be made in the activity of the Na^+/K^+ pump. As discussed, muscle fatigue was associated with a deterioration of the electrical properties of the muscle fiber membrane in the above studies. Therefore, muscle fatigue could be acting as a protective mechanism during prolonged contractions by reducing the activity of the Na^+/K^+ pump when demand is increased. However, this increase demand could be alleviated when glucose or lactate is infused resulting in the better maintenance of the electrical properties of the muscle fiber membrane. It is

apparent that muscle fatigue takes on the role of a "circuit breaker" that is essential for maintaining muscle viability. In support of this hypothesis, Ortenblad and Stephenson (2003) observed that a decrease in mitochondrial ATP-producing function with three different mitochondrial function antagonists under conditions in which the cytosolic ATP was maintained high and constant, consistently decreased the excitability of rat fibers. The authors suggested that mitochondria may regulate muscle cell function and have important implications for further understanding the differences between ATP utilization and ATP production during muscle contractions. This phenomenon seems to be involved, in particular, at the cellular level of skeletal muscle fibers. If this type of mechanism is operative, the question of the link between exercise performance and membrane function becomes even more intriguing. Nevertheless, despite the research that was completed in this thesis, the mechanism(s) that could explain the increase in muscle performance during prolonged exercise still remain(s) elusive.

It should be noted that some caution must be applied to the interpretation of the data presented in this thesis due to the limitations of the experimental model used. The similarity of the neuromuscular system in rat and human is well established. However, despite these similarities, the results obtained in the present experiment may be model-specific and not fully transferable to humans. Furthermore, the isolated in-situ nerve-muscle preparation employed throughout the experiment differs from the true in-vivo situation in many ways. For example, the effect of deep anaesthesia could act on the muscle preparation directly. In

addition, reflexes originating from the stimulated muscle are blunted. An alternative procedure could be a decerebration, which will remove the effect of deep anaesthesia and keep intact the spinal reflexes. Moreover, as muscles are stimulated to contract by imposing supramaximal stimulation through the sciatic nerve at a fixed stimulation frequency, the resulting pattern of muscle activation differs greatly from the physiological recruitment of muscle fibers.

We chose to use the plantaris muscle for our experiments because this muscle has been studied extensively in our laboratory and it is composed of a mixture of type I, IIa, IIx and IIb fibers (6, 14, 33, 47 %, respectively). Since a positive effect has been observed with the plantaris muscle, future studies may want to investigate other muscles with a different fiber type composition. It should be noted that the soleus muscle was examined in our laboratory using the same model. Results showed that the beneficial effect of glucose infusion during prolonged muscle contractions was greater in the soleus muscle than the plantaris muscle (Marcil et al. 2004). In addition, large amounts of glucose were giving in our experiments, which resulted in high plasma glucose concentrations (~10 mmol/l). We acknowledge that the high glucose concentrations are non-physiological, however it should be recognized that these were the first series of experiments that were performed. It would be interesting to see at which level of plasma glucose concentration the positive effect is no longer observed. Finally, in order to completely rule out a beneficial effect of insulin in our experiments the use of somatostatin, which inhibits endogenous insulin secretion, or the removal of the pancreas should be examined in future studies. This will confirm if glucose

only was responsible for the increase in muscle performance or if insulin, at least in part, was also involved.

In conclusion, it is hoped that the results of the above studies, which comprise this thesis, will stimulate more research into the realm of muscle physiology and metabolism. The present experimental model could be helpful in designing future studies of the causes of muscle fatigue during prolonged exercise. In addition, these results could serve to develop new mechanistic approaches for exerting control over the regulation of muscle fatigue. It should be clear that the etiology of muscle fatigue is complex, likely involving multiple factors acting at numerous cellular sites. Experiments concerning the identification of mechanism(s) of muscle fatigue will add a great deal to our understanding of how muscle functions during exercise. Once it is possible to identify the cause of defective muscle performance during prolonged exercise, we may be able to understand the mechanism by which CHO administration increases performance during prolonged exercise. This knowledge would have significant implications for improving exercise performance.

"Wise is the one who collects the wisdom of others".

- Juan Guerra Caceras

Chapter 8

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Appendix 1

Abstract of co-authored manuscript

Title: Glucose infusion attenuates fatigue without sparing glycogen in rat soleus muscle during prolonged electrical stimulation *in situ*.

Authors: Marcil M, Karelis AD, Péronnet F, and Gardiner PF

Journal: European Journal of Applied Physiology, 2004 (in press)

ABSTRACT

Carbohydrate administration increases endurance in man, and this could be associated with a reduction in muscle glycogen utilization in type I but not in type II fibres. Glucose infusion also attenuates fatigue in the rat plantaris muscle (94 % type II fibres) stimulated indirectly *in situ*, but this is not associated with a glycogen sparing effect. The aims of this study were to verify if glucose infusion would attenuate fatigue and would reduce glycogen utilization in a muscle predominantly composed of type I fibres. For this purpose, the soleus muscle (84 % type I fibres) was indirectly stimulated *in situ* on anaesthetized rats for 60 min while either infusing saline or glucose (1 $g \cdot kg^{-1}h^{-1}$; plasma glucose 7.7 $mmol \cdot L^{-1}$ vs ~5 $mmol \cdot L^{-1}$ with saline only). With and without glucose, dynamic force decreased by ~20% in the first minute of stimulation. With infusion of saline, dynamic force further decreased to 55% of the initial value at the end of the 60-min period of stimulation, but when glucose was infused for 60 min, dynamic force remained constant at 78% of the initial value. When glucose was infused starting at min 30, dynamic force was partially restored. However, muscle glycogen utilization was not significantly different with infusion of saline, or glucose. These results suggest that glucose infusion attenuates fatigue in type I muscle fibres, but that this is not associated with any muscle glycogen sparing.

Appendix 2

Published abstracts of presentations given at international conferences

Title: Glucose Infusion Delays Fatigue in Rat Skeletal Muscle In Situ.

Authors: A. Karelis, F. Péronnet and P. Gardiner

Journal: Canadian Journal of Applied Physiology, 26(5): 487, 2001

Abstract

Using an in-situ nerve-muscle preparation the plantaris muscles of 24 untrained rats was electrically stimulated (trains of stimuli at 50 Hz, for 200 ms, every 2 s) to perform concentric contractions for one hour while either infusing saline (Con) (2ml/h), glucose (1.07 g/kg/h) (Glu) or saline (2 ml/h) for the first 30 min followed by glucose (1.07 g/kg/h) (Swt). In the Con group mean peak force decreased by -70% after 5 min and stayed constant till the end, while in the Glu group mean peak force decreased only by -55%. In the Swt group mean peak force decreased by -70% for the first 30 min then increased by -8% for a final force loss of -62% by min 60 ($p < 0.01$). At the end of the procedure twitch force was decreased in all groups by -45%, while maximal tetanic force significantly decreased in the Con and Swt groups, however remained unchanged in the Glu group. The utilization of glycogen in all groups was not statistically different. These data indicate that muscle fatigue is delayed during prolonged electrical induced stimulation by infusing glucose in rats.

Title: Attenuation of fatigue in rat plantaris muscle stimulated *in situ*, associated with glucose infusion, is not mediated by an increase in insulin concentration

Authors: Antony D. Karelis, François Péronnet, and Phillip F. Gardiner

Journal: FASEB Journal, 17(4): D196, 2003

Abstract

Glucose infusion attenuates fatigue in rat plantaris muscle stimulated *in situ*, and this is associated with a better maintenance of electrical properties of the fibre membrane (Karelis et al. Exp Physiol 87:585, 2002). The purpose of the present study was to test the hypothesis that elevated plasma insulin concentration due to glucose infusion (~775 pmol/l), rather than high plasma glucose concentration (~10-11 mmol/l), could be responsible for this phenomenon, since insulin has been shown to stimulate the Na/K pump. The plantaris muscle was indirectly stimulated (50 Hz, for 200 ms, 5 V, every 2.7 s) via the sciatic nerve to perform concentric contractions for 60 min, while insulin (8 mU kg^{-1} min^{-1}) and glucose (6.2 mg kg^{-1} min^{-1} or 21.7 mg kg^{-1} min^{-1}) were infused in order to provide, either a hyperinsulinemic-euglycemic (HE) or hyperinsulinemic-hyperglycemic (HH) clamp condition (plasma glucose between 4-6 and 10-12 mmol/l, respectivley, plasma insulin: ~800 pmol/L) (6 rats/group). The reduction in submaximal dynamic force was significantly ($P<0.05$) less with HH (-53 %) than with HE and saline only (-66 and -70 %, respectively). M-wave characteristics were also better maintained in the HH than in HE and control groups. These results show that the increase in insulin concentration is not responsible for the increase in muscle performance observed following the elevation of circulating glucose.

Title: The attenuating effect of elevated plasma glucose on muscle fatigue is greater in rat slow-twitch soleus than fast-twitch plantaris

Authors: Mariannick Marcil, Antony D. Karelis, François Péronnet and Phillip F. Gardiner

Journal: FASEB Journal, 17(4): D197, 2003

Abstract

Karelis et al. (Exp Physiol 87:585, 2002) have shown that glucose infusion attenuates fatigue in rat plantaris muscle (PLN) stimulated *in situ*. The purpose of this study was to compare the effect of glucose infusion in slow-twitch soleus (SOL) and in fast-twitch PLN muscle. Electrical stimulation was conducted *in situ* on anaesthetized rats for 60 min while either infusing saline or glucose (1 g $kg^{-1}h^{-1}$; plasma glucose >11mmol/L vs ~5 mmol/L with saline only). In the control condition, submaximal dynamic force (-70% in PLN and -58% in SOL) as well as maximal isometric force (-21% in PLN, -13% in SOL) significantly decreased. The reduction in submaximal dynamic force was lower when glucose was infused, and the attenuation of fatigue was larger in the SOL (-24%) than in the PLN muscle (-55%). However, compared to control, the attenuation of fatigue in isometric maximal contraction was similar in both muscles (~19% higher in PLN vs ~15% higher in SOL). Glucose infusion did not have any direct effect on the contribution of the neuromuscular junction on muscle fatigue, either in the PLN nor SOL muscle, as assessed by direct vs. indirect stimulation at the end of the experiment. These results suggest that infusion of glucose has a larger protective role on fatigue in slow- than fast-twitch muscles.

Title: Fatigue in rat plantaris muscle during prolonged indirect stimulation *in situ*: effect of lactate infusion

Authors: Antony D. Karelis, Mariannick Marcil, François Péronnet, and Phillip F. Gardiner.

Journal: Canadian Journal of Applied Physiology, 28(Supplement): S70, 2003

Abstract

Glucose infusion attenuates fatigue in rat plantaris muscle stimulated *in situ*, and this is associated with a better maintenance of electrical properties of the fiber membrane (Karelis et al. Exp Physiol 87:585, 2002). The purpose of the present study was to test the hypothesis that increased circulating lactate availability during fatiguing contractions would have the same beneficial effects as increased glucose availability, since both are easily oxidizable substrates. The plantaris muscle was indirectly stimulated (50 Hz, for 200 ms, every 2.7 s, 5 V) *in situ* via the sciatic nerve to perform concentric contractions for 60 min while infusing intravenously either saline alone (7.25 mL kg^{-1} h^{-1}) or lactate (0.96 g kg^{-1} h^{-1}) (8 rats/group). Lactate infusion attenuated the reduction in submaximal dynamic force (~50 % decrease vs. ~70 % decrease in rats infused with saline alone, $P<0.05$). Maximum dynamic and isometric forces at the end of the period of stimulation were also significantly higher in rats infused with lactate compared to saline alone. The beneficial effect of lactate infusion on muscle force during prolonged stimulation was not associated with a reduction in muscle glycogen utilization, nor with a reduction of fatigue at the neuromuscular junction (as assessed through maximal direct muscle stimulation). M-wave characteristics were also better maintained with lactate infusion compared to the control group. These results suggest that, consistent with our hypothesis, any easily oxidizable substrate can serve to attenuate fatigue in this model.