METABOLIC STUDIES IN THYROID DISEASE

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ABSTRACT

The effects of thyroid hormones on intermediary metabolism have been investigated in hyperthyroid and hypothyroid man and in experimentally induced thyroid disease in the rat. Glucose metabolism was investigated by measurement of blood glucose, fasting and in response to meals and by using both ^{3}H-3-glucose and ^{14}C-1-glucose as tracers in kinetic studies. Analysis of glycerol clearance from blood following bulk infusions of glycerol permitted evaluation of gluconeogenesis. Circulating NEFA and glycerol levels and glycerol kinetic data permitted assessment of lipolysis. Ketogenesis was investigated in vivo by measurement of circulating ketone body concentrations and by ketone body kinetic studies using both ^{14}C-3-acetoacetate and ^{14}C-3-hydroxybutyrate. Influences of hyperthyroidism on ketone body production in vitro were examined in isolated rat hepatocytes. Sensitivity of glucose metabolism, lipolysis and ketogenesis in vivo in man were evaluated by the euglycaemic clamp technique.

Thyroid hormone excess in man caused fasting hyperglycaemia and a 50% increase in total glucose turnover. Much of this increase could be accounted for by increased cycling between glucose and glucose-derived 3-carbon intermediates with only a modest increase in irreversible glucose disposal. Although fasting blood lactate, pyruvate and alanine concentrations were normal, increased recycling and accelerated glycerol clearance suggested enhanced gluconeogenesis. Carbohydrate intolerance to meals was also evident. In hypothyroidism

fasting blood glucose concentrations were normal despite a decrease in glucose production and delayed glycerol clearance. Carbohydrate intolerance to meals was less marked than in thyrotoxic subjects. Lipolysis was increased in hyperthyroid man as evidenced by increased blood glycerol and plasma NEFA concentrations and an increase in endogenous glycerol production. Glycerol production was decreased in hypothyroidism although plasma NEFA concentrations were normal. Ketone body concentrations were increased in hyperthyroidism and ketone body production was increased 4-fold. Ketone body production estimates were normal in hypothyroidism. Experimental hyperthyroidism in rats increased ketone body production by isolated hepatocytes suggesting an increase in hepatic ketogenic potential.

In the rat hyperthyroidism was associated with hypoinsulinaemia. Hyperglycaemia, lipolysis and ketogenesis in hyperthyroid man occurred despite apparently normal peripheral insulin concentrations suggesting either relative hyposecretion of insulin and/or insensitivity to endogenous insulin. Euglycaemic clamp studies suggested normal sensitivity to exogenous insulin in terms of glucose metabolism but a decrease in sensitivity of lipolysis whilst insulin clearance was similar to controls.

Thyroid hormones exert stimulating effects on glucose, fatty acid and ketone body metabolism which are not associated with a compensatory increase in insulin secretion.

CONTENTS

ABBREVIATIONS

Acac, acetoacetate; A.T.P., adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ACT, acyl carnitine transferase; BOH, 3-hydroxybutyrate; BSA, bovine serum albumin; $CaCl_2$, calcium chloride; CoA, coenzyme A; cpm, counts per minute; C.V., coefficient of variation; DIT, diiodotyrosine; DNA, deoxyribonucleic acid; DHSS, Department of Health and Social Security; DHAP, dihydroxyacetone phosphate; d.p.m., disintegrations per minute; EGTA, ethylene glycol tetra acetic acid; EDTA, ethylene diamine tetra acetic acid; EPR, endogenous production rate; FAD(H), oxidised flavine adenosine dinucleotide (reduced form); αGPD, α glycerophosphate dehydrogenase; GTT, glucose tolerance test; G6PD, glucose-6-phosphate dehydrogenase; HCl, hydrochloric acid; HEPES, N_2-hydroxyethyl piperazine N_2 ethane sulphuric acid; H_2SO_4, sulphuric acid; $HgSO_4$, mercuric sulphate; IBW, ideal body weight; KB, ketone bodies; kiu, kallekrein inhibitory units; KOH, potassium hydroxide; KCl, potassium chloride; M, molar; MCR, metabolic clearance rate; MIT, monoiodotyrosine; $MgSO_4$, magnesium sulphate; mRNA, messenger ribonucleic acid; $Na^+K^+ATPase$, sodium-potassium dependent adenosine triphosphatase; N, Normal; NaOH, sodium hydroxide; $NaHCO_3$, sodium hydroxide; NaCl, sodium chloride; NAD(H), oxidised nicotinamide-adenine dinucleotide (reduced form); NADP(H) oxidised nicotinamide-adenine dinucleotide phosphate (reduced form); NEFA, non-esterified fatty acid; O.D., optical density; PCA, perchloric acid; PDH, pyruvate dehydrogenase; Ra, rate of appearance; Rd, rate of disappearance; RT, turnover; rT_3, reverse T_3; r.p.m., revolutions per minute; RNA, ribonucleic acid; S.A., specific activity; SSPG, steady state

plasma glucose; T_3, triiodothyronine; T_4, thyroxine; TBG, thyroid binding globulin; TBPA, thyroid binding prealbumin; TCA, tricarboxylic acid cycle; $^{99}T_c$, radioactively labelled technicium; TKB, total ketone bodies; TSH, thyroid stimulating hormone; TRH, thyrotrophin releasing hormone; V.D., volume of distribution; VLDL, very low density lipoprotein; w/v, weight per volume.

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1. ACKNOWLEDGEMENTS

2. DECLARATION

Study protocols were devised by myself under the supervision and guidance of Professor K. G. M. M. Alberti and Dr. D. G. Johnston. Recruitment and cannulation of patients were performed entirely by myself. Assistance with blood sampling and sample separation was provided by Mrs. Mavis Brown (Research Staff Nurse). All chemical methods included in glucose and ketone body turnover experiments were performed by myself. Isolated rat hepatocyte experiments were performed by myself with the help and supervision of Dr. J. M. Burrin. Glucose clamp experiments using the biostator were performed by myself with the help and guidance of Drs. P. Home and R. Heine. All metabolite assays on P. C. A. extracts were performed by the staff of the University Department of Clinical Biochemistry, Newcastle upon Tyne as were serum insulin determinations. Serum C-peptide estimations were performed by Mr. I. Hanning. Plasma glucagon measurements were performed by Dr. H. Ørskov (Aarhus, Denmark). Serum growth hormone was measured by Mrs. C. Waugh and serum thyroxine, triiodothyronine and thyrotrophin measurements by the Department of Clinical Biochemistry, Royal Victoria Infirmary, Newcastle upon Tyne. The thesis was composed entirely by myself.

3.   <u>AIMS</u>

1.   To investigate the effects of hyper and hypothyroidism
on circulating hormone and intermediary metabolite concentrations
in fed and fasted man.

2.   To investigate the effects of hyper and hypothyroidism
on glucose production, glucose utilisation and Cori (glucose-
lactate) cycle activity in man.

3.   To establish a simple test of gluconeogenic capacity and
apply it to patients with hyper and hypothyroidism.

4.   To investigate the cause(s) of previously reported glucose
intolerance in hyperthyroid subjects.

5.   To investigate the effects of thyroid hormones on ketone
body kinetics in man.

6.   To assess the effects of thyroid hormones on ketone body
production by isolated rat hepatocytes.

4. INTRODUCTION

4.1. THYROID HORMONE PHYSIOLOGY

The thyroid gland synthesises and secretes two metabolically active iodothyronine compounds (Sterling, 1974). L. Thyroxine was first identified in 1915 (Kendal, 1929) and subsequently purified and characterised by Harrington in 1926. Triiodo-L-thyronine, the second major hormone was identified in 1952 by Gross and Pitt-Rivers.

(a) Thyroid hormone synthesis and secretion

Inorganic iodide from dietary sources, water, fish and iodised salt is absorbed in the small intestine and accumulated by the thyroid against a concentration gradient. Within the thyroid iodide is oxidised and transferred to tyrosine by a haem containing particulate bound peroxidase the activity of which can be stimulated by thyroid stimulating hormone (TSH), cyclic adenosine monophosphate (cyclic a.m.p.), prostaglandins and adrenaline (Ahn & Rosenberg, 1970). Receptor tyrosine residues are in the macroglobulin protein thyroglobulin and are iodised first in position 3 then position 5 to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). Following this, secondary coupling occurs within the thyroglobulin molecule to form thyroxine (T_4)(DIT + DIT) and triiodothyronine (T_3) (DIT + MIT). The precise mechanism of this step is uncertain. The structures of tyrosine, DIT, MIT, T_4 and T_3 are shown in Figure 4:1.

Follicular colloid is the major store of thyroid hormones and about 1% of this store is released into the circulation each day.

Thyroglobulin is incorporated into the thyroid cell by pinocytosis, broken down by lysosomal proteases and peptidases and T_3 and T_4 are released from the gland (Seljelid, 1962). Any liberated MIT or DIT is deiodinated within the cell, the iodine being conserved and recycled. TSH stimulates both endocytosis and protease activity. Normal daily T_4 production rate lies between 80-100 μg (Nicoloff et al. 1972) and the T_3 production rate between 25-40 μg daily (Singer & Nicoloff, 1972). There is now also substantial evidence that 30-40% of the endogenous T_3 pool is derived from extrathyroidal conversion of T_4 to T_3 (Pitman et al. 1971).

(b)   Thyroid hormone transport in blood

T_3 and T_4 circulate in association with plasma proteins. Three thyroid hormone binding proteins have been described:

i) thyroxine binding globulin (TBG), a single chain glycoprotein with a high affinity for T_4 and a rather lower affinity for T_3. It is of low capacity with a single binding site for T_4 (or T_3) (Robbins, 1975) and is able to bind 15-30 μg of T_4/100 ml.

ii) thyroxine binding prealbumin (TBPA), a stable tetramer of identical subunits arranged round a central slot containing two identical binding sites for thyroid hormones (Blake et al. 1975). It is of higher capacity than TBG with a binding capacity of about 55 μg T_4/100 ml, but of lower affinity.

iii) Albumin. Thyroid hormones may be bound to albumin but its affinity for T_4 is very low although its capacity is large.

Approximately 70% of circulating T_4 and 77% of T_3 is bound

to TBG under normal conditions. The remainder is bound to TBPA (10% T_4, 8% T_3) and albumin (20% T_4 and 14% T_3). The concentration of total T_4 in serum normally lies between 60 and 150 nmol/l and total T_3 between 1.3 and 3.0 nmol/l. Thus the total T_4/T_3 ratio in blood is about 50 : 1. A small fraction of T_4 (0.015%) and T_3 (0.5%) circulate free and determine the metabolic status of the individual. Direct measurements of free T_4 give normal values between 10-22 pmol/l and free T_3 between 5-10 pmol/l (Ekins & Ellis, 1975) giving a free T_4 : T_3 ratio of 2 : 1. A rapid exchange equilibrium exists between bound and free hormones. Only the free forms of thyroid hormones can enter target tissues and exert their actions (Thorson et al. 1969). In addition to their function of transporting T_3 and T_4 from the thyroid gland to peripheral tissues, binding proteins also prolong hormone availability by retarding the rate of transcapillary diffusion, provide a buffering action to dampen the effects of transient perturbations of thyroid hormones, act as a reservoir for extra-thyroidal hormone storage and prevent loss of thyroid hormones in urine.

(c)   Thyroxine and Triiodothyronine metabolism

The half life of T_4 in plasma is 6-7 days and that of T_3 24-30h in euthyroid adults. Daily production of T_4 is 80-100 µg derived entirely from the thyroid. Daily T_3 production is 25-40 µg approximately 40% coming from extrathyroidal T_4 to T_3 conversion (Pittman et al. 1971). A number of tissues are capable of transforming T_4 to T_3 by monodeiodination including liver, kidney, heart and anterior pituitary

(Hoffenberg, 1973). Reverse T_3 (rT_3, 3 3'5'triiodothyronine) can also be formed by monodeiodination. Current evidence suggests that this may be a degradative step since rT_3 has negligible metabolic activity. Production of rT_3 is increased in a number of acute or chronic stressful states (acute illness [Harvey, 1971], starvation [Ingenbleek & Bekers, 1976] and cirrhosis [Chopra et al. 1974]). Concomitant with a rise in rT_3 is a fall in T_3 production. Thus monodeiodination of T_4 provides the body with either a highly active metabolite, T_3 (most if not all the effects of T_4 result from this interconversion) or one that is inert, rT_3. This may represent a control mechanism at the cellular level (Burr et al. 1975).

(d) Disposal of thyroid hormones

Deiodination is the major catabolic step in the degradation of both T_3 and T_4 (Soffer et al. 1973) (Figure 4:2). Iodine is lost in the urine (Berson & Yalow, 1954). The thyroxine nucleus may then be subjected to oxidative deamination or transamination leading to production of lactate, pyruvate and acetate. A small quantity (less than 20%) of hormone is conjugated in the liver and excreted in the bile (Taurog et al. 1952). Enterohepatic cycling probably does not occur Galton, 1972).

(e) Control of thyroid hormone secretion (Fig. 4:3)

The level of iodothyronine production by the thyroid is almost entirely determined by the circulating TSH concentration, and the negative feedback effect of thyroid hormones on TSH secretion is exerted mainly at the pituitary level. The effects of T_3 on thyrotrophin

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FIGURE 4:1.   Simplified diagram showing a synthesis of
              iodotyrosines and iodothyronines within the
              thyroglobulin molecule.

, FIGURE 4:2.   Peripheral Deiodination of Thyroid Hormones

FIGURE 4:3. Control of Thyroid Hormone Secretion
(after Evered, D.C. 1976)

releasing hormone (TRH) have been extensively studied (Snyder & Utiger, 1972). Small supplements of T_3 will block TSH release and this cannot be overcome by increased quantities of TRH. It has been observed in animals that the blocking of the effect of TRH does not occur unless T_3 is administered one hour or more before TRH and that prior administration of actinomycin D inhibits the blocking action of T_3. These observations suggest that T_3 blocks the effect of TRH by stimulating the synthesis of an intermediate compound, probably a peptide.

A reduction in thyroid hormone concentration leads to an increase in basal TSH levels and an enhanced TSH response to TRH although there is no evidence of enhanced TRH production. It seems likely that the response to the hypothyroid state is mediated by a reduction in a thyroid hormone dependent inhibitor of TSH release. These steps would depend on the presence of a basal level of TRH secretion.

(f)   Cellular actions of thyroid hormones

Insight into the cellular action(s) of thyroid hormones is limited compared to the understanding of the mechanisms of actions of other hormones with more sharply defined target tissues. This may be surprising since the overall importance of the thyroid has been recognised for almost 100 years (Kocher, 1883). The lack of specific target tissue suggests that thyroid hormones control processes that are common to all target tissues and underlie their diverse metabolic effects.

i)   Mitochondria and Mitochondrial Receptors

Increased oxygen consumption in human hyperthyroidism was

first noted in 1895 (Magnus Levy). After in vivo administration of

T_4 or T_3 to animals, oxygen consumption is increased in heart,

liver, muscle, kidney and white blood cells (Barker & Schwartz, 1953)

although not all tissues behave in this way. Gonadal tissue, lymph

nodes, spleen and notably brain do not increase their oxygen

consumption when exposed to thyroid hormones (Barker & Klitgaard,

1952). Once the function of the mitochondria (which are involved in

over 90% of total body oxygen consumption) became known, investigation

of the mitochondria as sites of action of thyroid hormones naturally

proceeded. Three separate groups reported that T_4 could uncouple

oxidative phosphorylation both in vitro and in vivo (Hoch & Lipman,

1954; Lardy & Feldatt, 1951; Martino & Hess, 1955). Several problems

emerged however. Firstly the doses of T_4 required to produce this

phenomenon were extremely large (4-8 mg/day for several days).

Secondly, D thyroxine, was equally effective an uncoupling agent in vitro

despite its lack of effect in the whole animal. Thirdly, mitochondria

isolated from skeletal muscle of thyrotoxic humans showed tightly

coupled oxidative phosphorylation (Stocker et al. 1968). Clearly

therefore this effect is unphysiological.

Thyroid hormones clearly do however have effects on

mitochondria. Increased rates of cytochrome synthesis (Booth &

Holloszy, 1975), increased amounts of mitochondrial cristae (Reith

et al. 1973), increased mitochondrial turnover (Gross, 1971) and

increased incorporation of amino acids into mitochondrial proteins

(Herd et al. 1974) have all been reportedly induced by thyroid

hormones in vitro or in vivo. It is as yet unclear whether these

effects are primary or secondary to an effect of thyroid hormones elsewhere, particularly on the nucleus. However, the demonstration of high affinity T_3 binding sites in mitochondria (Sterling & Milch, 1975) suggests the possibility of a physiological action of thyroid hormones at the level of this organelle.

ii) Nuclei and Nuclear Receptors

There has been a spate of publications describing binding sites for T_3 (and T_4) on nuclei following the original report by Oppenheimer (1972) and there has been growing interest in these sites as a possible point of initiation of thyroid hormone action (De Groot & Strausser, 1974; Samuels et al. 1974; Spindler et al. 1974).

Nuclear receptors have been characterised as non-histone proteins associated with nuclear chromatin (Surks et al. 1973) which exhibit a limited capacity and a high affinity as well as a high degree of specificity for iodothyronines.

Five general lines of evidence lend support to the idea that nuclear T_3 receptors may be the site of initiation of thyroid hormone action.

1. Studies of various rat tissues have clearly indicated the widespread nature of nuclear T_3 receptor sites. The binding capacity per mg. DNA varies from tissue to tissue but the percentage of total available sites occupied is remarkably constant under physiological conditions. This would suggest that the association constant is also identical and that the receptor sites from various tissues are chemically identical.

2.   There is a strong correlation between nuclear binding of
thyroid hormone analogues and their known thyromimetic effects
(Koerner et al. 1975).

3.   There is a correlation between the number of nuclear receptors
per gram of tissue or per gram of DNA and the responsiveness (in
terms of oxygen consumption) of the tissue concerned.   Tissues
considered responsive, such as liver, pituitary, heart and kidney
have a higher density of receptor sites than those considered
unresponsive such as testis and spleen.   Brain however has a density
of binding sites intermediate between responsive and unresponsive
tissues yet does not increase its oxygen consumption in response to
T_3 (Barker & Klitgaard, 1952).   The role of thyroid hormones on
central nervous system function is clearly established however.

4. T_3 appears to stimulate transcription of DNA (Tata & Widnell,
1966) perhaps through increased activity of RNA polymerase (Dillman
et al. 1977) although this is not clearly defined.   Enhanced formation
of two species of mRNA coding for proteins known to be stimulated by
T_3, pituitary growth hormone and α 2u globulin (Seo et al. 1977;
Kurtz et al. 1976) have been reported.   This is compatible with the
view that the primary effect of T_3 is to stimulate an as yet undefined
number of genes.   The concept that thyroid hormone action depends on
subsequent protein synthesis is supported by experiments showing
inhibition of T_3 effects by agents known to block protein synthesis at
the transcriptional or translational level (Sellinger & Lee, 1964;
Samuels & Schapiro, 1976; Dillman et al. 1977).

5.   The relationship between nuclear occupancy and response,
both in vivo and in vitro, are compatible with a 'nuclear' hypothesis
(Oppenheimer et al. 1977; Samuels et al. 1976) although the expected
proportional relationship has not been shown in all instances. It is
also known that the principal of negative self-regulation (down-
regulation), clearly defined for the interaction between insulin and
other polypeptides and their respective membrane binding sites
(Gavin et al. 1974) may apply to T_3 and its nuclear receptor (Samuels
et al. 1976).

It appears most likely therefore that thyroid hormones,
principally T_3, act on nuclear receptors to increase protein synthesis.
Many of the changes observed in mitochondria may be secondary to this.
However, the demonstration of mitochondrial receptors and the
observation that some 'in vitro' effects on mitochondrial function
cannot be inhibited by agents which block protein synthesis (Segal &
Gordon, 1977) raises the possibility that some thyroid hormone action
may be mediated by non-nuclear mechanisms.

iii) Post-receptor mechanisms

It remains unclear whether thyroid hormones induce an
overall increase in protein synthesis or induce synthesis of one or a
very few key proteins which underlie the developmental, physiological
and toxic effects attributed to thyroid hormones. Studies of the
mechanisms of induction by T_3 have been confined to only a few
selected proteins. In rat liver, the proteins which have received most
attention are α-2u globulin, malic enzyme and α-glycerophosphate

dehydrogenase (α GPD).

The physiological role of α-2u globulin is unknown. It is synthesised in the male rat liver and found in increased concentrations in the hepatic cytosol in experimental hyperthyroidism, exported into the plasma and excreted in the urine (Roy, 1953). There are no indications that α 2u gobulin is essential for thyroid hormone actions since in rats there are no established sex differences with regard to tissue response.

Synthesis of both mitochondrial α glycerophosphate dehydrogenase and malic enzyme in rat liver are known to be dependent on thyroid hormone status (Lee & Lardy, 1965; Lardy et al. 1960). Both these enzymes function as components of hydrogen shuttles which transfer reducing equivalents into mitochondria to be oxidised in the phosphorylating system. This allows glycolysis to continue without accumulation of lactate. Thus hormonally induced changes in these enzymes could stimulate both oxidative phosphorylation and glycolysis. However thyroid hormone induced changes are both species and tissue specific. Some mammals show no changes in mitochondrial α GPD despite thyroid hormone induced increases in basal metabolic rate (Lee et al. 1970). In man although hepatic α GPD may not increase in response to T_3 administration (Nolte et al. 1972) adipose tissue α GPD does (Bray, 1969). The precise role played by these enzymes in thyroid hormone effects is uncertain.

Because of the tight coupling of oxidation and phosphorylation in man consideration has been given to ATP consuming processes which could account for the increased rate of oxygen consumption seen

in hyperthyroidism. The energy consumption of active sodium-potassium transport has therefore attracted much attention. Several studies have suggested that T_3 injection in rats enhances the synthesis of Na^+/K^+ dependent ATPase (Ismail-Beigi & Edelman, 1970; Lo & Edelman, 1976) which is responsible for maintaining the sodium-potassium gradients between cellular and extracellular compartments. Based on experiments with tissue fragments and tissue slices, Ismael-Beigi and Edelman (1971) proposed that the extra uptake of oxygen in hyperthyroid liver and skeletal muscle was almost entirely due to increased energy expenditure by Na^+/K^+ ATPase. However, in perfused rat liver studies, Folke and Sestoft (1977) suggested that T_3 treatment caused only a modest increase in Na^+/K^+ transport and could not account for any major part of increased hepatic oxygen uptake. Similar findings have also been reported for adipose tissue (Fain & Rosenthal, 1971), kidney (Silva et al. 1976) and muscle (Biron et al. 1979). Reasons for the discrepancies are uncertain but may relate to experimental design. It is known that intracellular sodium concentrations are increased in incubated liver slices, thus stimulating Na/K ATPase towards maximal activity while in isolated perfused livers, physiological ion concentrations are maintained (Clariet & Mazet, 1972).

More recently, the concept of increased substrate cycle activity being responsible for increased ATP consumption has been proposed (Sestoft, 1980). The unidirectionality of flow through metabolic pathways is ensured by a few so called "key" reactions which are

displaced far from equilibrium. During reverse conversion these energy barriers are bypassed by other reactions which are also irreversible and catalysed by different enzymes. If the antagonistic reactions operate simultaneously there is no net flux of metabolites but only a "futile" recycling, the net balance of which is the wasteful hydrolysis of ATP. Such a 'futile' or substrate cycle is shown in Figure 4:4. Since these processes are characterised by energy dissipation (i.e. heat production), they have a possible role in the regulation of thermogenesis (Newsholme, 1978). Also substrate cycles might provide amplification mechanisms for allosteric control of metabolism. This theory, which stemmed from analysis of the cycle shown in Figure 4:4 (Newsholme & Crabtree, 1976) allows the prediction that small changes in an effector might provide much larger changes in net flux, the extent of amplification depending on the rate of futile cycling. Changes in substrate cycle activity in hyper or hypothyroid man could thus account for some of the clinical features and metabolic abnormalities encountered in these disease states. There is indirect evidence, particularly with regard to lipid metabolism, that this may occur in hyperthyroidism. Lipolysis and triglyceride synthesis are known to proceed simultaneously in adipocytes from hyperthyroid subjects (Eaton et al. 1965; Fisher & Ball, 1967). Increased futile cycle activity in the glycolytic pathway has also been recently described in hyperthyroid rats with decreased activity in hypothyroidism (Okajima & Ui, 1979). Futile cycle activity has not however been examined directly in hyper or

FIGURE 4:4.   Illustration of futile cycle activity here
represented by the fructose 6 phosphate-fructose
1-6 diphosphate cycle.

hypothyroid man.

However, whether these postulated changes in substrate cycle activity represent specific enzyme induction by thyroid hormones is unknown as is the degree to which they determine overall changes in energy conservation.

4. 2.   REGULATION OF INTERMEDIARY METABOLISM

The purpose of this section is to describe the basic reactions of fat, carbohydrate and protein metabolism in both the fed and starved states, together with an account of the major actions of the main anabolic and catabolic hormones. The main emphasis will be on tissue-tissue interactions rather than on intracellular mechanisms. This outline is intended to serve as a basis for subsequent chapters and also as an introduction to the role of thyroid hormones in the regulation of metabolism.

(a)   Carbohydrate Metabolism

i)   Post absorptive man

Glucose occupies a central role in carbohydrate metabolism (Johnston & Alberti, 1976). It serves as an optimal fuel for many tissues and as an obligatory fuel for brain (except during starvation) red cells and renal medulla. Reserves are found in the form of glycogen in many tissues but only liver can use this glycogen to provide new glucose in the circulation. Other tissues lack glucose-6-phosphatase and, although they can provide fuel for their own use which may be extremely important in, for instance, exercising muscle, they cannot directly provide glucose for other

tissues. Liver glycogen stores are limited, and, if no other source
of glucose were available, would be exhausted after about 12 hours.
In fasted man there is a daily requirement of about 160 g glucose per
70 kg body weight (Cahill & Owen, 1968). After an overnight fast
about 25% of this comes from glycogen whilst the rest is made de novo.
This also occurs primarily in the liver. The kidney produces about
10% of new glucose during short-term fasting although this may increase
to 50% as fasting proceeds.

Lactate, pyruvate, glycogenic amino acids (mainly alanine),
and glycerol are the main glucose precursors. Lactate provides some
15% of glucose in post-absorptive man. This is one limb of the Cori
cycle. Glucose produced by the liver enters different tissues and is
metabolised through glycolysis to pyruvate. A proportion of pyruvate
enters the tricarboxylic acid cycle while some, through the lactate
dehydrogenase reaction, forms lactate. Any lactate and pyruvate
not metabolised is released into the circulation and returns to the
liver to be reconverted to glucose thus completing the Cori cycle
(Fig. 4:5). The Cori cycle therefore serves as a means of preserving
carbohydrate not required in a particular tissue.

Amino acids are the other major glucose precursors and
quantitatively the most important is alanine which provides 5-10% of
glucose after an overnight fast (Felig, 1973). It is derived from the
amination of pyruvate in peripheral tissues, particularly muscle and
approximately half of the amino groups released from muscle are in
the form of alanine (Fig. 4:6). Alanine is in equilibrium with lactate
and pyruvate and may be considered not only as a transport form of

amino groups but also as a transport form of carbon, much of which is derived from the carbon skeletons of amino acids. A certain, important fraction of alanine is also derived from glutamine metabolism in the gut. Once in the liver, alanine is transaminated with oxaloacetate yielding pyruvate and aspartate. Pyruvate passes through the normal gluconeogenic pathway while aspartate passes its amino group into the urea cycle. Other amino acids may also act as glucose precursors but are quantitatively less important.

The other main glucose precursor is glycerol released from adipose tissues (Fig. 4:7). Under normal conditions this is a minor contributor (1-3%) to glucose production and only assumes importance in chronic starvation.

In short term starvation glycogen and gluconeogenesis combine to produce about 160g glucose per day. If starvation continues glycogen stores are exhausted and gluconeogenesis becomes the sole source of glucose. Tissue glucose utilisation falls as a result of inhibition by non-esterified fatty acids and ketone bodies (Newsholme 1976). The brain adapts to ketone body utilisation and glucose requirements fall even further (Owen et al. 1967). Glucose production falls and amino acids are therefore conserved with lactate, pyruvate and glycerol contributing a greater proportion of the carbon skeletons. Blood glucose concentrations fall slightly.

ii)   Fed Man

Even after ingestion of large amounts of carbohydrate there is remarkably little fluctuation in blood glucose concentrations with values remaining between 4 and 8 mmol/1 in normal individuals.

FIGURE 4:5.   The Glucose-Lactate (Cori) Cycle.
              G-6-P = Glucose-6-Phosphate.
              TCA = Tricarboxylic Acid Cycle.

FIGURE 4:6.   The Glucose-Lactate-Alanine Cycle.
G-6-P = Glucose-6-Phosphate.
TCA =   Tricarboxylic Acid Cycle

FIGURE 4:7.   Integrated carbohydrate, fat and protein metabolism

The liver is the most important organ of glucose disposal and all glucose absorbed must first enter the liver by way of the portal circulation. Approximately 60% of a glucose load is disposed of in the liver (Felig et al. 1975), some going to glycogen and some being converted to fatty acids and triglycerides. Glucose metabolised peripherally serves to replenish glycogen stores as well as providing immediate fuel requirements.

(b)   Lipid Metabolism

Lipids provide two   potentially useful fuels which are used directly by tissues, non-esterified fatty acids (NEFA) and ketone bodies. In addition triglycerides are distributed throughout the body by the lipoprotein carrier system and are either stored for future use (the only important long term storage form of calories) or used immediately once degraded into fatty acids and glycerol.

   i)   Post absorptive man

In the post absorptive state there is a requirement for non-carbohydrate calorie sources so that glycogen stores are not depleted too rapidly and protein reserves needlessly converted to glucose. Fatty acids, directly and indirectly, provide these calories. Fatty acid release from triglyceride stores is controlled by glucose availability and the prevailing hormone environment (vide infra). Normally there is continual cycling in the adipocyte whereby triglycerides are broken down (lipolysis) by hormone-sensitive lipase and resynthesised (reesterification). Lipolysis results in the formation of NEFA and glycerol. This glycerol cannot be re-utilised

because of low glycerol kinase activity in adipose tissue (Arner et al. 1976) and is used in the liver for gluconeogenesis. For reesterification to occur α-glycerophosphate is required and this is synthesised from glucose. If glucose is not available either due to a low extracellular concentration (e.g. during starvation) or to inability to enter the cell (e.g. in insulin deficiency) then there will be insufficient α-glycerophosphate synthesis and NEFA will be released into the circulation. Progressively more NEFA are released as starvation continues and NEFA themselves form a useful fuel. Most tissues, apart from red cells and nervous tissue have the capacity to use them. They provide a rich source of calories (9 kcal/g compared to 4 kcal/g for carbohydrate) and may be used in preference to glucose by some tissues. Resting muscle derives 90% of its energy requirements from fatty acids and ketone bodies and only during exercise do glucose and/or glycogen become significant fuels for muscle.

NEFA also serve as precursors in the liver for ketone body synthesis. NEFA enter the liver down a concentration gradient. Once in the cytosol they can either be reesterified to triglyceride or be transported into the mitochondria via the acyl carnitine transferase system (McGarry & Foster 1976). Once with the mitochondria, NEFA are oxidised to acetyl CoA and may be metabolised to acetoacetate and 3-hydroxybutyrate via the 3-hydroxy-3-methyl-glutaryl CoA pathway. In post absorptive man the preferred route of disposal of NEFA is towards ketone body synthesis. Ketone bodies cannot be used for fuel in the liver. They enter the circulation for utilisation elsewhere and

in prolonged starvation may be oxidised by brain.

The main importance of fats as fuels is their role in preserving glucose in the starved state for use by obligatory tissues. This indirectly spares protein which would otherwise become severely depleted.

ii) Fed Man

In fed man blood levels of usable fat fuels fall to very low concentrations and metabolism is directed towards storage. Fatty acids derived from ingested triglyceride reach the liver via the portal and systemic circulation but are now directed towards reesterification rather than to mitochondrial β oxidation. Newly synthesised triglyceride is released into the circulation as very low density lipoprotein (VLDL). When VLDL enter adipose tissue capillaries, they are partially degraded by lipoprotein lipase derived from capillary endothelial cells. Released fatty acids enter adipocytes and are encorporated into fat stores. In contrast, in muscle capillaries, lipoprotein lipase is inhibited in the fed state so that fatty acids are not released in muscle. During fasting the reverse situation holds and fatty acids are released from VLDL in muscle but not in adipose tissue.

Chylomicrons derived from the diet which bypass the liver in the thoracic duct are subject to similar treatment to VLDL.

This seemingly complex series of pathways whereby fats are used or stored is due to the chemical properties of triglycerides and NEFA. Triglycerides are water insoluble and must be transported as lipoproteins to make them water soluble. Alternatively, if all fats

were transported as NEFA, problems may arise as fatty acids are toxic and in high concentrations may cause haemolysis, cardiac arrhythmias and cell wall destruction.

(c)   Protein Metabolism

Tissue proteins are continually synthesised and degraded. In a normal adult on a protein free diet there are obligatory nitrogen losses of 50-60 mg/kg/day. The recommended daily intake of protein for an adult is 0.6 g/kg/day. Requirements are influenced by the specific aminoacid content of protein in that lack of an essential aminoacid can prevent protein synthesis. The essential amino acids in man are leucine, isoleucine, lysine, methionine, cystine, phenylalanine, tyrosine, threanine, valine and tryptophan. Overall protein synthesis rates in man range from 2 to 5 g/kg/day.

In any tissue the aminoacids released by protein degradation can follow one of three pathways. They can be reincorporated into protein, released from the tissue or metabolised. Branched chain amino acids (valine, leucine and isoleucine) are metabolised mainly in muscle. Their amino groups are donated to pyruvate to form alanine which is then released. Other amino acids also donate amino groups to pyruvate and also to glutamate forming glutamine. Carbon skeletons enter the glycolytic or tricarboxylic acid pathways and contribute to the formation of lactate and pyruvate. The amount of amino acid released or oxidised in tissues depends on the relative activities of the relevant pathways with decreased amino acid availability causing an increase in the activity of amino acid activating enzymes and a decrease in activity

of amino acid oxidases. Protein breakdown also appears to be responsive to changes in ketone body concentrations. When the latter are high, such as in starvation, protein breakdown is inhibited.

The liver is able to oxidise all natural amino acids, except the branched chain amino acids. Amino groups are used for urea formation which is controlled primarily by amino acid availability. Some of the amino acids arriving at the liver are also used for plasma protein synthesis and some of the amino acid mobilisation seen in starvation may be directed towards maintainance of plasma protein concentrations rather than to providing precursors for gluconeogenesis.

(d)   Hormone Action

The complex series of reactions described requires careful regulation. The system is designed to suit the normal human state; that is periods of feeding, or anabolism, are interdigitated with periods of starvation or catabolism. A group of hormones is responsible for the regulation and coordination of these processes. Insulin is the prime anabolic hormone while cortisol, catecholamines and glucagon are hormones of starvation or fuel mobilisation (catabolism). Growth hormone has a mixed role in that it is geared mainly to promote protein synthesis and to achieve this it has certain catabolic effects on lipid and carbohydrate metabolism. The role of thyroid hormones within this system is unknown.

i)   Insulin (Table 4:1)

All the actions of insulin are directed towards anabolism (Steiner & Frankell, 1972). In carbohydrate metabolism insulin promotes the entry of glucose into tissues such as muscle and adipose tissue,

TABLE 4:1.   Metabolic Effects of Hormones

INSULIN		
	Increased	Decreased
Carbohydrate Metabolism	Glucose Transport (muscle, adipose tissue) Glucose Phosphorylation Glycogenesis Glycolysis PDH* activity	Gluconeogenesis Glycogenolysis
Lipid Metabolism	Lipoprotein lipase activity (adipose tissue) Triglyceride synthesis Fatty acid synthesis (liver)	Lipoprotein lipase activity (muscle) Lipolysis
Protein Metabolism	Amino acid transport Protein synthesis	

*   Pyruvate dehydrogenase.

but not liver. Insulin has, however, a major effect on glucose prosphorylation in many tissues through stimulation of hexokinase and glucokinase activity. Glycogen synthesis is stimulated partly by an inhibition of cyclic AMP formation. Insulin also stimulates glycolysis mainly through activation of phosphofructokinase and pyruvate kinase. Pyruvate conversion to acetyl CoA is also enhanced. Although these would appear catabolic rather than anabolic, the acetyl CoA formed is directed, in the liver, to fatty acid synthesis. At the same time, insulin inhibits gluconeogenesis thus sparing the incoming gluconeogenic precursors such as amino acids. The net effect is a sharp reduction of hepatic glucose output when insulin concentrations rise. These hepatic processes are very sensitive to small increments in insulin concentrations and are more sensitive than the uptake of glucose by extrahepatic tissues.

As in other tissues, insulin promotes glucose uptake and metabolism in the adipocyte. Increased reesterification of fatty acids will result and fatty acid release from the adipocyte will decrease. At the same time insulin inhibits lipolysis so blood glycerol release will also decrease. Insulin stimulates adipose tissue lipoprotein lipase so that more fatty acids enter the adipocyte and are stored as triglyceride. These fatty acids will derive from dietary fats and from de novo hepatic fatty acid synthesis. Muscle lipoprotein lipase is inhibited and fatty acid release in muscle is inhibited.

Insulin promotes protein anabolism. Amino acid transport is increased and the intracellular amino acid pool increases. Insulin also directly stimulates protein synthesis but it is uncertain whether insulin

has any direct inhibitory effect on protein degradation.

Insulin secretion is stimulated primarily by glucose and amino acids, these effects being enhanced by gut hormones, particularly GIP. After meals, insulin will direct metabolism towards anabolism. Insulin effects are mediated through cell surface receptors which have been identified in many tissues such as liver, adipose tissue, muscle, lung, monocyte and lymphocyte. Most of the actions of insulin can be accounted for by these cell surface interactions, although recent work suggests that insulin may also have actions within the cell.

ii) Glucagon (Table 4:2)

In carbohydrate metabolism glucagon exerts its main effects in the liver. Glucagon acutely raises hepatic glucose production and hence blood glucose concentrations through stimulation of glycogenolysis. This is mediated by cyclic AMP, glucagon being one of the polypeptide hormones whose cell surface receptors are linked to adenyl cyclase. This effect of glucagon is short lived and persists for only one to two hours. Glucagon has a more prolonged effect on gluconeogenesis and stimulates it both directly, by an effect on cyclic AMP, and indirectly, by enhanced hepatic uptake of the major amino acid precursor, alanine. This is associated with increased urea synthesis. There is no evidence that glucagon has any effect on extra hepatic glucose metabolism.

In lipid metabolism glucagon again has relatively minor extrahepatic effects under normal conditions. It can stimulate lipolysis thereby increasing NEFA release from adipose tissue but this is significant only when insulin deficiency coexists (Alberti &

Nattrass, 1977). In contrast to insulin glucagon promotes fatty acid entry into mitochondria, perhaps by inhibiting acetyl CoA carboxylase, thus decreasing malonyl CoA levels in the liver and hence increasing ketogenesis (McGarry et al. 1978). The balance between insulin and glucagon appears to determine the fate of fatty acids in the liver, their relative proportions determining whether ketogenesis or triglyceride synthesis predominates (Parilla et al. 1974). Although glucagon has effects to increase hepatic protein synthesis and amino acid uptake, the metabolic significance of these actions is unclear.

iii) Cortisol (Table 4:3)

Despite earlier ideas that cortisol had only a "permissive" role in metabolic control, it is now clear that cortisol has an important role in metabolic homeostasis (Alberti et al. 1977). While most of the 'anti-insulin' effects of glucagon are situated in the liver cortisol has a major extra hepatic role as well. Cortisol inhibits glucose uptake by peripheral tissues and this has a glucose sparing effect. In addition cortisol is the most important proteolytic hormone and increases the flow of amino acids to the liver. Simultaneously the flow of carbon skeletons, lactate and pyruvate is increased. Cortisol enhances gluconeogenesis in the liver and induces several key gluconeogenic enzymes as well as enzymes of amino acid metabolism.

In adipose tissue reesterification of fatty acids is reduced because of diminished glucose entry. NEFA circulate to the liver where they will be incorporated into triglycerides or, when insulin is deficient, will increase ketogenesis.

TABLE 4:2.   Metabolic Effects of Hormones

GLUCAGON

	Increased	Decreased
Carbohydrate Metabolism	Gluconeogenesis Glycogenolysis (liver)	
Lipid Metabolism	Lipolysis Ketogenesis	Liver lipoprotein release
Protein Metabolism	Amino acid transport (liver)	Protein synthesis (liver)

TABLE 4:3. Metabolic Effects of Hormones

CORTISOL

	Increased	Decreased
Carbohydrate Metabolism	Gluconeogenesis Glycogenesis Gluconeogenic precursor supply	Glucose transport
Lipid Metabolism	Lipolysis Triglyceride formation (liver)	Reesterification
Protein Metabolism	Protein synthesis (liver) Proteolysis (periphery) Amino acid uptake and degradation (liver)	

iv) <u>Catecholamines</u> (Table 4:4)

The prime importance of catecholamines is to increase circulating fuel supplies regardless of the metabolic status of the body at any specific time (Young & Lansberg, 1977). They have a potent effect on hepatic glycogenolysis, providing more circulating glucose, and increase peripheral glycogen breakdown, providing immediate fuels within tissues. They have a weaker effect on gluconeogenesis although by stimulating peripheral glycolysis, they increase the flow of lactate back to the liver.

Catecholamines also stimulate glycolysis, regardless of the presence of insulin, acting through cyclic AMP. As a result of increased flow of NEFA to the liver ketone body formation is increased. Catecholamines may also have a direct effect on hepatic ketogenesis per se as well as the indirect effect of the increase in the supply of NEFA.

The relationship between thyroid hormones and catecholamines has been widely investigated and will be discussed later in this chapter.

vi) <u>Growth Hormone</u> (Table 4:5)

Growth hormone has both anabolic and catabolic properties which are directed towards the preservation of proteins. In addition, it has long and short term effects which may differ, some being mediated through the somatomedins which are formed in the liver under the influence of growth hormone. Growth hormone may cause an acute rise of glucose uptake in muscle but chronic elevation of growth hormone concentration results in inhibition of glucose entry and enhanced

TABLE 4:4.   Metabolic Effects of Hormones

CATECHOLAMINES

	Increased	Decreased
Carbohydrate Metabolism	glycogenolysis gluconeogenesis muscle lactate release glycolysis (muscle)	glycogenesis
Lipid Metabolism	lipolysis ketogenesis	

TABLE 4:5.   Metabolic Effects of Hormones

GROWTH HORMONE

	Increased	Decreased
Carbohydrate Metabolism	gluconeogenesis glucose transport (short term)	glucose transport (long term)
Lipid Metabolism	lipolysis NEFA uptake (peripheral) ketogenesis triglyceride synthesis	
Protein Metabolism	protein synthesis amino acid transport	

hepatic gluconeogenesis. At the same time alternative fuels to glucose are provided through stimulation of lipolysis, enhanced ketogenesis and increased peripheral uptake and oxidation of fatty acids. The effects on protein metabolism are more straightforward with growth hormone stimulating both amino acid uptake and protein synthesis.

(e)   Hormone-Hormone Interactions

Apart from the individual effects of hormones on metabolism, each of the hormones has effects on the secretion of others. Glucagon in physiological concentrations stimulates the secretion of insulin, growth hormone and perhaps cortisol. The effect on insulin secretion means that many of the effects of glucagon are masked unless insulin secretion is impaired.

In contrast catecholamines inhibit insulin secretion and promote glucagon secretion so that lipolysis, ketogenesis, glycogenolysis and gluconeogenesis will all be increased. Cortisol increases insulin secretion, although this may be secondary to hyperglycaemia, and also mobilises the other catabolic hormones. These various interactions are finely regulated in normal man and designed to maintain appropriate fuel supply at all times.

(f)   Hormone-Metabolite Integration

The metabolic adjustments to the fasted state described above can largely be explained in hormonal terms. As blood glucose concentration falls so insulin secretion decreases and glucagon concentration rises slightly. There is a relative excess of catabolic

hormones over insulin. Lipolysis therefore increases and ketogenesis occurs partly as a result of this and partly because of a more ketogenic 'set' of the liver. The brain responds to increased ketone body concentrations by adapting to ketone body utilisation and glucose utilisation falls. Simultaneously, through the 'glucose-fatty acid' cycle, peripheral glucose utilisation decreases. Glucose production falls and amino acids are spared.

In the fed state the hormonal response to a mixed meal comprises an increase initially in insulin and glucagon secretion. The latter is secreted in response to protein feeding and serves to inject glucose into the system so that hypoglycaemia is prevented. Insulin causes its well known anabolic effects. Later, growth hormone secretion occurs which further conserves amino acids and stimulates protein synthesis.

4.3. KNOWN EFFECTS OF THYROID HORMONES ON THE REGULATION OF INTERMEDIARY METABOLISM

This section describes the known effects of thyroid hormones on intermediary metabolism. Much of the work referenced has been published since work on this thesis was begun in 1979 and has been included in this section to give a complete and up to date review of the literature.

(a)   Carbohydrate Metabolism

i)   Post-Absorptive Man

a)   Hyperthyroidism

Mild elevation of fasting blood glucose concentrations have been observed in most, (Ikejeri et al. 1978; Doar et al. 1969;

Lamberg, 1965) but not all studies (Saunders et al. 1980) in
hyperthyroid man. Fasting hyperglycaemia may result from increased
glucose production, decreased glucose utilisation or a combination of
the two. Most studies have suggested that hepatic glucose production
in hyperthyroidism is increased (Saunders et al. 1980; Perez et al.
1981) although conflicting data have recently been reported (Wahren
et al. 1981). Increased glucose production by the liver may result
from increased glycogenolysis and/or increased gluconeogenesis.
Hepatic glycogen stores are decreased in fasting hyperthyroid man
(Movitt et al. 1963; Pipher & Poulsen, 1947) and animals (Battarbee,
1974). Whether this reflects increased glycogenolysis or decreased
glycogen synthesis is uncertain. However, depletion of glycogen
stores occurs more rapidly in perfused hyperthyroid rat livers than
in control livers (Laker & Mayes, 1981) suggesting that glycogenolysis
is increased.

The effect of thyroid hormone excess on gluconeogenesis in man
is uncertain. Studies using the isolated perfused rat liver preparation
have yielded conflicting results. Some have shown that the uptake of
all the major gluconeogenic precursors (lactate [Menahan & Weiland,
1969], alanine [Singh & Snyder, 1978] and glycerol [Krebs &
Freedland, 1965]) is increased while hepatic glucose output is also
increased.

These results are consistent with data showing increased
activity of key enzymes in the gluconeogenic pathway (Maley, 1957;
Tata et al. 1963) particularly pyruvate carboxylase (Böttiger et al. 1970).

Other studies have shown that although uptake of lactate and pyruvate is increased, hepatic glucose output is normal (Laker & Mayes, 1981) and it has been suggested that these metabolites are directed more towards oxidation than gluconeogenesis. In addition, recent studies by Bartels and Sestoft (1980) have suggested that gluconeogenesis from lactate in hyperthyroid rat livers is not increased when physiological concentrations of lactate (1 mmolar) in the perfusate are used whilst, when supraphysiological (10 mmolar) concentrations are used (as in previous studies) an increase is observed.

In the intact rat, isotopic kinetic studies have shown an increase in hepatic glucose output together with an increase in the rate of Cori cycle (glucose-lactate) activity (Okajima & Ui, 1979), suggesting a primary increase in gluconeogenesis. These studies have also shown increased futile cycle activity in the gluconeogenesis-glycolysis pathway at the glucose-glucose-6-phosphate and fructose-1-phosphate-fructose 1,6-diphosphate levels. Such increases in substrate/futile cycle activity provide a potential source of ATP utilisation and, may be partly responsible for thyroid hormone induced calorigenesis. These avenues have not been explored in hyperthyroid man. Splanchic balance studies have been performed (Wahren et al. 1981) and have shown that despite increased hepatic uptake of all the gluconeogenic precursors, suggesting increased gluconeogenesis, hepatic glucose production is reduced. This may have been due to diminished hepatic glycogen stores.

b)   Hypothyroidism

Fasting hypoglycaemia has previously been reported in

hypothyroid man, particularly those in myxoedema coma (Nickerson et al. 1960), but this is uncommon (Lamberg, 1965). Most studies in man have shown a 10-15% reduction in fasting blood glucose in hypothyroid subjects compared to controls (McDaniel et al. 1977; Lamberg, 1965; Levy et al. 1970; Shah et al. 1975; Aranda et al. 1972). Hepatic glucose production, assessed by isotopic kinetic studies, is normal in hypothyroid man (Saunders et al. 1980) but reduced in hypothyroid rats (Okajima & Ui, 1979). Few data are available concerning glycogen metabolism in hypothyroid man. In the intact hypothyroid rat, hepatic glycogen stores are normal (Battarbee, 1974) and the rate of depletion of glycogen stores in the perfused hypothyroid rat liver (Laker & Mayes, 1981) is also normal. Incorporation of glucose into glycogen however is reduced in hypothyroid rats (Okajima & Ui, 1979).

Similarly, little is known about gluconeogenesis in hypothyroid man, although gluconeogenesis from alanine has been shown to be normal in infants with congenital hypothyroidism (Hayek, 1979). In perfused rat liver studies, again conflicting results have been reported. Diminished glucose production from lactate has been reported (Menahan & Weiland, 1969) perhaps because of decreased pyruvate carboxylase activity (Weinberg & Utter, 1979). Laker and Mayes (1981) found that hepatic glucose production and glycogenolysis (and therefore, by implication, gluconeogenesis) were normal whilst hepatic uptake of lactate and pyruvate was diminished. They postulated that this decreased precursor uptake was the result of decreased

hepatic energy requirement. Glucose production from glycerol (Sestoft et al. 1977) may also be decreased in hypothyroidism. In the intact rat, isotopically determined hepatic glucose production is decreased as is Cori cycle activity (Okajima & Ui, 1979). This again suggests a decrease in gluconeogenesis.

ii) Fed Man

a) Hyperthyroidism

Intolerance to oral glucose has been reported in up to 57% of patients with hyperthyroidism (Krienes et al. 1965) depending on criteria used. A number of mechanisms have been proposed. Increased absorption of hexoses from the gastrointestinal tract (Althausen & Stockholm, 1938) and rapid gastric emptying (Holdsworth & Besser, 1968) have been suggested as causes of the early and exaggerated blood glucose responses seen during oral glucose tolerance tests. Alternatively, abnormalities of insulin secretion and/or action have been proposed. However, detailed measurements of plasma insulin levels during oral glucose tolerance tests have failed to clarify the issue. Insulin secretion has been found to be normal (Hales & Hyams, 1964; Holdsworth & Besser, 1968) increased (Field et al. 1966; Doar et al. 1969) or decreased (Levy et al. 1969; Andreani et al. 1970). Holdsworth and Besser (1968) also pointed out that although insulin release in hyperthyroid subjects may be comparable to that of controls, it may be insufficient in proportion to the blood glucose stimulus. It has been suggested that thyroid hormone excess may cause pancreatic damage (Holst, 1921). Such damage may be temporary after short term

exposure or permanent following prolonged exposure to thyroid

hormones (Houssay, 1946). More recent studies in the rat have

suggested that insulin secretory capacity is decreased in hyperthyroidism

(Lenzen et al. 1975) and that this may be the result of increased

β-adrenergic tone (Watchenberg et al. 1978). An alteration in the

pattern of insulin secretion has also been reported by Sestoft and Heding

(1981) in hyperthyroid subjects. They have demonstrated that a

larger proportion than normal of "insulin" measured by radioimmunoassay

both basally and during oral and intravenous glucose challenge may

in fact be proinsulin which cross reacts in the assay. Thus "true"

insulin secretion may be decreased in hyperthyroidism. In addition it

is conceivable that proinsulin may cause some degree of insulin

resistance by competing with "true" insulin for its receptor.

Insulin sensitivity has variously been reported as normal

(Elrick et al. 1961), decreased (Ikejiri et al. 1977; Doar et al. 1969)

or increased (Hagen, 1960; Elgee & Williams, 1955; Maracek et al.

1973). These studies have however based their conclusions on the

rate of fall of blood glucose concentrations following oral glucose or

intravenous insulin administration. No studies of insulin sensitivity

at physiological blood glucose levels and in the absence of counter -

regulatory hormone secretion have appeared in the literature.

Insulin clearance has been reported to be increased (Elgee &

Williams, 1955; Mariacek et al. 1973) or normal (Wajchenberg, 1978).

Increased insulin clearance would not however explain glucose

intolerance as insulin clearance is inextricably linked to insulin action

(Terris & Steiner, 1975).

Recently, suggestions have been made that glucagon may be

an important mediator of both fasting hyperglycaemia and glucose

intolerance in hyperthyroidism. Fasting plasma glucagon levels

have been reported to be increased with impaired suppression after

oral glucose (Kabadi & Eistenstein, 1980a). Insensitivity of the

pancreatic alpha cells has been proposed as the mechanism of these

phenomena, and support for this theory comes from the finding of an

impaired rise in plasma glucagon following protein feeding (Kabadi &

Eisenstein, 1980b). The reason for impaired alpha cell responsiveness

is unknown.

b)   Hypothyroidism

Blood glucose concentrations following oral glucose

challenge have been found to be decreased in hypothyroidism (Holdsworth

& Besser, 1968) as a result of a decrease in gastric emptying rate or

of decreased hexose absorption from the gastrointestinal tract

(Stockholm & Althausen, 1938). Glucose intolerance has also been

reported (Gutman et al. 1972; Wilder 1926; Jackson et al. 1970). The

available data regarding insulin secretion in hypothyroid man is

conflicting with low (Shah & Cerchio, 1973; Jolin et al. 1970), normal

(Hales & Hyams, 1964; Holdsworth & Besser, 1968) or increased

(Jackson et al. 1970) serum insulin responses to oral glucose challenge

being reported. The presence of normal or elevated blood glucose

concentrations with normal or increased plasma insulin levels suggests

insulin resistance (Holdsworth & Besser, 1968; Jackson et al. 1970;

Andreani et al. 1970; Elrick et al. 1961). Other studies have however

concluded that insulin sensitivity in hypothyroid man is normal, based

on a normal rate of development of hypoglycaemia during insulin stress tests (Iwatsubo et al. 1967; Brauman & Courvilain, 1968). These studies do not however take into account possible impairment of counter regulatory hormone responses which could also explain these findings. Conversely, the finding of normal or low serum insulin levels in the face of impaired glucose tolerance suggests hyposecretion of insulin (Shah & Cerchio, 1973; Malaise et al. 1967; Milcu et al. 1975). This may be a result of decreased insulin release (Malaise et al. 1967) or synthesis (Milcu et al. 1975). The pattern of insulin secretion, as regards insulin : proinsulin ratio, in hypothyroid man is unknown. The role of glucagon in carbohydrate metabolism in hypothyroidism has not previously been investigated.

(b)   Lipid Metabolism

     i)   Post absorptive man

     a)   Hyperthyroidism

Hyperthyroidism is accompanied by marked increases in circulating levels of non-esterified fatty acids (NEFA) (Rich et al. 1959; Harlan et al. 1963) and glycerol (Tibbling et al. 1969) during fasting. These findings suggest increased lipolysis and NEFA release from adipose tissue. In addition the turnover of both glycerol (Tibbling et al. 1969) and NEFA (Saunders et al. 1980) are increased in fasting hyperthyroid man. Not only is NEFA turnover increased but also more is oxidised as determined by increased production of $^{14}CO_2$ in expired air following intravenous ^{14}C-palmitate administration. In vitro experiments have also suggested that, even during fasting, triglyceride synthesis as well as breakdown is increased in the adipocyte (Fisher & Ball, 1967). The ATP requirement for reesterification of fatty acids

may account for the increased rate of oxygen uptake in vitro (Fisher & Ball, 1967). This increased rate of reesterification in hyperthyroid adipose tissue continues even under conditions with a high rate of lipolysis (Deykin & Vaughan, 1963). Thus lipolysis and reesterification constitutes a substrate cycle in adipose tissue which works at increased rate in hyperthyroidism.

The mechanism of this increase in lipolysis is probably multifactorial. Considerable attention has been focused on the interrelationship between catecholamines and thyroid hormones in the regulation of lipolysis. Infusion of catecholamines into hyperthyroid subjects leads to an enhanced lipolytic response (Harlan et al. 1963) as judged by the increase in circulating NEFA levels. The finding of decreased circulating catecholamine levels in hyperthyroid subjects (Christensen, 1973) lead to the hypothesis of increased 'sensitivity' to catecholamines as the underlying mechanism. Adipose tissue from hyperthyroid rats had been shown to demonstrate both increased adenyl cyclase activity (Brodie et al. 1966) and an increased lipolytic response to catecholamines (Debons & Schwartz, 1961; Fisher & Ball, 1967). In adipose tissue from hyperthyroid man, cyclic AMP levels are increased both basally and during incubation with adrenaline (6 μmol/1) and noradrenaline (6 μmol/1) (Arner et al. 1979). Basal glycerol release is normal but increases by 50% after incubation with the catecholamines. These results suggest an enhanced lipolytic response to catecholamines which may be due to increased β adrenergic sensitivity.

The failure of plasma NEFA and blood glycerol levels to suppress in hyperthyroid subjects following propranolol administration (Beylot et al. 1980; Saunders et al. 1980; Ortigosa et al. 1976) is therefore surprising but may reflect inadequate β blockade (the mean daily dose of propranolol in these studies was only 120 mg) or an additional α component (Arner et al. 1979).

An alternative explanation for increased lipolysis in hyperthyroidism may lie in a reduction of the peripheral effects of insulin (Wennlund et al. 1981). Using isolated segments of adipose tissue from hyperthyroid subjects, these authors have shown decreased sensitivity of adipocytes to the antilipolytic effects of insulin with a normal maximal response (indicating a right shift of the dose-response curve). They have also demonstrated a simultaneous and significant increase in lipogenesis again suggesting the possibility of enhanced futile cycle activity.

Glycerol released during lipolysis is taken up almost exclusively by the liver for gluconeogenesis and its turnover is proportional to its concentration in blood (Bortz et al. 1972).

In addition to reesterification, NEFA may also be oxidised peripherally in increased amounts (Saunders et al. 1980) or alternatively may be transported to the liver for further metabolism. Recent studies in hyperthyroid man have shown enhanced ketonaemia during prolonged fasting (Bartels et al. 1979; Beylot et al. 1980; Carter et al. 1975). This may be a consequence of increased hepatic NEFA supply (Hagenfeldt et al. 1981), enhanced hepatic ketogenic capacity or a

combination of these two processes. Enhanced hepatic ketogenic capacity has been demonstrated in isolated livers from thyrotoxic rats (Laker & Mayes, 1981; Keyes & Heimberg, 1979). It has been suggested that this ketogenic 'set' of the liver may be mediated by a β adrenergic mechanism (Beylot et al. 1980). Increased activity of carnitine acyl transferase (necessary for the transfer of long chain fatty acids into the mitochondria) has also been suggested as a mechanism (Van Tol, 1971). The rate of ketogenesis from medium chain NEFA, which is independent of carnitine acyl transferase activity, is not influenced by thyroid status (Bartel & Sestoft, 1980). Although production and utilisation of ketone bodies in normal man is proportional to ketone body concentrations in blood, no studies of ketone body kinetics in hyperthyroid or hypothyroid man have been made.

b) Hypothyroidism

In hypothyroidism, plasma NEFA levels have been reported as decreased (Harlan et al. 1963; Nikkila & Kekki, 1972) or normal (Hamburger et al. 1963; Saunders et al. 1980) with decreased (Lin 1977) or normal (Saunders et al. 1980) blood glycerol levels. NEFA turnover is normal with normal NEFA oxidation (Saunders et al. 1980). The interrelationship between thyroid status and catecholamines and their effects on lipolysis has also been examined within the context of hypothyroidism. Circulating levels of both adrenaline and noradrenaline are increased in hypothyroidism (Christensen, 1973). However, most studies have shown decreased lipolysis in response to

catecholamines both in vivo (Rosenqvist & Hylander, 1981) and in isolated human adipose tissue (Hamburger et al. 1963; Rosenqvist, 1972). These results suggest that hypothyroidism is associated with decreased sensitivity of adipose tissue to the β adrenergic effect of catecholamines. The underlying mechanism(s) is uncertain.

In vitro rat studies have also suggested that lipogenesis in adipocytes is also decreased (Bray & Goodman, 1968; Bates et al. 1955). The combination of decreased lipolysis and lipogenesis in the adipocyte may reflect decrease adipose tissue futile cycle activity in hypothyroidism.

Little is known concerning intrahepatic fatty acid metabolism in hypothyroid man. Perfused rat liver studies however have shown that hypothyroid livers oxidise and esterify fatty acids at normal rates (Laker & Mayes, 1981). These findings are consistent with the findings of normal fatty acid turnover in hypothyroid man (Saunders et al. 1981). Rates of production and utilisation of ketone bodies in hypothyroid man are unknown although in vitro rat studies have suggested that ketogenesis may be either increased (Laker & Mayes, 1981) or decreased (Keyes & Heimberg, 1979). Circulating blood ketone body levels in hypothyroid man after an overnight fast are normal (Saunders et al. 1980).

ii) Fed Man

a) Hyperthyroidism

Little data is available concerning the response of lipid metabolism to feeding in either hyper or hypothyroidism in man.

Following oral glucose, circulating NEFA levels fail to return to normal in hyperthyroidism (Ortigosa et al. 1976) suggesting that lipolysis may not be completely suppressed. This may be because of resistance to insulin action in the adipocytes of hyperthyroid subjects (Wennlund et al. 1981). These and other authors (Fisher & Ball, 1967) have demonstrated impaired insulin mediated suppression of glycerol release from hyperthyroid human adipocytes. It has been proposed that this decreased insulin action may be the result of decreased insulin sensitivity (either decreased receptor number or receptor affinity) as maximal responsiveness is normal.

Thyroid hormones are known to have "anabolic" effects on lipid metabolism, although these findings have not necessarily been obtained from feeding studies. In hyperthyroid man triglyceride concentrations in blood and triglyceride turnover are increased, although proportional clearance is decreased (Nikkila & Kekki, 1972). Decreased clearance may result from decreased post-heparin lipolytic activity in adipose tissue (Nikkila & Kekki, 1972; Arons et al. 1979). In the rat adipocyte, triglyceride synthesis may be increased (Fisher & Ball, 1967) or decreased (Roncari & Murthy, 1975) whilst in the rat liver triglyceride synthesis may be enhanced (Roncari & Murthy, 1975) or diminished (Laker & Mayes, 1981; Keyes & Heimberg, 1980). Other studies have suggested that although net triglyceride synthesis in hyperthyroid rat liver is decreased, hepatic triglyceride turnover is increased (Glenny & Brindley, 1978).

Evidence, again from animal studies, suggests that hyperthyroidism

is associated with increased fatty acid synthesis both in liver and
adipose tissue (Dayton et al. 1960; Diamant et al. 1972; Correze et al.
1977) with increased activity of both acetyl CoA carboxylase and fatty
acid synthetase (key enzymes in fatty acid synthesis) (Diamant et al.
1972). However, diminished activity of these two enzymes in the
adipocyte has also been reported in hyperthyroid rats (Roncari &
Murthy, 1975).

b) Hypothyroidism

In hypothyroid subjects, again plasma NEFA levels show
impaired suppression following oral glucose (Gutman et al. 1972).
The reason for this is uncertain but in the presence of diminished
peripheral glucose utilisation (Okajima & Ui, 1979) it may reflect
impaired reesterification of NEFA by hypothyroid adipocytes.

Circulating triglyceride concentrations in hypothyroid man
have been reported to be either increased (Nikkila & Kekki, 1972;
Kirkeby, 1968) or normal (Kutty et al. 1968). Triglyceride synthesis
in hypothyroid rat liver (Keyes & Heimberg, 1979; Laker & Mayes, 1981)
and rat adipocyte (Fisher & Ball, 1967) is normal and it has been
postulated that increased circulating triglyceride levels (above) may
result from diminished triglyceride clearance. Decreased post
heparin lipolytic activity may be an important factor (Nikkila &
Kekki, 1972; Porte et al. 1966). Similarly, raised blood levels of
cholesterol particularly in low density and very low density
lipoproteins (LDL and VLDL) (Ballantyne et al. 1979) may be the
product of normal production and diminished clearance (Walton et al.
1965).

(c)   Protein Metabolism

Available evidence suggests that thyroid hormones exert a biphasic effect on protein metabolism. Small amounts of thyroid hormones are necessary for protein synthesis whilst larger amounts induce overall protein catabolism (Hoch, 1974).

Skeletal muscle weakness and wasting are common features of the hyperthyroid state (Satayoshi et al. 1963; Fitch, 1968). Several studies have suggested that hyperthyroidism increases protein degradation in skeletal muscle in man (Carter et al. 1980; Bayley et al. 1981; Burman et al. 1979; Burini et al. 1981) and this may be a major factor in promoting muscle wasting. Thyroid hormones are known to increase lysosomal proteases in skeletal muscle (De Martino & Goldberg, 1978) and thyrotoxicosis is associated with creatinuria and negative nitrogen balance (Carter et al. 1975; Carter et al. 1977). Animal studies have suggested that thyroid hormones may play an essential role in the mobilisation of body proteins during starvation (Li & Goldberg, 1976) causing enhanced release of amino acids from muscle for use in gluconeogenesis. In prolonged starvation however, urine nitrogen excretion falls even in severely thyrotoxic rats. The basis of this inhibition of protein degradation during long term starvation is unknown.

In experimental hypothyroidism, depressed growth rates and depressed protein synthesis have been reported (Flaim et al. 1978) and these can be reversed by thyroxine (Goldberg et al. 1980). Decreased protein degradation has also been reported in hypothyroidism (Goldberg et al. 1980) and can be stimulated by thyroid hormones.

These sequential changes are paralleled by changes in muscle

proteolytic enzyme activity (De Martino & Goldberg, 1978; De Martino

et al. 1977).

4.4.   METHODS OF INVESTIGATING METABOLISM

Several different techniques have been employed to investigate

the effects of thyroid hormone excess and deficiency on intermediary

metabolism in man in this thesis.   This section is intended to give

an overall assessment of the advantages and limitations of these

techniques which will be described in detail in the appropriate

chapters.

(a)   Diurnal Hormone-Metabolite Profiles

This method has been used in chapters 7 and 8.   It provides

data concerning the blood levels of various hormones and metabolites

after an overnight fast and during a 12 h period of normal meals and

activity.   Measurement of the concentration of a substrate in blood is

an indirect method of assessing its metabolism.   Blood is easily

accessible and the method simple.   The concentration of a substrate

in blood is the balance between its rate of production and utilisation

and changes in substrate concentrations may reflect changes in either

of these processes.   For some substrates, during health, conconcentration

in blood is proportional to turnover (glycerol [Tibbling, 1969],

nonesterified fatty acids [Saunders et al. 1980]) but for many

important metabolites this relationship does not hold.   The proportional

relationship between turnover and blood concentrations may also not

hold in disease states.   However, this type of integrated approach may

highlight areas of abnormal metabolism (i. e. meal intolerance) which

merit further investigation. Although mechanisms of production of

abnormalities may be suggested (elevated blood glucose concentrations

with elevated plasma insulin suggesting insulin resistance) specific

studies must be designed to test these hypotheses.

(b)   Tolerance (clearance) Tests

This type of test involves the administration of a substrate load

directly (intravenously) or indirectly (orally) to the substrate pool. The

substrate can be given directly as a bolus and the subsequent clearance

assessed by the decline in concentration in serial blood samples. This

may allow assessment of metabolic clearance rate (MCR) back to basal

values. (MCR = Dose administered/ $\int_{0}^{\infty} \Delta$ [substrate] dt). Substrate

can also be directly administered as a continuous infusion until a

steady equilibrium concentration is achieved when the infusion rate is

equal to the rate of utilisation. Metabolic clearance then equals infusion

rate/(Equilibrium concentration - basal concentration). These techniques

are relatively simple and are safe. They give information concerning the

rate at which added substrate is removed and reveal more about maximal

metabolic capacity rather than subtle alterations in regulatory processes.

Interpretation of data obtained using these studies must be made

with caution. Administration of a substrate load may alter its metabolism

by several methods. For example, infusion of ketone bodies (Bradley

et al. 1981) may alter ketone body utilisation (Reichard et al. 1974)

simply because of increased blood concentrations, by inducing insulin

secretion (Balasse & Neef, 1975; Madison et al. 1964; Miles et al. 1981),

by directly inhibiting lipolysis (Bjorntop, 1966) and also ketone bodies

may be lost in the urine. Also no direct quantification of ketone body

or other substrate production can be made from this type of experiment

although it can be inferred from clearance rates and basal concentrations.

Of the oral tolerance tests, the most commonly used in clinical medicine

is the glucose tolerance test (GTT). The GTT is used primarily as a

screening and diagnostic procedure for diabetes mellitus. It has several

drawbacks amongst which is relatively poor reproducibility (McDonald

et al. 1965). Despite its long usage there is no universally accepted

way of standardising the test methodology or the manner of evaluating

test results (Meinert 1972; Duffy et al. 1973; Billewicz 1974). In

addition, the GTT is highly unphysiological and bears little resemblance

to carbohydrate tolerance as it affects individual subjects from day to

day. Free glucose is not present in normal food and ingestion of

50-100 g of carbohydrate without any accompanying protein or lipid is

extremely rare in normal life. For clinical investigation 'meal'

tolerance tests therefore provide a more physiological model for

assessment of metabolic abnormalities produced by hormone excess

or deficiency (Le Febvre & Luyckx, 1976; Owens et al. 1979;

Lenner, 1976).

(c)   Catheterisation Techniques including "Arterialisation" of
         Venous Blood

The above methods for investigating metabolism give no

direct measurements of production or utilisation of a substrate by any

particular organ or tissue. By placing a catheter in the artery

serving and the vein draining a particular organ or tissue the net

amount of substrate produced or utilised can be determined. A

measurement of arterial and venous substrate concentrations together

with an estimate of blood flow through the organ or tissue is required

and the production or utilisation is given by the expression;

Production/Utilisation = A-V difference x Blood Flow

The technique of arterial-venous sampling has been successfully

applied to determine metabolic balance across human forearm

(Hagenfeldt & Wahren, 1968; Foster et al. 1972; McGuire et al. 1976).

However, arterial puncture is not always successful and is associated

with a small but significant morbidity (Machleder et al. 1972;

Campion et al. 1971). An alternative method is retrograde blood

sampling from 'arterialised' hand veins where local heat increases

blood flow whilst, because the hand contains little muscle, oxygen and

substrate uptake are not significantly increased. A method for this

technique is described in this thesis. Arterialisation may assume

particular importance in determining whole body turnover of substrates,

particularly ketone bodies (Keller 1981). Venous sampling reflects

local exchange and may thus lead to an overestimate of whole body

turnover. A similar technique has recently been validated for glucose

and alanine kinetics (Adumrad et al. 1981).

Alternatively, knowledge of splanchnic metabolism of substrates

may be obtained using catheters in the hepatic vein and an artery

(Hagenfeldt et al. 1981; Wahren et al. 1981). This technique is invasive

and ethical considerations limit its application. There are also

technical difficulties with sampling. Streaming of blood may occur

within the hepatic vein such that, if the catheter is not perfectly sited, samples may not reflect splanchnic balance accurately. Also, estimatic of splanchnic blood flow, frequently estimated using dyes such as indocyanine green (Hagenfeldt et al. 1981) is fraught with difficulty and misleading results may be obtained.

(d)   Isotope Dilution Studies

The precise definition of disorders of carbohydrate and lipid metabolism together with the elucidation of the homeostatic mechanisms that maintain circulating concentrations of glucose, glycerol and NEFA and ketone bodies are areas in which isotope turnover studies have played a significant role.

i)   Tracer considerations

An ideal tracer should have the following properties:

1)   It should be negligible in quantity with respect to tracee (so that the administration of tracer will not of itself produce changes in the system under study).

2)   It should be similar to the tracee in all physical, chemical and biological properties such that its metabolism would be identical with that of the tracee.

3)   Where radioactive isotopes are used, they should not be sequestered in critical portions of cells as is thymidine (Quastler & Sherman, 1959) or in critical organs as is radioiodine in the thyroid (Baumann 1895) and the radiation dose should not constitute an unreasonable risk to the subject. Cumulative dose experiments following the administration of such compounds as ^{14}C-glycine (Berlin et al.

1973) together with shorter term estimates of $^{14}CO_2$ excretion following administration of such compounds as ^{14}C glucose and lactic acid (Searle, 1976) indicate that those considerations can be reasonably met with ^{14}C and ^{3}H labels used in the study of carbohydrate and lipid metabolism in man.

ii) Tracer Assay

In dealing with metabolising systems it must always be remembered that the instant a tracer is introduced into the system, it becomes subject to all the biological transformations the tracee is undergoing. The primary measurement in turnover studies involves, for the single injection experiment, the rate of loss of tracer and for the continuous infusion experiment the degree of dilution of tracer by tracee. A prime consideration then is that the assay for tracer activity or specific activity is measuring the activity (^{14}C, ^{3}H-common radioactive labels; or ^{13}C, ^{2}H, - common stable isotope labels) that is only associated with the injected tracer metabolite. For example, in the measurement of glucose and/or lactate turnover rates in man, the injected glucose or lactate tracers are rapidly interconverted in appreciable quantities. It therefore becomes necessary to isolate physically those metabolites one from another, and from other circulating compounds into which their carbon skeletons may be transformed prior to any assessment of isotopic content. Techniques which have been used for the physical separation of glucose from its circulating metabolite derivatives have included ion exchange chromatography to exclude charged molecules (Reichard et al. 1963),

IRREVERSIBLE TRACER

$$^3H-3 \text{ Glucose} \xrightarrow{\quad ^3H_2O \quad} \text{Lactate} \longrightarrow CO_2 + H_2O$$

REVERSIBLE TRACER

$$^{14}C-1-\text{Glucose} \xrightarrow{\quad H_2O \quad} {}^{14}C \text{ Lactate} \longrightarrow {}^{14}CO_2 + H_2O$$

FIGURE 4:8.   Recycling of radioactive label illustrated by the use
of the reversible tracer ^{14}C-1-glucose.

and isolation of specific chemical derivatives of glucose such as
the phenyl glucose osazone (Searle & Chaikoff, 1952), the osotriozole
(Steel et al. 1957) and the potassium salt of gluconic acid (Blair &
Segal, 1960). Hydrogen labels on the glucose molecule (Katz et al.
1974) are metabolised to water and may be excluded from specific
activity assay by a much simpler technique, i.e. by freeze drying.

    iii) Recycling of Label (Fig. 4:8)

        As already described, glucose and lactate are constantly
being interconverted. This cyclic phenomenon begins with the
metabolism of glucose via the Embden-Meyerhoff pathway and
terminating with the synthesis of glucose from lactic acid is
formally known as the Cori cycle (Cori 1933). In addition to the
problem of assaying tracer activity that is associated with and only
with the tracer molecule, it is necessary to confirm that the tracee
entering the system does not contain label that would distort the
accuracy of the measurement. These problems arise if estimates of
turnover are made with carbon labelled glucose. When hydrogen
labelled glucose is used the label is oxidised to water as the glucose is
metabolised. This not only simplifies the assay of isotope associated
with tracer molecule but also eliminates recycling (Katz et al. 1974).
If however, an estimate of Cori cycle activity is required, the
combination of carbon (reversible) and hydrogen (irreversible)
glucose tracers can then be used, differences in determined turnover
rates being due to recycling of carbon label (Streja et al. 1977).

iv) Stable versus Radioactive Isotope Tracers

The stable isotopes of ^{13}carbon and other elements have been available in limited quantity for many years and indeed their use in the biological sciences predates the use of ^{14}carbon. Until recently, little use has been made of stable isotopes as tracers. Firstly they are difficult and expensive to prepare; secondly, instrumentation necessary for their detection and assay (not widely available) has been expensive and difficult to maintain; and thirdly, until recently the detection of stable isotope tracers has been considerable less sensitive than those available for detecting radioactive tracers. This latter problem has meant that the amount of "tracer" injected in previous studies may have perturbed the system under investigation (Bier et al. 1973; Shreene, 1973). Countering all these arguments is the advantage that they are not radioactive. For studies in children and pregnant women and repetitive studies in any subject they are the only method of choice. Recent advances in methods of detection of the stable isotopes and their increased availability (Haig et al. 1981) offers the hope that they will be increasingly used in clinical medicine and research.

v) Experimental Techniques

1. Steady state systems

Input equals output (Ra = Rd) and the concentration of substrate in the sampling pool remains constant over the period of the study in a steady state system. Two basic experimental techniques for the study of turnover have evolved over the years.

1) Single injection (bolus) technique

A simple injection of isotopically labelled substrate (tracer)

is administered at zero time of the experiment. Subsequent analysis of blood or plasma samples collected at specific time intervals thereafter provides data relative to the time course of the decay in the specific activity (ratio of tracer concentration to that of tracee, c.p.m./mg or atom per cent excess) of the tracer as shown in Figure 4:9. For glucose kinetics, the area under the glucose specific activity (SA) decay curve can be computed and knowing the quantity of tracer injected, glucose turnover may be calculated:

$$\text{Glucose turnover} = \frac{\text{c.p.m. injected}}{\int_{0}^{\infty} \text{glucose SAdt}}$$

$$= \frac{\text{cpm}}{\text{cpm/mmol glucose/min}}$$

$$= \text{mmol/glucose/min.}$$

The derivation of this formula is as follows (Fig. 4:10). If a dose of tracer (D^*) is injected into a pool with constant appearance (Ra) and disappearance (Rd) of tracer (i.e. a steady state system) from which glucose concentration and radioactivity (y^*) can be measured, the tracer will be removed from the pool at the same rate as the tracee (Rd). The dose of tracer injected must equal the total amount of tracer removed in time;

$$D^* = \int_{0}^{\infty} Rd \; \frac{y^*}{[\text{glucose}]} \; dt$$

$$= Rd \int_{0}^{\infty} SA \; dt$$

$$Rd = \frac{D^*}{\int_{0}^{\infty} SAdt}$$

64.

FIGURE 4:9.   Schematic representation of a glucose specific
activity time curve following a simple bolus
dose of tracer activity.

FIGURE 4:10. Schematic representation of a glucose pool to
which a quantity of tracer (D^*) is administered.
Ra = rate of appearance of substrate,
Rd = rate of dissappearance. [glucose] =
concentration of glucose and y^* = c. p. m. in
a sample taken from the glucose pool.

$$Rd = Ra = Rt = \frac{D^*\ (cpm)}{\int_0^\infty SAdt}$$

where Rt = glucose turnover.

Several methods can be used to measure further kinetic parameters, e. g. glucose **pool**, mean residence time and volume of distribution. Pool size may be calculated from the known quantity of tracer injected and the zero time intercept of the final slope of the course, thus

$$POOL = \frac{cpm}{cpm/mmol} = mmol \quad (Searle, 1976)$$

The volume of distribution occupied by the substrate pool (an apparant volume) may then be obtained by dividing the mass of the pool by its concentration at the sampling site (usually blood or plasma)

$$SPACE = mmol/mmol/l = litres$$

This quantity is usually represented as a fraction of the body weight. Alternatively pool size may be estimated by the methods of Shipley and Clarke 1972.

$$\text{MEAN RESIDENCE TIME (MRT)} = \frac{\int_0^\infty (t \times SA)\ dt}{\int_0^\infty SAdt}$$

$$POOL = RT \times MRT$$

$$\text{VOLUME OF DISTRIBUTION} = \frac{POOL \times 100}{BODY\ WT \times [GLUCOSE]}$$

Single injection techniques have the advantages of being shorter and thus the steady state is more likely to be maintained throughout. Errors may however arise because interpreting the data requires extrapolation of the SA decay curve to time $(t) = 0$ and to $SA \rightleftharpoons 0$. Accuracy of the method is thus affected by the number and weighting

of the points on the curve. Accuracy will be improved if a large number of samples are taken at the rapidly changing points on the specific activity decay curve (Hetenyi & Norwich, 1974).

2.   Continuous Infusion Techniques

A continuous infusion of tracer (with or without a priming dose) is administered throughout the experiment. Analysis of blood or plasma samples taken over the period of the experiment provide the data relative to the time course of the establishment of an asymptotic tracer specific activity curve, (as shown in Fig. 4:11.) from which, as in the case of the single injection technique, pool size, space and turnover rate may be calculated. Fig. 4:11a represents a continuous infusion without a priming dose. Turnover rate may be calculated directly from the final asymptotic portion of the curve and the known rate of isotope infusion.

$$\text{Turnover rate} = \frac{\text{cpm infused/min}}{\text{cpm/mmol}} = \frac{\text{mmol}}{\text{min}}$$

Then, by extrapolating the asymptote to zero time and subtracting the ascending specific activity from that value, one can construct a curve $(T\frac{1}{2})$ that describes the turnover characteristics of the system. The remaining indices of the system, pool size and space can be determined taking turnover rate and substrace concentration into account. An alternative method which considers the dilution kinetics of the priming dose associated with a continuous infusion procedure may be employed (Wall et al. 1957) Fig. 4:11b. Errors in determining volume of distribution may still occur from extrapolating SA to t = 0.

68.

FIGURE 4:11(a). Schematic representation of the plasma substrate
specific activity time curve during a continuous
infusion of tracer activity. Decay curve labelled
$t\frac{1}{2}$ derived from specific activity data as
described in the text.

FIGURE 4:11(b). Schematic representation of a plasma substrate
specific activity time curve during a primed
continuous infusion of tracer activity.

3. Non-steady state systems

Input is greater than or less than output and in such a system the concentration of tracee, by definition, must change. The most commonly used method for estimating Ra and Rd in these circumstances is the primed continuous infusion technique as described above. This method however assumes that steady state conditions are observed for at least the first 90-120 minutes of the infusion while steady state tracer values are achieved (i.e. the method is not completely non-steady state). Changes in Ra and Rd are then predicted by the formulas

$$Ra = \frac{I - pV \left(\frac{[SA_1 + SA_2]}{2} \times \frac{\Delta C}{\Delta t} \right)}{\frac{1}{2}(SA_1 + SA_2)}$$

$$Rd = Ra - \left(pV \, \frac{\Delta C}{\Delta t} \right)$$

where
I = infusion rate of tracer

p = pool fraction

V = volume of distribution

c = concentration of tracee

ΔC = change in tracee concentration between sample times $(t_2 - t_1)$

ΔSA = change in specific activity between sample times $(SA_2 - SA_1)$

Δt = $t_2 - t_1$

Note that these formulae relate the combined observations for C and SA to predict changes in Ra and Rd.

The effect of non-steady state systems on tracer kinetics is best conceptualised by considering a single pool undergoing constant infusion of tracer. The pool contains unlabelled solute in continually varying amount, Q, dissolved in water occupying an assumed constant

volume. Thus as Q changes, the concentration (C) changes proportionally. Assume initially that the system is in steady state until a plateau is reached. If the rate of input of solute is then reduced abruptly, one may predict that C will fall (thus also Q) and SA will increase. If input rate were increased above output, the reverse effect would be expected. If a constant input rate is now assumed but output rate is reduced, SA will not change but C will increase. An increase in output rate likewise will not affect SA, however C will decline. Thus the combined observations for C and SA versus time should predict a rise or fall in either input or output rate.

Note that where SA and C are constant, Ra (i. e. the steady state exists), RA predicted by these formula

$$= \quad \frac{I}{\frac{1}{2}(SA_1 + SA_2)} \quad = \frac{I}{SA} \quad \left(\equiv \quad \frac{D^*}{\int_0^\infty SA dt} \quad \right)$$

and Ra = Rd (i. e. the same formulae for steady state kinetics).

The methods described for investigating steady and non steady state kinetics make no assumptions about the number of compartments into which tracee equilibrates (i. e. they are non-compartmental analyses), and have been commonly used for the investigation of glucose (Streja et al. 1977; Reichard et al. 1963) and ketone body (Keller et al. 1981; Balasse & Neef, 1975) kinetics in man.

vi)   Ketone Body Kinetics - the growing debate

Assessment of the kinetics of ketone body metabolism by isotope experiments poses problems since acetoacetate (Acac) and β hydroxybutyrate (BOH) are interconverted too rapidly to be considered

as independent substrates but too slowly for their specific

radioactivities to become equal following the administration of either

$3-^{14}C$-Acac or $3-^{14}C$-BOH (i. e. isotopic disequilibrium occurs). All

investigators (Balasse, 1978, 1979; Keller et al. 1978, 1981) except

Barton (1976, 1980) and Bates (1971) have attempted to circumvent

this problem by using the combined SA of Acac and BOH to quantify

total ketone body (TKB) turnover. It is now not clear whether this

approach is valid.

It has recently been suggested that this method may lead to

incorrect estimates of disposal and plasma clearance rates for ketone

bodies. It has been proposed by Barton (1980) that either separate

injections of ^{14}C-Acac and ^{14}C-BOH on separate occasions or

simultaneous administration of ^{14}C-Acac and ^{3}H-BOH could provide a

more accurate means of estimating the individual plasma clearance

rates and more generally, the kinetics of the two ketones. BOH can be

tritiated in either the 2 or 4 position. However, when converted to

acetoacetate (either in vivo or during the assay procedure) tritium

labels in either of those positions become unstable and may interchange

with hydrogen ions in any aqueous medium. Using tritiated BOH as

tracer would thus yield misleading results (Nosadini & McCulloch -

Unpublished observations). Administration of ^{14}C Acac on separate

occasions to the same subjects was therefore performed in this thesis.

Using the data from these experiments, Cobelli and coworkers

(Cobelli et al. 1982) have described a 4 compartment model of ketone

body kinetics. The principal drawbacks with this method are

a) two experiments must be performed on each subject to complete one

study and b) the subject must be in the same metabolic condition during each experiment, particularly with regard to ketone body concentrations in blood. Several studies have proved unsuitable because of this latter prerequisite.

Since the 4 compartment model has been entirely developed by others, the data presented in this thesis will be those derived using the combined specific activities of Acac and BOH.

(e)   Assessment of insulin sensitivity in man

Many of the metabolic abnormalities noted in hyper and hypothyroid subjects may be related to sensitivity or resistance to endogenous or exogenous insulin. There is however marked variability in the reported findings which may be related to experimental design.

The most common method for assessing insulin sensitivity in man is the insulin stress test. This test involves the administration of exogenous insulin and subsequent analysis of the rate of fall of blood glucose or the nadir of blood glucose achieved (Abrams et al. 1934; Fraser et al. 1941; Himsworth & Kerr, 1939). Such tests may evoke unpleasant or even dangerous symptoms of hypoglycaemia and certainly invoke the release of counter-regulatory hormones including catecholamines, cortisol, glucagon and growth hormone. The use of relatively high doses of insulin may also complicate interpretation of the results (Norgaard et al. 1929). Alternatively the rate of clearance of a glucose load may be used as an index of insulin sensitivity (Heller et al. 1968) but such tests may induce unphysiologically high blood glucose values and glycosuria. In addition, possible insulin

hyposecretion or the secretion of biologically inactive insulin may confuse the results. Recently, the euglycaemic clamp technique has been developed and applied to several disease states (Olefsky & Kolterman, 1981; De Fronzo et al. 1979). Two basic methods of glucose clamping have evolved, although the underlying principals of the two methods are similar. In both, insulin is infused intravenously to achieve steady state insulin levels in blood. Glucose is infused to maintain blood glucose at basal values. The amount of glucose (M) infused is taken to be equal to the amount of glucose metabolised and is used as the index of insulin sensitivity.

The first method uses a closed-loop feed back glucose-controlled glucose infusion by means of the Miles biostator (Artificial Pancreas). The biostator is programmed to deliver glucose in response to a fall in blood glucose values and to maintain blood glucose at the selected (basal) value (Home et al. 1981). Blood glucose values are continuously monitored using a glucose oxidase/oxygen electrode.

The second technique in common use is that developed by De Fronzo (De Fronzo et al. 1979). Again insulin is infused exogenously but blood glucose is monitored intermittantly (every 5 minutes) by any rapid method. Blood glucose is maintained constant by infusion of glucose the rate of which is altered to maintain euglycaemia as guided by a pre-set formula which takes account of direction and rate of change of blood glucose values and body weight. Both types of clamp measure glucose in "arterialised" blood. The latter technique has the advantage that it can be performed without a biostator and is therefore considerably cheaper.

A further technique for assessment of insulin sensitivity has recently been described (Reaven et al., 1977). This technique involves the quadruple infusion of adrenalin, propranolol, insulin and glucose. Adrenalin is administered to inhibit endogenous insulin secretion by its α stimulating effects and the β blocker to inhibit the β effects of the infused adrenalin. Insulin and glucose are infused in fixed amounts and the index of sensitivity is the final steady state plasma glucose (SSPG) achieved. The lower the SSPG, the greater the insulin sensitivity. The infusion of insulin and propranolol may have direct effects however on the sensitivity of peripheral tissues to insulin and the technique does not allow quantification of endogenous glucose production as the other techniques do when combined with administration of labelled glucose.

(f)   Animal Models (Isolated Hepatocyte Techniques)

Although extrapolation of results achieved using animal studies to the situation in man may be misleading, certain types of experiments for ethical reasons can only be performed using animal tissues. The liver occupies a central position in body metabolism and its size, softness and relative homogeneity have made it a favourite organ for biochemical investigation. For the study of intact liver functions under controlled conditions, perfused rat liver preparations have been extensively used. This experimental system is excellent for many purposes but has several major shortcomings: (1) the liver as a organ is not completely homogenous containing up to 40% non parenchymal

cells (Daoust, 1958); (2) it is difficult to test experimental treatments simultaneously; and (3) the viability of an isolated liver can be maintained only for a limited period of time. Numerous attempts have been made to overcome these problems by the isolation and purification of intact parenchymal rat liver cells.

The early mechanical and chemical methods for liver cell preparation were relatively successful in converting liver to a suspension of isolated cells, but unfortunately, nearly all such cells were damaged. The introduction of collagenase as a liver dispensing enzyme (Howard et al. 1967) greatly facilitated the preparation of intact cells and the use of physiological liver perfusion to make the tissue uniformly accessible to the action of collagenase (Berry & Friend, 1969) made it possible to prepare intact liver cells in high yield. Isolated parenchymal cells are now increasingly being used as an experimental tool and the method of preparation is described in detail in the relevant chapter. It should be pointed out however that cell function in such preparations may not be identical with cell function in the intact liver model where cells are intimately related, one to another. Isolated cells are exposed on all sides to incubation medium, unlike the situation in perfused liver studies.

In this thesis the use of the hepatocyte method to investigate ketogenesis from palmitic acid is particularly appropriate. Changes in ketogenesis may occur because of events in adipose tissue or events within the liver. The observation of increased ketogenesis in hyperthyroidism (Beylot et al. 1980; Bartels & Sestoft, 1979) in man

gives no information about the role the liver may play in such a process. Experiments using isolated animal tissues are required to resolve this question.

5. APPROACHES

5.1.   Diurnal hormone-metabolite profiles have been examined in

hyper and hypothyroid subjects before and during appropriate therapy

and in matched controls.

5.2.   Glucose turnover (assessed using ^{3}H-3 glucose as tracer)

and recycling from glucose derived 3-carbon intermediates

(assessed by analysis of decay of specific activity of simultaneously

administered ^{14}C-1-glucose) has been examined in hyperthyroid and

hypothyroid subjects before and during therapy and in matched

controls.

5.3.   Gluconeogenic capacity from glycerol has been assessed in

hyper and hypothyroid subjects and controls by analysing clearance

of glycerol (0.1 mmol/kg) administered by bolus intravenous

injection.

5.4.   Insulin sensitivity has been assessed in hyperthyroid and

control subjects using a euglycaemic clamp technique by means of

the biostator (Miles) ("artificial endocrine pancreas").

5.5. Ketone body kinetics have been assessed in hyperthyroid and

hypothyroid subjects before and during therapy and in controls by

analysis of decay of specific activity of ^{14}C-3-hydroxybutyrate and

^{14}C acetoacetate.

5.6. The effects of thyroid hormones on ketogenesis by isolated rat

hepatocytes have been assessed by

a) incubating hepatocytes from normal fed and starved rats

with 10^{-8}M triiodothyronine in the presence and absence of 1mM

palmitic acid substrate.

b) incubating hepatocytes from normal and thyrotoxic fed

and starved rats in the presence and absence of 1 mM palmitic acid

substrate.

c) measuring blood concentrations of ketone bodies,

intermediary metabolites and insulin in fed and starved normal and

thyrotoxic rats.

6.   MATERIALS AND METHODS

Only those methods or techniques common to most or all

experiments are described here.   Special techniques are described in

detail in individual results chapters (glucose turnover, ketone body

turnover, glucose clamping, isolated hepatocytes etc.) (see Declaration).

6.1.   CANNULAE

17 and 19 gauge teflon cannulae (Venflon, Viggo, Helsinborg)

were used in all experiments and were inserted intravenously using

1% lignocaine as local anaesthetic.

6.2.   METABOLITES

i.   Collection

1-2 ml. blood for glucose, lactate, pyruvate, alanine,

glycerol, 3-hydroxybutyrate and acetoacetate were taken into 5 ml.

chilled 5% perchloric acid for deproteinisation.   Samples were

centrifuged immediately, separated and frozen at -20°C.   All samples

were analysed within 24h.

Blood for non-esterified fatty acid estimation was taken into

lithium heparin tubes, centrifuged, separated and the plasma frozen at

-20°C until assayed.

ii.   Assay

For metabolites a-f below, enzymatic fluorimetric

continuous flow assays were used on Technicon Autoanalyser II

Equipment (Technicon Instrument Co. Ltd. , Basingstoke, Hants, U.K.)

(Lloyd et al. , 1978).

These assays depend on the change in NAD/NADH or NADP/

NADPH measured fluorimetrically at 340 n metres. The specific

reactions are listed below.

a)   Glucose

Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose-6-Phosphate + ADP

Glucose-6-Phosphate + NADP + $\xrightarrow{\text{G6PD}^{*}}$ 6-Phosphogluconate
+ NADPH + H^{+}

* Glucose 6-phosphate dehydrogenase

b)   Lactate

Lactate + NAD^{+} $\xrightarrow[\text{Dehydrogenase}]{\text{Lactate}}$ Pyruvate + NADH + H^{+}

c)   Pyruvate

Pyruvate + NADH $\xrightarrow[\text{Dehydrogenase}]{\text{Lactate}}$ Lactate + NAD^{+}

d)   Alanine

L-Alanine + NAD^{+} $\xrightarrow[\text{Dehydrogenase}]{\text{L-alanine}}$ Pyruvate + NAD^{+}

e)   Glycerol

Glycerol + ATP $\xrightarrow[\text{kinase}]{\text{Glycerol}}$ L-Glycerol-1-Phosphate + ADP

L-Glycerol-1-Phosphate + NAD^{+} $\xrightarrow[\substack{\text{Phosphate} \\ \text{Dehydrogenase}}]{\text{Glycerol-3-}}$ Dihydroacetone Phosphate + NADH

f)   3 Hydroxybutyrate

D-(-) 3 Hydroxybutyrate + NAD^{+} $\xrightarrow[\text{Dehydrogenase}]{\text{3 Hydroxybutyrate}}$ Acetoacetate + NADH

Within batch and between batch coefficients of variation were between

0.4 and 4.4% for all these metabolites except 3-hydroxybutyrate where

CV's were higher at lower concentrations.

Acetoacetate in blood was measured using a kinetic spectrophotometric

method (Price et al. 1977) which allows low concentrations of

acetoacetate to be measured with good precision using a reaction rate

analyser (LKB 8600, LKB Instruments, Addington Road, Croydon,

Surrey). The reaction (which is monitored for 60 seconds) is shown below.

g) Acetoacetate + NADH $\xrightarrow[\text{Dehydrogenase}]{\text{3 Hydroxybutyrate}}$ 3 Hydroxybutyrate + NAD

Acetoacetate is particularly unstable and if left at room temperature

in PCA extracts, 60% will decarboxylate to acetone within 24 h. Samples

were frozen at -20°C and assayed within 24h when expected loss is

less than 5%. Within and between batch variation ranged from 4-6%

in PCA.

h)   Non esterified fatty acids (NEFA)

Plasma non esterified fatty acids were measured by a modification

of the method of Ho and Meng, described by Turnell et al. 1980. This

method was a Dole type extraction with formation of ^{57}Cobalt soaps.

Between batch coefficient of variation at 1.12 mmol/l was 7%.

6.3.   HORMONES

i.   Collection

Blood for insulin, growth hormone, c.-peptide, thyroxine,

triiodothyronine and thyroid stimulating hormone were taken into plain

glass tubes. The serum was separated and frozen at -20°C until

assayed. For plasma glucagon 2.25 ml blood was taken into tubes

containing 2500 kiu trasylol and 9.3 mg ethylendiaminetetracetate

(EDTA) in 0.25 ml. Samples were centrifuged and separated

immediately and stored at -20°C until assayed.

ii.   Assays

a)   Insulin

Serum insulin was measured using a double antibody

radioimmunoassay technique (Soeldner & Stone, 1965). Within and between batch variation were 5% and 12-14% respectively.

b) Glucagon

Plasma glucagon was measured by a double antibody radioimmunoassay employing wick chromatography (Ørskov et al. 1968). Between batch CV for this assay is 9%.

c) Growth Hormone

Serum growth hormone was measured by double antibody radioimmunoassay based on the method of Hartog et al. (1964).

d) C-peptide

C-peptide concentrations were measured by radioimmuno-assay with ethanol precipitation using a 'kit' supplied by Novo Industries, Copenhagen. Intraassay CV for this method is 7%.

e) Thyroxine

Serum thyroxine was measured by radioimmunoassay using a commercially available kit (Malinkrodt, RIA-MAT$^{(R)}$ T$_4$).

f) Triiodothyronine

Serum triiodothyronine was measured by a double antibody radioimmunoassay method described by Hesch & Evered (1973).

g) Thyroid Stimulating Hormone

Serum thyroid stimulating hormone was measured by double antibody radioimmunoassay (Hall et al. 1971).

6.4.   STATISTICAL ANALYSES

Statistical analyses were performed using Students paired and unpaired t-tests as appropriate. Measurements which were normally

distributed are shown in the text as mean $\pm$ standard error of the
mean (SEM). Certain measurements, ketone bodies, insulin and
glucagon were log normally distributed and statistical analyses were
therefore performed on log transformed data. These measurements
are shown in the text as mean and range. Correlations were sought
by the method of least squares or by the Spearman ranking method
as appropriate.

6.5. INFORMED CONSENT AND ETHICAL APPROVAL

Oral consent was obtained from all patients and control
subjects after full explanation of the aims, procedures and risks
involved in each study. The patients general practitioners were
informed by letter of any patients inclusion in a study and an entry was
made in the patients case records.

All studies had the approval of the Ethical Committee of
Newcastle Area Health Authority (Teaching). Where radioisotopes
were used, study design conformed with the regulations set down by
the DHSS Isotopes Advisory Panel.

7.   DIURNAL METABOLIC PROFILES IN HYPERTHYROIDISM

7.1.   INTRODUCTION

Numerous, often conflicting, metabolic abnormalities have been

described in hyperthyroid man.   Fasting blood glucose concentrations

have been reported to be normal (Kabadi & Eisenstein, 1980;

Saunders et al. 1980) or elevated (Doar et al. 1969; Levy et al. 1970)

with normal (Macho, 1958) or abnormal tolerance to oral (Hales &

Hyams,1964; Marks et al. 1960) or intravenous glucose (Doar et al.

1969).   Peripheral insulin responses to oral glucose have also been

described as normal (Andreani et al. 1970), enhanced (Doar et al. 1969)

or impaired (Malaisse et al. 1967).   Plasma non-esterified fatty acid

(NEFA) concentrations (Doar et al. 1969; Harlan et al. 1963) and

turnover (Sanders et al. 1980) may be increased with enhanced ketosis

during starvation (Beylet et al. 1980).   A fuller account of metabolic

abnormalities is given in the Introduction (4.3.).   Little is known however

about the effects of thyroid hormone excess on hormone and

intermediary metabolite levels during fasting and following mixed meals.

The purpose of this study was to investigate these effects in

hyperthyroid subjects before and after treatment and in euthyroid

controls.

7.2.   SUBJECTS AND METHODS

Subjects

Nine (eight female, one male) patients with hyperthyroidism

were compared with sixteen (thirteen female, three male) normal

controls (Table 7:1). No subject had a personal or family history of

diabetes and none was on any medication at the time of the initial study. Patients had Graves' disease on the basis of clinical features, such as ophthalmopathy, and an absence of thyroid nodules on ^{99}Tc scanning.

Hyperthyroid subjects were restudied when clinically euthyroid and when thyroid function tests had been normal for at least 2 months (Table 7:1). Treatment consisted of carbimazole 30-45 mg daily with subsequent addition of thyroxine to maintain normal circulating serum thyroxine and serum thyroid stimulating hormone concentrations. Antithyroid therapy was taken on the day of repeat study. The mean interval between studies was 7 months (range 5-10).

Protocol

Intravenous teflon cannulae were inserted at 08.00 hours after an overnight (10 h) fast and subjects remained recumbent until 08.30 hours. Basal samples for hormone and metabolite estimations were withdrawn at 08.25 and 08.30 hours when breakfast was taken. Lunch was at 12.00 hours and dinner at 18.00 hours with snacks at 10.00 and 15.30 hours. Blood samples were withdrawn at 30 min intervals until 20.00 hours. Meals were standard hospital meals containing approximately 2500 calories with 45% carbohydrate 40% fat and 15% protein by weight. All subjects consumed a daily intake of 250 g of carbohydrate for at least 48 h before study. After breakfast, subjects were mobile and encouraged to take gentle exercise throughout the test but remained at rest for at least 10 min before each sample time.

TABLE 7:1. Clinical details of patients before and during therapy and of controls

| Patient | Age | Sex | Before therapy | | | | During therapy | | | | Interval between studies (months) |
			Serum thyroxine (nmol/l)	Serum triiodo-thyronine (nmol/l)	Serum TSH (mU/l)	% ideal body weight	Serum thyroxine (nmol/l)	Serum triiodo-thyronine (nmol/l)	Serum TSH (mU/l)	% ideal body weight	
1	38	F	283	11.2	0.1	114	94	1.4	1.6	119	5
2	63	F	347	12.3	0.1	110	84	1.4	2.8	115	10
3	61	F	189	3.9	0.1	138	89	1.2	3.1	136	6
4	33	F	327	12.1	0.1	105	87	1.7	3.2	110	5
5	57	F	222	3.8	1.8	121	106	1.4	8.0	122	9
6	30	F	312	12.4	1.2	100	95	2.1	4.2	105	5
7	58	F	172	5.3	1.4	98					
8	43	M	225	6.6	1.6	102					
9	43	F	164	3.0	1.4	110					
Controls	41 (23-61)	13F 3M	98 (77-112)	1.6 (1.2-2.3)	(>7)	109 (94-122)					

For controls mean (range) is given and for serum thyroid stimulating hormone (TSH) the normal range is quoted.

Chemical Methods

Hormone and metabolite concentrations were measured as described in Chapter 6.2. and Chapter 6.3. Possible interference in metabolite assays by carbimazole was excluded by addition of this drug in a concentration of 10 mg/1, far in excess of expected plasma values, to paired blood samples.

Statistical analyses were performed using Student's paired and unpaired t-tests as appropriate. Certain measurements (ketone bodies, insulin and growth hormone) were log-normally distributed and statistical analysis was performed on log transformed data. Correlations were sought by the least squares method or the Spearman Rank method where appropriate. Values in the text are given as the mean $\pm$ standard error of the mean (SEM) or mean (range).

7.3. RESULTS

1. Fasting metabolite and hormone levels in untreated hyperthyroidism

(a) Metabolite concentrations. Fasting blood glucose concentrations were elevated in untreated hyperthyroidism (5.5 $\pm$ 0.1 mmol/1, hyperthyroid subjects; 4.8 $\pm$ 0.1 mmol/1, controls; $P < 0.01$). Concentrations of the gluconeogenic precursors, lactate, pyruvate and alanine were similar in hyperthyroid and control subjects (Table 7:2).

Fasting blood glycerol concentrations were elevated in hyperthyroidism (0.15 $\pm$ 0.02 v. 0.08 $\pm$ 0.01 mmol/1, $P < 0.001$) as were plasma non-esterified fatty acid levels (0.91 $\pm$ 0.06 v. 0.03 mmol/1, $P < 0.001$). Blood ketone body concentrations were variable and did

TABLE 7.2. Fasting and mean 12h values for hormones and metabolites in hyperthyroid subjects before and during treatment and in controls

	Fasting values						12 h values					
	Hyperthyroid	Hyperthyroid during therapy	Controls	P_1	P_2	P_3	Hyperthyroid	Hyperthyroid during therapy	Controls	P_1	P_2	P_3
Glucose (mmol/l)	5.5±0.1	5.4±0.5	4.8±0.1	NS	<0.01	NS	6.1±0.1	6.0±0.1	5.5±0.1	NS	<0.01	<0.05
Lactate (mmol/l)	0.87±0.12	0.75±0.16	0.69±0.06	NS	NS	NS	0.98±0.07	1.01±0.1	0.77±0.03	NS	<0.05	<0.05
Pyruvate (mmol/l)	0.08±0.01	0.08±0.01	0.07±0.01	NS	NS	NS	0.09±0.01	0.09±0.01	0.07±0.003	NS	<0.05	<0.05
Lactate : pyruvate	11.4±0.6	10.4±0.4	10.2±0.3	NS	NS	NS	10.4±0.1	10.9±0.4	10.3±0.2	NS	NS	NS
Alanine (mmol/l)	0.31±0.04	0.36±0.04	0.29±0.02	NS	NS	NS	0.35±0.01	0.39±0.01	0.33±0.02	NS	NS	NS
3-Hydroxybutyrate (mmol/l)	0.10 (0.03-0.37)	0.06 (0.02-0.1)	0.06 (0.02-0.12)	NS	NS	NS	0.09 (0.04-0.21)	0.04 (0.01-0.06)	0.04 (0.02-0.08)	NS	NS	NS
Acetoacetate (mmol/l)	0.10 (0.01-0.22)	0.03 (0.02-0.05)	0.04 (0.01-0.09)	NS	NS	NS	0.05 (0.03-0.09)	0.04 (0.03-0.045)	0.04 (0.01-0.08)	<0.05	NS	NS
Total ketone bodies (mmol/l)	0.20 (0.03-0.06)	0.09 (0.06-0.15)	0.10 (0.02-0.25)	NS	NS	NS	0.14 (0.06-0.3)	0.07 (0.06-0.08)	0.09 (0.04-0.2)	NS	NS	NS
Glycerol (mmol/l)	0.15±0.02	0.06±0.01	0.08±0.01	<0.001	<0.001	NS	0.12±0.01	0.05±0.004	0.08±0.01	<0.01	<0.01	<0.01
NEFA (mmol/l)	0.91±0.06	0.64±0.07	0.58±0.03	<0.001	<0.001	NS	0.71±0.03	0.44±0.08	0.53±0.04	<0.001	<0.01	<0.05
Insulin (mU/l)	6 (1-10)	2 (1-6)	6 (1-13)	NS	NS	NS	18 (11-33)	19 (13-34)	18 (9.7-29)	NS	NS	NS
Growth hormone (miu/l)	2.2 (0.4-4.6)	10.5 (0.2-41.0)	7.4 (0.4-19.1)	NS	NS	NS	2.1 (0.5-6.1)	4.3 (0.9-5.9)	2.8 (0.6-7.4)	NS	NS	NS

Values are mean ± standard error of the mean except for 3-hydroxybutyrate, acetoacetate, total ketone bodies, insulin and growth hormone where mean (range) is given.

P_1 = significance on Student's paired t-test between untreated and treated hyperthyroid subjects.

P_2 = significance on Student's unpaired t-test between untreated hyperthyroid subjects and controls.

P_3 = significance on Student's unpaired t-test between treated hyperthyroid subjects and controls.

t-tests were performed on straight or log transformed data as in the text.

not differ between hyperthyroid and control subjects.

(b) Hormone concentrations. Fasting serum insulin and growth hormone concentrations were similar in hyperthyroid subjects and controls (Table 7:2).

2.   Diurnal hormone and metabolite profiles in untreated hyperthyroidism

(a) Metabolite concentrations. Blood glucose profiles over 12 h are shown in Fig. 7:1. The peak blood glucose responses to breakfast (7.3 $\pm$ 0.4 v. 6.0 $\pm$ 0.2 mmol/l, P $<$ 0.01) and lunch (8.0 $\pm$ 0.4 v. 6.1 $\pm$ 0.3 mmol/l, P$<$ 0.001) although synchronous in both groups were exaggerated in hyperthyroidism. The mean blood glucose concentration over the 12 h was also increased (Table 7:2). Similarly, blood lactate and pyruvate concentrations after breakfast and lunch were increased in untreated hyperthyroidism (Fig. 7:2) as were the 12 h mean values for lactate and pyruvate (Table 7:2). Blood alanine concentrations were similar in hyperthyroid subjects and controls (Fig. 7:2, Table 7:2).

In control subjects, blood total ketone body, plasma NEFA and blood glycerol concentrations fell with feeding and rose with fasting before meals (Fig. 7:3). This diurnal pattern was preserved in hyperthyroidism but the premeal peaks for blood ketone body, plasma NEFA and blood glycerol levels were exaggerated. As a result, the 12 h mean values for blood glycerol and plasma NEFA were elevated in hyperthyroidism (Table 7:2).

(b) Hormone concentrations. Serum insulin and growth hormone concentrations were similar in hyperthyroid and control

subjects over the 12 h (Fig. 7:1 and Table 7:2).

3.   Effects of antithyroid therapy

(a)   Fasting hormones and metabolites. Blocking-
replacement treatment had no effect on fasting blood glucose
concentrations (Table 7:2). Blood glycerol and plasma NEFA levels
decreased to normal, however, with therapy. Concentrations of
other metabolites, insulin and growth hormone were not influenced by
treatment.

(b)   Diurnal hormone and metabolite profiles. Blood glucose
concentrations remained elevated in hyperthyroid subjects after
treatment (Fig. 7:1), with 12 h mean values higher than in controls
(6. 0 $\pm$ 0. 1 v. 5. 5 $\pm$ 0. 2 mmol/l, P < 0. 05) (Table 7:2). Similarly,
hyper lactataemia and hyperpyruvicaemia persisted during antithyroid
therapy (Fig. 7:2 and Table 7:2). Treatment also resulted in a
significant increase in blood alanine concentrations, not evident in
the untreated state (Fig. 7:2).

Circulating ketone body, NEFA and glycerol concentrations
decreased with antithyroid therapy (Fig. 7:3) although the meal
related fluctuations were preserved. Plasma NEFA and blood glycerol
levels were suppressed to values below those found in controls
(Fig. 7:3 and Table 7:2).

Insulin and growth hormone concentrations were unaffected
by therapy. In untreated hyperthyroidism neither serum thyroxine nor
triiodothyronine levels correlated with fasting, peak or mean 12 h

FIGURE 7:1.   Blood glucose and serum insulin profiles in
hyperthyroid subjects before (●————●) and during
(●------●) therapy and in controls (■————■).   The
first sample was taken after an overnight fast.
Thereafter meals were taken at 08.30, 12.00 and
18.00 hours with snacks at 10.00 and 15.30 hours.
*P ∠ 0.05 hyperthyroid subjects before therapy v.
hyperthyroid subjects during therapy (**P ∠ 0.01)
+ P ∠ 0.05 hyperthyroid subjects before therapy v.
controls (++ P ∠ 0.01).   ● P ∠ 0.05 hyperthyroid
subjects during therapy v. controls (●● P ∠ 0.01).

FIGURE 7:2.   Blood alanine, lactate and pyruvate profiles in
hyperthyroid subjects before and during therapy and
in controls.   See legend to Figure 7:1 for symbols
and conditions.

FIGURE 7:3. Diurnal profiles of blood ketone bodies, plasma
non-esterified fatty acids and blood glycerol in
hyperthyroid subjects before and during therapy and
in controls. See legend to Figure 7:1 for symbols
and conditions.

values for any of the hormones or metabolites measured.

7.4.   DISCUSSION

The present study showed fasting hyperglycaemia in

hyperthyroid subjects.   This could be a consequence of diminished

utilization or increased hepatic glucose production.   The latter has

been noted by some (Saunders et al. 1980; Okajima & Ui, 1979), but

not all (Wahren et al. 1947) workers.   Increased glucose production

may result from increased gluconeogenesis and/or increased

glycogenolysis.   Hepatic glycogen stores are decreased in hyperthyroid

man (Pipher & Paulsen, 1947) although whether this is because of

increased utilization or decreased synthesis is uncertain.   Recently,

studies in overnight fasted hyperthyroid man have shown increased

splanchnic uptake of the gluconeogenic precursors, lactate, pyruvate,

glycerol and alanine, largely due to increased fractional extraction

of these precursors but also, to a smaller extent, to increased hepatic

blood flow (Wahren et al. 1981).   Similarly, the finding of increased

glucose production from glucose-derived 3-carbon intermediates in

experimental hyperthyroidism in animals (Okajima & Ui, 1979) suggests

an increase in gluconeogenesis.   Confirmatory evidence has been

obtained from studies with perfused rat livers at high concentrations

of gluconeogenic precursors (Menahan & Weiland, 1969; Freedland &

Krebs, 1967) although not at physiological levels (Bartels & Sestoft,

1980).

In the present study, postprandial as well as fasting

hyperglycaemia was noted.   Peak blood glucose concentrations

occurred at the same time as in controls indicating that rapid absorption of carbohydrate was not the major factor; a suggestion made by others on the basis of glucose tolerance tests (Holdsworth & Besser, 1968).

Normal serum immunoreactive insulin levels were also found. The combination of fasting hyperglycaemia, meal intolerance and normal circulating insulin levels suggests insulin resistance. Studies of insulin sensitivity in hyperthyroid man have been equivocal showing a normal (Elrich et al. 1961), increased (Macho, 1958) or decreased (West et al. 1975) blood glucose response to exogenous insulin. Enhanced insulin degradation in hyperthyroidism, noted by some (Elgee & Williams, 1955) but not all workers (Wajchenberg et al. 1978), may not explain the coexistence of normal insulin and elevated blood glucose concentrations as insulin degradation and biological effect are inextricably linked (Terris & Steiner, 1975).

The important recent demonstration of elevated circulating proinsulin concentrations with low C-peptide levels suggests that true insulin secretion may be diminished in hyperthyroid man (Sestoft & Heding, 1981). Proinsulin cross reacts with insulin in the assay used in our radioimmunoassay as in many other studies. Thus 'true' insulin levels may have been decreased in our patients. However, even if proinsulin secretion were normal, the fact that insulin levels were not raised in the presence of hyperglycaemia suggests undersecretion of insulin. The mechanism of insulin hyposecretion is uncertain but has been attributed to increased adrenergic tone (Wajchenberg et al. 1978).

Recent work has suggested a role for glucagon in glucose intolerance of hyperthyroidism (Kabadi & Eisenstein, 1980), some hyperthyroid subjects showing elevated basal plasma glucagon levels with impaired glucagon suppression following oral glucose challenge. In addition, increased sensitivity to the cyclic AMP mediated effects of glucagon has been proposed (Lamberg, 1965). This may be important in decreasing glycogen stores in the liver of hyperthyroid man and animals.

The elevated blood lactate and pyruvate responses to meals is not readily explained. Blood lactate levels are also increased in response to exercise in hyperthyroid man (Nazar et al. 1978). This hyperlactataemia is abolished by β-adrenergic blockade and increased adrenergic tone has been suggested as a mechanism (Kaciuba-Uscilko & Brezinska, 1975). Our subjects were resting and a thyroid hormone mediated increase in peripheral glycolysis is a more likely mechanism. Such an effect has been demonstrated in vivo (Araki et al. 1968).

The elevated circulating concentrations of glycerol and NEFA after an overnight fast and before lunch and dinner reflect increased adipose tissue lipolysis. Increased lipolysis in hyperthyroidism has long been recognized (Marks et al. 1960; Hesch & Evered, 1973) and NEFA (Saunders et al. 1980) and glycerol turnover (Tibbling, 1969) are elevated in fasting hyperthyroid man. This increase in lipolytic activity could reflect either a direct effect of thyroid hormones or potentiation of the lipolytic action of other hormones,

particularly the catecholamines. Although plasma catecholamine

levels are not increased in hyperthyroidism (Christensen, 1973)

increased lipolysis may be due to increased β adrenergic responsiveness

(Arner et al. 1980). Enhanced ketogenesis with fasting has been

recognized recently in hyperthyroidism (Beylot & Rioux, 1980).

Increased NEFA supply combined with relative insulin deficiency are

important and an additional direct ketogenic effect of thyroid hormone

excess on the liver has recently been described (Keyes & Heimberg, 1979).

Antithyroid treatment with carbimazole and thyroxine

replacement therapy did not restore blood glucose or gluconeogenic

precursor concentrations to normal. Similarly Kreines et al. (1965)

and Cavagnini et al. (1974) reported persistent intolerance to oral

glucose after antithyroid therapy while Saunders et al.(1980)

reported normalization of fasting glucose turnover by carbimazole

but blood lactate levels remained elevated. The reasons for the

persisting abnormalities are not apparent. All patients were clinically

and biochemically euthyroid and the decline in blood glycerol, ketone

body and plasma NEFA levels (to subnormal levels in the case of

glycerol) suggests that therapy adequately suppressed lipolysis and

ketogenesis. No direct effects of carbimazole on intermediary

metabolism, independent of its effect on thyroid function have been

reported and the effects of methimazole, a related drug, on glucose

turnover in the rat are restored to normal by thyroxine (Okajima &

Ui, 1979). Insulin secretion, assessed by peripheral insulin

concentrations did not alter although the proportion of 'true' insulin

98.

to proinsulin may have increased. It is still possible, however, that

there may have been residual pancreatic hyposecretion or, particularly

had 'true' insulin secretion increased, persistent insulin resistance.

These may have contributed to the persistent carbohydrate intolerance

but do   not explain the elevated post-prandial lactate and pyruvate

concentrations during antithyroid therapy. Finally, the duration of

therapy (6. 7 months) may have been inadequate. Studies in the rat,

however, suggest that normalization of glucose metabolism occurs after

5 weeks (Okajima & Ui, 1979). Longer-term follow-up studies will be

necessary to resolve this question.

8.   DIURNAL METABOLIC PROFILES IN HYPOTHYROIDISM

8.1.   INTRODUCTION

Animal studies have shown that experimental hypothyroidism is

associated with decreases in gluconeogenesis (Freedland & Krebs, 1967),

glycogenolysis (Battarbee, 1974), peripheral glucose utilisation

(Scow & Cornfield, 1954), lipolysis (Bray & Goodman, 1968),

lipogenesis (Bates et al. 1955) and protein turnover (Hoberman &

Graff, 1951).

In man fasting hypoglycaemia (Lamberg, 1965), impaired

tolerance to oral glucose (Crawford, 1940) with normal (Holdsworth

& Besser, 1968) or impaired (Shah & Cerchio, 1973) peripheral insulin

responses have all been reported. Decreased lipolysis, both in vivo

(Hamburger et al. 1963) and in isolated human adipocytes (Rosenqvist,

1972) has been demonstrated but little is known about the effects of

hypothyroidism on ketone body metabolism. (A fuller account of

metabolic abnormalities accompanying hypothyroidism is given in

the Introduction (4.3)).

The effects of hypothyroidism on circulating hormone and

metabolite concentrations under normal circumstances are unknown and

the results of subsequent thyroid hormone replacement have not

previously been described. Hormone-metabolite profiles during a 12h

period of normal meals and activity have therefore been examined in

hypothyroid subjects before and during thyroxine replacement therapy

and in normal euthyroid controls.

8.2.   PATIENTS AND METHODS

Eight (six female and two male) patients with hypothyroidism were compared with sixteen control subjects of similar age, sex and ideal body weight. (Table 8:1). No subject had a personal or family history of diabetes and none were on any medication at the time of initial study. Liver function tests were normal. Hypothyroidism was secondary to Hashimoto's thyroiditis in six subjects (on the basis of spontaneous hypothyroidism occurring in subjects with circulating antibodies to thyroid tissue), partial thyroidectomy for colloid goitre in one subject and radioiodine therapy in one subject.

The protocol for the study was identical to that in Chapter 7. Hypothyroid subjects were restudied when clinically euthyroid and conventional thyroid function tests were normal. Treatment consisted of L-thyroxine 100-200 μg daily to maintain normal circulating serum thyroxine and serum thyroid stimulating hormone concentrations. The mean interval between studies was 5.9 months.

Hormone and metabolite concentrations were measured as described in 6.2. and 6.3.

Statistical analyses were performed using Student's paired and unpaired t tests as appropriate. Certain measurements (ketone bodies, glucagon and insulin) were log-normally distributed and statistical analysis was performed on log-transformed data.

Correlations were sought by the method of least squares or the Spearman ranking method where appropriate. Values in the text are given as the mean ± the standard error of the mean or as mean (range).

TABLE 8:1. Clinical details of patients before and during therapy and of controls. For controls, mean + range is quoted and for serum thyroid stimulating hormone (TSH) the normal range is quoted

Patient no.	Age	Sex	Before therapy				During therapy				Interval between studies (months)
			Serum triiodo-thyronine (nmol/1)	Serum thyroxine (nmol/1)	Serum TSH (mu/1)	% ideal body weight*	Serum triiodo-thyronine (nmol/1)	Serum thyroxine (nmol/1)	Serum TSH (mu/1)	% ideal body weight	
1	18	F	1.3	30	29	104	2.1	98	4.6	103	6
2	57	F	0.4	13	30	109	2.6	121	4.6	106	5
3	66	F	0.8	42	21	110	1.2	81	2.8	111	7
4	45	F	0.8	28	20	108	1.5	122	4.0	103	5
5	48	F	0.8	30	40	105	1.5	120	6.6	93	4
6	49	F	0.4	40	40	139	1.7	121	3.3	128	8
7	56	M	1.0	46	18	99	1.5	131	6.4	100	6
8	39	M	1.1	52	28	110	1.4	60	3.8	102	6
Mean ± SEM	46±5	6F2M	0.8±0.1	34±8	28±3	111±4	1.7±2	106±9	4.5±0.5	106±4	5.9±0.5
Controls 41		14F2M	1.6 (1.3-2.3)	98 (77-112)	>7	109 (94-122)					

* Assessed from Metropolitan Life Assurance Tables.

8.3.   RESULTS

Thyroid function tests

Results for serum thyroxine (T_4) serum triiodothyronine (T_3)

serum thyroid stimulating hormone (TSH) and percent ideal body weight

(% IBW) are shown in Table 8:1.   Serum T_4 and T_3 concentrations were

lower in hypothyroidism (P $<$ 0.001) than in controls and increased

to normal with thyroid hormone replacement.   Serum TSH levels

were higher in hypothyroidism (P $<$ 0.001) and fell, with treatment,

to normal values.   Although there was a significant fall with thyroxine

therapy (P $<$ 0.05), percentage of ideal body weight in hypothyroid

subjects before and after therapy was similar to controls.

Fasting metabolites and hormones

Metabolite concentrations.   Fasting concentrations of blood

glucose and the gluconeogenic precursors, lactate, pyruvate, and alanine,

blood ketone bodies and plasma NEFA were similar in hypothyroid

patients and controls (Table 8:2).   Blood glycerol concentrations were

however decreased in hypothyroidism (patients, 0.06 $\pm$ 0.01; controls,

0.08 $\pm$ 0.01 mmol/l; P $<$ 0.001).

Hormone concentrations.   There were no significant differences

between fasting serum insulin, growth hormone and plasma glucagon

concentrations in hypothyroid and control subjects.

Effects of thyroxine therapy.   Fasting blood concentrations of

glucose, lactate, pyruvate, alanine and 3-hydroxybutyrate were

unchanged by thyroxine replacement therapy.   Blood acetoacetate levels

rose significantly [during treatment 0.08 (0.03-0.16), untreated 0.04

(0.02-0.07) mmol/l; P $<$ 0.05].   Blood glycerol levels also rose

(during treatment 0. 08 ± 0. 01 , untreated 0. 06 ± 0. 01 mmol/1;

P < 0. 05). Serum insulin, growth hormone and plasma glucagon

levels were unchanged by thyroxine replacement.

Diurnal profiles

Metabolite concentrations. Mild hyperglycaemia was

apparent after breakfast in hypothyroid subjects (Fig.8:1) although the

glycaemic response to lunch and evening meal was normal. As a

result the mean 12-h blood glucose value was unaltered by hypothyroidism.

Blood lactate and pyruvate responses to meals were exaggerated and

mean 12h values for blood lactate (hypothyroid 1. 08 ± 0. 08; control

0. 77 ± 0. 03 mmol/1; P < 0. 001) and blood pyruvate (hypothyroid 0.10 ±

0. 01; control 0. 08 ± 0. 003 mmol/1; P< 0. 01) were elevated in

hypothyroidism (Fig. 8:2) (Table 8:2). The ratio of blood lactate to

blood pyruvate in control subjects fell after each main meal (Table 8:3).

In hypothyroid subjects, lactate:pyruvate ratios rose with meals and

were significantly higher than in control subjects.

Blood alanine concentrations were similar in hypothyroid and

control subjects in the morning and early afternoon but were significantly

higher in hypothyroidism over the last 4 h of the study (Fig. 8:2). As a

result mean 12 h blood alanine levels (Table 8:2) were significantly

higher in hypothyroidism (0. 40 ± 0. 02 v. 0. 33 ± 0. 02 mmol/1, P < 0. 05).

In control subjects, blood total ketone body, glycerol and plasma

non-esterified fatty acid (NEFA) showed a characteristic diurnal pattern

with rises in circulating levels of each metabolite before meals (Fig. 8:3).

In hypothyroidism, this diurnal pattern for blood glycerol and blood

ketone bodies was lost. Blood glycerol levels were decreased

throughout the study period (0. 04 $\pm$ 0. 01 v. 0. 08 $\pm$ 0. 01 mmol/1,

P $<$ 0. 001). A similar, though less striking decrease was also

evident for blood ketone bodies before the evening meal. The rise in

ketone body concentrations between lunch and the evening meal,

assessed as the area under the ketone body curve, was decreased in

hypothyroidism (P $<$ 0. 05). Plasma NEFA levels were comparable

in patients and controls.

Hormone concentrations. Serum insulin, growth hormone and

plasma glucagon levels did not differ in hypothyroid and control

subjects at any time.

Effects of thyroxine therapy. Thyroxine therapy did not restore

the blood glucose response to breakfast to normal but serum insulin

levels after meals were increased particularly in the evening, and

the mean 12 h serum insulin concentration was elevated (Table 8:2).

Plasma glucagon concentrations were not affected.

Mean 12 h values for blood lactate, pyruvate or alanine concentrations

(Table 8: 2) were also unaltered by thyroxine therapy, but blood glycerol

concentration rose significantly (untreated 0. 04 $\pm$ 0. 01, during

treatment 0. 06 $\pm$ 0. 01 mmol/1, P $<$ 0. 01) although they remained lower

than in controls (0. 08 $\pm$ 0. 01 mmol/1, P $<$ 0. 05). Total ketone body

concentrations were unaltered by replacement therapy although a normal

diurnal pattern was restored.

Serum T_4, T_3 and TSH concentrations did not correlate with

blood levels of any of the hormones or metabolites measured in

105.

TABLE 8:2. Fasting and 12h mean hormone and metabolite concentrations in control subjects and hypothyroid patients before and after replacement thyroxine therapy

Hormone or metabolite	Fasting values						Mean 12 h values					
	Hypothyroid	Hypothyroid during therapy	Control	P_1	P_2	P_3	Hypothyroid	Hypothyroid during therapy	Control	P_1	P_2	P_3
Glucose (mmol/l)	4.5 ± 0.5	4.8 ± 0.1	4.8 ± 0.1	NS	NS	NS	5.6 ± 0.3	5.8 ± 0.2	5.5 ± 0.1	NS	NS	NS
Lactate (mmol/l)	0.88±0.11	0.78±0.14	0.69±0.06	NS	NS	NS	1.08±0.06	1.0 ± 0.1	0.77±0.03	NS	<0.001	<0.01
Pyruvate (mmol/l)	0.08±0.01	0.08±0.01	0.07±0.05	NS	NS	NS	0.10±0.01	0.10±0.01	0.08±0.003	NS	<0.01	<0.01
Lactate : pyruvate	10.3±1.0	10.1±1.0	10.2±0.3	NS	NS	NS	10.8±0.3	10.6±0.3	10.3±0.2	NS	NS	NS
Alanine (mmol/l)	0.30±0.02	0.29±0.04	0.29±0.02	NS	NS	NS	0.40±0.02	0.38±0.05	0.33±0.02	NS	0.05	NS
3-hydroxybutyrate (mmol/l)	0.06(0.01-0.14)	0.07(0.04-0.18)	0.06(0.01-0.12)	NS	NS	NS.	0.04(0.02-0.05)	0.03(0.01-0.05)	0.04 (0.02-0.08)	NS	NS	NS
Acetoacetate (mmol/l)	0.04(0.02-0.07)	0.08(0.03-0.16)	0.04(0.01-0.21)	<0.05	NS	<0.05	0.04(0.03-0.06)	0.06(0.01-0.09)	0.04 (0.01-0.08)	NS	NS	<0.05
Total ketone bodies (mmol/l)	0.10(0.04-0.18)	0.15(0.09-0.28)	0.10(0.02-0.25)	NS	NS	NS	0.08(0.06-0.10)	0.09(0.08-0.11)	0.09 (0.04-0.20)	NS	NS	NS
Glycerol (mmol/l)	0.06±0.01	0.08±0.01	0.08±0.01	<0.05	<0.001	NS	0.04±0.005	0.06±0.01	0.08±0.01	<0.01	<0.001	<0.05
NEFA (mmol/l)	0.73±0.04	0.64±0.06	0.58±0.03	NS	NS	NS	0.52±0.05	0.48±0.04	0.53±0.04	NS	NS	NS
Insulin (mu/l)	4 (1-14)	6 (1-12)	6 (1-13)	NS	NS	NS	14 (3-33)	33 (12-54)	18 (8-29)	<0.05	NS	<0.05
Glucagon (ng/l)	20 (0-40)	31 (0-55)	26 (5-50)	NS	NS	NS	23 (4-44)	22 (1-35)	22 (10-40)	NS	NS	NS
Growth hormone (miu/l)	9.5 (0.9-31.0)	4.8(0.5-9.8)	7.4(0.4-19.1)	NS	NS	NS	2.4(0.5-3.0)	2.2(0.7-5.0)	2.8(0.8-4.6)	NS	NS	NS

Values are mean ± standard error of the mean except for 3-hydroxybutyrate, acetoacetate, total ketone bodies, insulin and glucagon where mean (range) is given.

P_1 = significance on Student's paired t test between untreated and treated hypothyroid subjects.

P_2 = significance on Student's unpaired t test between untreated hypothyroid subjects and controls.

P_3 = significance on Student's unpaired t test between treated hypothyroid subjects and controls.

t tests were performed on straight or log transformed data as in the text.

FIGURE 8:1.   Blood glucose and serum insulin profiles in
hypothyroid subjects before (●———●) and during
(●-----●) thyroxine therapy and in controls
(■———■).   The first sample was taken after an
overnight fast.   Thereafter meals were taken at
0830, 1200 and 1800h with snacks at 1030 and
1530 h.   *P < 0.05 hypothyroid subjects before
therapy v. hypothyroid subjects during therapy.
(**P < 0.01).   +P < 0.05 hypothyroid subjects
before therapy v. controls (++ = P < 0.01).
● = P < 0.05 treated hypothyroid subjects v.
controls (●● = P < 0.01).

FIGURE 8:2.   Blood alanine, lactate and pyruvate profiles in
             hypothyroid subjects before and during thyroxine
             therapy and in controls.   See legend to Figure 8:1
             for key and conditions.

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FIGURE 8:3.   Diurnal profiles for plasma non-esterified fatty
acids, blood ketone bodies and blood glycerol in
hypothyroid subjects before and during therapy
and in controls.   For conditions and symbols
see Figure 8:1.

09.

TABLE 8:3. Blood lactate : pyruvate ratios before and 2 h after the three main meals in hypothyroid subjects before and during therapy and in controls.
(For symbols and conditions see legend to Table 8:2)

	Hypothyroid before therapy	Hypothyroid during thyroxine therapy	Controls	P_1	P_2	P_3
Before breakfast	10.3 ± 0.7	10.1 ± 0.6	10.2±0.3	ns	ns	ns
2h after breakfast	11.7 ± 0.6	10.6 ± 0.4	9.7±0.4	ns	<0.05	ns
Before lunch	10.3 ± 0.4	10.2 ± 0.6	10.5±0.4	ns	ns	ns
2h after lunch	11.5 ± 0.5	10.9 ± 0.6	10.2±0.4	ns	<0.05	ns
Before supper	10.6 ± 0.3	10.5 ± 0.7	11.2±0.3	ns	ns	ns
2h after supper	12.1 ± 0.8	11.6 ± 0.3	10.1±0.4	ns	<0.05	<0.05

hypothyroid subjects before or during therapy or in controls.

8.4.   DISCUSSION

Previous studies in hypothyroid subjects have shown either

normal (Saunders et al. 1980) or decreased (Lamberg, 1965; Levy et al.

1970; Shah et al. 1972) fasting blood glucose concentrations. The

decrease in blood glucose has been attributed to both impaired hepatic

glycogenolysis (Battarbee, 1974) and impaired gluconeogenesis

(Bondy, 1949; Freedland & Krebs, 1967; Menahan & Weiland, 1969;

Sestoft et al. 1977). In the present study, fasting blood glucose

concentrations were normal. Glucose turnover is decreased in

hypothyroid rats (Okajima & Ui, 1979) but in hypothyroid man, glucose

turnover has been reported to be normal (Saunders et al. 1980).

The blood glucose response to breakfast was mildly elevated

and the peak blood glucose value delayed in hypothyroidism. Absorption

of glucose from the gastrointestinal tract is delayed both in man

(Holdsworth & Besser, 1968) and experimental animals (Althausen &

Stockholm, 1980). This may explain the delay in blood glucose peak but

does not explain the exaggerated glycaemic response. This suggests

impaired peripheral utilization of glucose. A similar intolerance to oral

glucose has previously been noted (Gutman et al. 1978; Crawford 1940).

Fasting blood concentrations of the gluconeogenic precursors,

lactate, pyruvate and alanine were normal in the present and previous

(Saunders et al. , 1980) but not all (McDaniel et al. , 1977) studies.

Peripheral release of lactate (McDaniel et al. , 1977) and alanine

(Bondy, 1949; Ness et al. , 1969) may be reduced in hypothyroidism but

the normal circulating levels suggest that any impairment of

gluconeogenesis is not secondary to a reduction in precursor supply.
This suggests that lactate, pyruvate and alanine uptake by the liver is
decreased in hypothyroidism in proportion to the decrease in hepatic
glucose output.

The exaggerated lactate and pyruvate responses to meals may
be secondary to increased peripheral production or diminished clearance.
Most studies in hypothyroidism suggest diminished peripheral glucose
utilization and diminished lactate (Shamer et al., 1971; Okajima & Ui,
1979) and pyruvate output (McDaniel et al., 1977) from peripheral tissues.
Increased production of lactate and pyruvate after meals is therefore
unlikely as the cause of the hyperlactataemia and hyperpyruvicaemia,
making a defect in hepatic uptake more likely.

The ratio of blood lactate to blood pyruvate was elevated after
meals. This ratio may reflect the prevailing cytosolic redox state
which is in turn dependent on the transfer of reducing equivalents across
the mitochondrial membrane (Krebs et al., 1975; Alberti & Nattrass,
1977). Thyroid hormones are important in this transfer process
(Mullhoffer & Loy, 1974; Rognstadt, 1977; Sestoft, 1980), which may
therefore be deficient in hypothyroidism.

Fasting blood alanine concentrations were normal in
hypothyroidism and were only significantly greater than control values
during the last 4 h of the study. Hyperalaninaemia may be due either
to diminished alanine uptake for gluconeogenesis or alternatively to
a loss of the normal protein sparing effects of ketone bodies. Ketone
bodies are known to inhibit protein degradation and peripheral alanine

output (Sherwin et al., 1976). The lower ketone body concentrations, particularly during the afternoon period, could possibly be responsible for the elevated blood alanine concentrations observed.

Alone among the gluconeogenic precursors, circulating blood glycerol levels were diminished in hypothyroidism. This finding, and the findings of other studies (Goodman & Bray, 1966; Fisher & Ball, 1967) suggests decreased lipolysis. Thyroid hormones have lipolytic actions in vitro (Vaughan, 1967), and a permissive effect for the lipolytic actions of other hormones, particularly the catecholamines both in vitro (Arner et al., 1979; Debons & Schwartz, 1961) and in vivo (Hamburger et al., 1963). In hypothyroidism plasma catecholamine levels may be increased (Christensen, 1972) and sensitivity to their peripheral effects reduced (Goodman & Bray, 1966; Rosenqvist, 1972; Reckless et al., 1976). These changes may be mediated by altered phosphodiesterase activity (Armstrong et al., 1974). The presence of normal rather than low plasma non-esterified fatty acid levels is not readily explained but has previously been reported (Saunders et al., 1980). It is possible that despite diminished lipolysis, decreased NEFA uptake by the hypothyroid liver balances the diminished release.

The hypoketonaemia observed may be secondary to diminished lipolysis although substrate supply as judged by plasma NEFA measurements appears adequate. Recent studies have suggested that intrahepatic fatty acid oxidation and ketone body production may be thyroid hormone dependent (Keyes & Heimberg, 1979; Bartels & Sestoft, 1980).

Serum insulin levels in hypothyroidism have been reported to be decreased (Okajima & Ui, 1979; Jolin et al., 1970; Shah & Cerchio, 1973), normal (Holdsworth & Besser, 1968), or increased (Andreani et al. 1968; Renauld et al. 1979), with a marked species variation. Thyroxine is necessary for normal insulin secretion by isolated pancreatic cells in vitro (Milcu et al., 1975). In the present study serum insulin concentrations were similar to control values in the presence of elevated ambient blood glucose concentrations suggesting insulin hyposecretion. The mechanism of this is uncertain. Elevated blood glucose levels with insulin concentrations similar to controls also suggest insulin resistance in hypothyroidism. Alternatively the insulin measured by radioimmunoassay may be inactive. The pattern of insulin secretion has not been examined however in hypothyroidism. Sensitivity to exogenous insulin at physiological blood glucose concentrations has previously been reported to be either normal (Iwatsubo et al., 1967; Brauman & Corvilain, 1968), or reduced (West et al., 1975) in hypothyroid man.

Some of the abnormalities described in hypothyroidism, in particular the mild elevation in blood glucose and gluconeogenic precursor concentrations have previously been described in obese subjects (Doar & Wynn, 1970). The patients outlined in the present study were within 10% ideal body weight with one exception. Abnormalities in intermediary metabolite concentrations are not noted in patients with such mild obesity (Johnston et al., 1981). Similarly weight change with thyroxine therapy was small and could not explain any metabolic

changes noted.

Thyroxine therapy had no effect on circulating blood glucose concentrations but did cause a significant elevation in the serum insulin response to meals. This may reflect a permissive effect of thyroid hormones on pancreatic insulin secretion. Blood lactate and pyruvate responses to meals were not normalized by thyroxine therapy and it is difficult to explain the persistent abnormality of glucose or gluconeogenic precursor concentrations with treatment.

Thyroxine therapy did cause a significant elevation in blood glycerol concentrations although levels remained lower than those obtained in control subjects. This change presumably reflects a thyroid hormone-induced effect on peripheral lipolysis.

Plasma glucagon levels were unaltered in hypothyroidism and no effect of thyroid hormone therapy was seen. Plasma glucagon response to protein meals has been shown to be impaired in hyperthyroid subjects but no previous information on plasma glucagon levels in hypothyroidism has been published. Growth hormone deficiency has previously been demonstrated in hypothyroidism (Eisenberg et al., 1972; Peake et al. 1973) but in the present study growth hormone levels were normal and were unaffected by thyroid hormone replacement.

9. GLUCOSE TURNOVER AND RECYCLING : EFFECTS OF THYROID HORMONES

9.1. INTRODUCTION

In the preceding chapters elevated fasting blood glucose concentrations were described in hyperthyroid subjects whilst the blood glucose response to mixed meals was exaggerated. In hypothyrodism, fasting blood glucose concentrations were normal with mild hyperglycaemia after breakfast although meal tolerance thereafter was normal. In addition blood levels of the gluconeogenic precursors lactate and pyruvate were elevated after meals in both conditions suggesting the possibility of altered Cori cycle activity whilst fasting blood glycerol concentrations were increased in hyperthyroidism and decreased in hypothyroidism. These studies however give no quantitative information about glucose production and utilisation in hyper and hypothyroidism. Administration of pharmacological amounts of thyroid hormones to animals has produced more clearcut results. Thus increases in glycogenolysis (Coggeshall & Green, 1933) gluconeogenesis (Svedmyr, 1966) and glucose turnover (Okajima & Ui, 1979) have all been reported. Such experiments have also demonstrated increased activity of certain substrate cycles, such as those between glucose and glucose-6-phosphate (Okajima & Ui, 1979) and between triglycerides and non-esterified fatty acids (Sestoft, 1980). In man increased glucose turnover has been reported in hyperthyroidism and normal turnover in hypothyroidism using ^{3}H-3-glucose as tracer (Saunders et al. 1980), and paradoxically decreased splanchnic glucose output in hyperthyroidism using catheterisation techniques

(Wahren et al. 1981), but substrate cycling between glucose and glucose-derived 3-carbon intermediates has not been examined.

In the present study glucose turnover has been investigated in fasting hyperthyroid and hypothyroid man before and during treatment using ^{3}H-3-glucose as tracer to indicate total glucose turnover. In addition the possibility of cycling of glucose derived 3-carbon intermediates has been investigated by simultaneous analysis of decay of ^{14}C-1-glucose specific activity.

9.2.   SUBJECTS AND METHODS

Six hyperthyroid subjects, mean $\pm$ SEM percent ideal body weight (% IBW) 90 $\pm$ 3 before therapy, 99 $\pm$ 1% IBW after therapy (5 subjects), were compared with six age-matched normal controls, 95 $\pm$ 2% IBW (Table 9:1).   Hyperthyroidism was caused by Graves' disease in all subjects, on the basis of clinical features and antibodies to thyroid tissue components.   No subject had a thyroid nodule on Tc99 scanning. Hyperthyroid subjects were restudied after 5-8 months (Mean 5.9) of antithyroid therapy (carbimazole 30-40 mg daily from diagnosis, with L-thyroxine 100-150 μg daily added after 4-6 weeks).   Circulating thyroxine (T_4). triiodothyronine (T_3) and thyroid stimulating hormone (TSH) concentrations were normal at the time of reinvestigation.

Seven hypothyroid patients, 121 $\pm$ 3% IBW before therapy and 110 $\pm$ 2% IBW after therapy (six subjects only) were compared with six normal controls (111 $\pm$ 4% IBW) (Table 9:1).   Hypothyroid subjects were older than controls although the difference was not significant. Hypothyroidism was secondary to Hashimoto's thyroiditis in all cases.

TABLE 9:1. Characteristics of hyper and hypothyroid patients before and during therapy and of controls

	Hyperthyroid	Hyperthyroid during therapy	Control	Hypothyroid	Hypothyroid during therapy	Control
Sex	3M 3F	3M 2F	4M 2F	2M 5F	2M 4F	4M 2F
Age	46 ± 3	44 ± 3	43 ± 5	59 ± 3	58 ± 4	45 ± 6
% Ideal body weight	90 ± 3	99 ± 1	95 ± 2	122 ± 3	110 ± 2	111 ± 4
Serum thyroxine (nmol/l)	197 ± 17 ***	84 ± 8 •••	90 ± 8	24 ± 7 ***	110 ± 5 •••	104 ± 10
Serum triiodothyronine (nmol/l)	6.2 ± 0.7 ***	2.4 ± 0.2 •••	1.6 ± 0.2	0.8 ± 0.1 ***	2.0 ± 0.1 •••	2.0 ± 0.2
Serum thyroid/stimulating hormone (mu/l)	1.3 ± 0.6	3.3 ± 1.5	2.9 ± 0.5	26.0 ± 2 ***	3.7 ± 0.9 •••	2.9 ± 0.5

*** $p < 0.001$ untreated patients vs. appropriate controls.

••• $p < 0.001$ untreated vs. treated patients.

TABLE 9:2. Fasting metabolite and hormone concentrations in hyperthyroid and hypothyroid subjects and controls shown as mean ± SEM or mean (and range)

Hormone or Metabolite	Hyperthyroid before therapy	Hyperthyroid during therapy	Controls	Hypothyroid before therapy	Hypothyroid during therapy	Controls
Blood glucose mmol/l	5.4 ± 0.3	5.3 ± 0.5	5.2 ± 0.9	5.1 ± 0.1	5.0 ± 0.2	4.9 ± 0.2
Blood lactate mmol/l	0.67 ± 0.10	0.84 ± 0.11	0.91 ± 0.08	0.91 ± 0.05	0.79 ± 0.04	0.75 ± 0.05
Blood pyruvate mmol/l	0.06 ± 0.01	0.07 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Lactate:Pyruvate ratio	11.3 ± 0.2	12.1 ± 2.3	10.7 ± 2.6	12.4 ± 0.6	11.35 ± 0.8	9.2 ± 1.2
Blood Alanine mmol/l	0.27 ± 0.03	0.29 ± 0.03	0.34 ± 0.02	0.36 ± 0.01	0.34 ± 0.02	0.33 ± 0.02
Blood glycerol mmol/l	0.12 ± 0.02 *	0.05 ± 0.01 ●	0.06 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Serum insulin mu/l	5 (1-10)	7 (0-12)	6 (1-8)	7 (1-14)	5 (2-11)	7 (0-11)
Plasma glucagon pg/l	28 (12-40)	30 (18-50)	26 (13-36)	21 (10-30)	28 (2-57)	26 (20-31)

* $p < 0.05$ hyperthyroid subjects vs. controls

● $p < 0.05$ hyperthyroid subjects before vs. during therapy

Patients were re-studied after 6 months therapy with L-thyroxine, 100-200 μg daily, when circulating T_4, T_3 and TSH concentrations were within the normal range.

No subject was on any medication at the time of initial study and none had any other known disease nor family history of diabetes. All subjects consumed in excess of 250 g of carbohydrate daily for at least 48 h before study.

Protocol

After a 10 h (overnight) fast, indwelling intravenous teflon cannulae (Venflon, Viggo, Helsinborg) were positioned in both antecubital veins at 0800 h. Subjects remained recumbent throughout the test and basal samples for hormones and metabolites were taken at 0825 and 0830 h. Bolus intravenous injections of 50 μCi (1.85 mBq) of $[^3H]$-3-glucose and 25 μCi (0.925 mBq) of $[^{14}C]$-1-glucose (Radiochemical Centre, Amersham, Bucks, U.K.) were given at 0830 h. Blood samples (10 ml) were taken at 1, 2, 3, 4, 5, 6, 8, 10, 12.5, 15, 20, 30, 40, 50, 60, 75, 90 and 120 minutes after injection for estimation of glucose specific activity, intermediary metabolites and hormones.

Chemical Methods

Hormone and metabolite concentrations were measured as described in 6.2. and 6.3. For measurement of radioactivity in glucose samples were taken into heparinized tubes and the plasma separated and stored at -20°C until assay. Plasma (0.5 ml) was deproteinised using 1 ml 5.36% (w/v) barium hydroxide and 1 ml 5% (w/v)

zinc sulphate (Somogyi, 1945) and 7.5 ml distilled water. The

supernatant was passed through a column containing 5 ml ion exchange

resin (AG2-x 8/B, Biorad Lab., Richmond, California). The column

was washed with 10 ml distilled water and the eluate was freeze-dried,

resuspended in a toluene based scintillant (Cocktail T, Hopkins and

Williams, Chadwell Heath, England) and counted in a scintillation

counter (Intertechnique, Middlesex, England) using an external standard

to correct for efficiency and overlapping. Recovery of standards was

93% (range 90-100%) for $[^3H]$-3-glucose and 94% (91-100%) for

$[^{14}C]$-1-glucose.

Calculations

Curves for $[^3H]$ glucose and $[^{14}C]$ glucose specific activity (SA)

against time were constructed. Values for glucose turnover, mean

residence time, glucose pool and glucose space were calculated

according to Shipley & Clark (1972) by the formulae:

$$\text{Glucose turnover (RT)} = \frac{\text{injected dose}}{\int_0^\infty \text{glucose SAdt}}$$

$$\text{Mean Residence Time (MRT)} = \frac{\int_0^\infty (t \times \text{glucose SAdt})}{\int_0^\infty \text{glucose SAdt}}$$

Glucose Pool = Glucose RT x MRT

$$\text{Glucose Space} = \frac{\text{Glucose Pool} \times 100}{\text{body wt} \times \text{glucose concentration}}$$

Metabolic clearance rates (MCR) were given by the formula

$$MCR = \frac{\text{Glucose RT}}{\text{Glucose Concentration}}$$

Values for glucose mean residence time, pool size, space and

metabolic clearance rates quoted in the text are those calculated from

the data given by analysis of $[^3H]$-3-glucose specific activity.

Radioactive glucose "recycling", as defined by Streja <u>et al</u>.

was estimated by the difference between glucose turnover calculated

by ^3H-3-glucose and that by ^{14}C-1-glucose (Recycling = $RT^3H - RT^{14}C$).

"Recycling" was also expressed as a percentage of total (RT^3H) glucose

turnover (% "recycling" = $\dfrac{RT^3H - RT^{14}C}{RT^3H} \times 100$)

Double exponential functions were fitted to the specific activity (SA) -

time (t) curves for ^3H and ^{14}C glucose by linear least squares

regression using an iterative program on a mini-computer (Apple II

Europlus, Cupertino, USA). For the number of data points (n = 17)

polyexponential regression provided a better estimate of t than the

trapezoidal rule where SA approximated towards zero. The correlation

of curve fit was 0.95 (0.92 - 0.99).

Fuller consideration of the mathematical concepts are given in

the Introduction (4.4.d). Statistical analyses were performed using

Student's paired and unpaired t-tests as appropriate. Serum insulin

and plasma glucagon levels showed a logarithmic distribution and

statistical analyses were performed on log-transformed data.

Correlations were sought by the least squares method or the Spearman

Rank method as appropriate. Values in the text are given as mean ±

standard error of the mean (SEM) or mean and range.

9.3. RESULTS

In both hyperthyroid and hypothyroid subjects serum thyroxine,

triiodothyronine and thyroid stimulating hormone concentrations

returned to normal with treatment (Table 9:1). Fasting blood glucose

concentrations and blood concentrations of the gluconeogenic

precursors lactate, pyruvate and alanine were similar in hyper-

and hypothyroid subjects and control subjects and were unaltered by

therapy (Table 9:2). In hyperthyroidism fasting blood glycerol

concentrations were elevated compared with control values ($0.12 \pm$

0.02 vs 0.06 ± 0.02 mmol/l, $p < 0.05$) and fell to normal with therapy.

Blood glycerol concentrations were normal in hypothyroidism. Serum

insulin and plasma glucagon concentrations were similar in all groups.

Total glucose turnover (RT [^{3}H]) was increased in hyperthyroidism

(18.2 ± 1.0 vs 12.4 ± 0.9 μmol/kg/min, $p < 0.01$) and decreased in

hypothyroidism (8.2 ± 0.3 vs 11.7 ± 1.2 μmol/kg/min, $p < 0.01$)

(Tables 9:3 and 9:4) and returned to normal with treatment. Glucose

turnover rates derived from the [^{14}C]-1-glucose data (RT[^{14}C]) were

similar in hyperthyroid and control subjects (hyperthyroid, 13.4 ± 1.1

vs control, 11.2 ± 0.9 μmol/kg/min) although a small but significant

decrease was seen after antithyroid therapy (12.3 ± 1.2 μmol/kg/min,

$p < 0.05$). In hypothyroid subjects RT[^{14}C] was decreased (6.9 ± 0.4

vs 10.2 ± 0.8 μmol/kg/min, $p < 0.01$) and increased with thyroxine

therapy (8.2 ± 0.8 μmol/kg/min).

The difference between RT[^{3}H] and RT[^{14}C] was increased

four fold in hyperthyroidism (4.7 ± 0.6 vs 1.3 ± 0.3 μmol/kg/min,

$p < 0.001$) with a 2.7 fold increase in the recycling index (RT[^{3}H]-

RT[^{14}C] x 100) (Table 9:3). Both returned to normal with therapy.

By contrast, apparent recycling was not significantly altered in

hypothyroidism (Table 9:4). Metabolic clearance rate for glucose was

TABLE 9.3. Glucose turnover rates, "recycling", mean residence time, pool, space and metabolic clearance rates in hyperthyroid subjects before and during therapy and in controls.

Subject No. Patient	Absolute Glucose Turnover (RT^3H) $\mu mol\ kg^{-1} min^{-1}$ Before	During	^{14}C derived glucose Turnover $(RT^{14}C)$ $\mu mol\ kg^{-1} min^{-1}$ Before	During	$RT^3H-RT^{14}C$ [a] $\mu mol\ kg^{-1} min^{-1}$ Before	During	$\frac{RT^3H-RT^{14}C}{RT^3H} \times 100$ [b] Before	During	Mean Residence Time (min) Before	During	Glucose Pool $(\mu mol/kg)$ Before	During	Glucose Space (% body wt) Before	During	Metabolic Clearance Rate $(ml\ kg^{-1} min^{-1})$ Before	During
1	16.6	11.1	10.8	10.5	5.8	0.6	35	5	60	74	969	818	20	20	3.5	2.5
2	18.5	13.5	13.7	13.4	4.8	0.1	26	1	83	89	1535	1210	33	28	3.8	3.0
3	17.7	13.3	12.7	11.8	5.0	1.5	28	11	81	80	1431	1169	21	19	2.8	2.2
4	21.6	18.5	18.4	16.5	3.2	2.0	15	11	71	72	1530	1332	34	28	4.7	4.0
5	14.4	11.9	11.4	9.4	3.0	2.5	27	21	79	88	1141	1046	20	19	2.4	1.7
6	19.9		13.5		6.4		32		69		1375		29		3.8	
Mean	18.1 ●●	13.7 ***	13.4	12.3 *	4.7 ●●●	1.3 *	27 ●●●	10 *	74 ●●	81	1330	1113 **	26	23	3.5 ●	2.7 **
SEM	1.0	1.3	1.1	1.2	0.6	0.4	3	3	4	3	93	87	3	2	0.3	0.4
Control 1	14.9		12.4		2.5		17		85		1265		25		2.7	
2	9.6		8.3		1.3		14		116		1109		22		1.6	
3	13.6		12.5		1.1		8		101		1363		27		2.5	
4	13.6		13.6		0		0		93		1261		25		2.8	
5	13.1		11.5		1.6		12		84		1121		22		2.6	
6	9.7		8.7		1.6		10		101		981		20		1.9	
Mean	12.4		11.2		1.3		10		97		1183		24		2.1	
SEM	0.9		0.9		0.3		2		5		56		1		0.2	

* $p < 0.05$ untreated patients vs treated patients
** $p < 0.01$ "   "   "   "
*** $p < 0.001$ "   "   "   "

● $p < 0.05$ untreated patients vs controls
●● $p < 0.01$ "   "   "
●●● $p < 0.001$ "   "   "

a   amount of glucose "recycled"

b   % "recycled"

TABLE 9.4. Glucose turnover, "recycling", mean residence time, pool, space and metabolic clearance rates in hypothyroid subjects before and during therapy and in controls

Subject No. Patient	Absolute Glucose Turnover (RT^{3}H) μmol kg^{-1} min^{-1}		^{14}C derived glucose turnover (RT^{14}C) μmol kg^{-1} min^{-1}		RT^{3}H-RT^{14}C [a] μmol kg^{-1} min^{-1}		$\dfrac{\text{RT}^3\text{H-RT}^{14}\text{C}}{\text{RT}^3\text{H}}$ x 100 [b] %		Mean Residence Time (mins)		Glucose Pool (μmol/kg)		Glucose Space (% body wt)		Metabolic Clearance (ml kg^{-1} min^{-1})	
	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During	Before	During
1	9.5	9.7	8.3	7.9	1.2	1.8	13	19	95	151	909	1460	19	27	1.9	2.0
2	7.8	8.6	6.5	6.5	1.3	2.1	17	24	193	156	1511	1335	28	26	1.5	1.7
3	7.2	8.5	6.1	7.1	1.1	1.4	16	17	105	75	756	678	16	18	1.6	1.7
4	8.5	12.3	5.6	11.1	2.9	0.6	34	5	153	101	1307	1204	25	26	1.5	2.5
5	7.0	9.2	6.2	8.5	0.8	0.7	11	8	184	120	1288	1100	28	24	1.5	2.1
6	8.8	11.3	8.2	10.7	0.6	0.6	7	5	159	108	1395	1223	28	23	1.4	1.8
7	8.7		7.7		1.0		7		138		1196		21		1.5	
Mean	8.2••	9.9*	6.9•••	8.7	1.3	1.2	16	13	146•	119	1195	1172	24	24	1.6•••	2.0*
SEM	0.3	0.6	0.4	0.8	0.8	0.3	3	3	14	13	102	110	2	1	0.1	0.1
Control																
1	9.1		8.2		0.9		10		82		747		19		1.8	
2	12.6		11.3		1.3		10		116		1462		25		2.5	
3	16.1		13.0		3.1		19		83		1337		27		2.8	
4	9.7		8.5		1.2		12		95		921		21		2.1	
5	13.4		11.7		1.7		13		111		1596		30		2.7	
6	9.2		8.5		0.7		7		117		1078		22		2.7	
Mean	11.7		10.2		1.5		12		101		1190		24		2.3	
SEM	1.2		0.8		0.4		2		7		134		2		0.2	

*   p < 0.05 untreated patients vs treated patients

•   p < 0.05 untreated patients vs controls

••   p < 0.01   "   "   "   "

••• p < 0.001   "   "   "   "

a   amount of glucose "recycled"

b   % "recycled"

also increased (3.5 ± 0.3 vs 2.4 ± 0.2 ml/kg /min , $p < 0.05$)

and decreased (1.6 ± 0.1 vs 2.3 ± 0.2 ml/kg /min , $p < 0.001$) in

hyper- and hypothyroidism respectively. Glucose space was similar

in all groups and was unaffected by treatment. In hyperthyroid

subjects the glucose pool was not significantly different from control

values although a 15% decrease ($p < 0.01$) was observed after

antithyroid therapy. Values for the glucose pool were normal in

hypothyroid subjects. Mean residence time was decreased in

hyperthyroid subjects (74 ± 4 vs 97 ± 5 mins, $p < 0.01$) and increased

in hypothyroid subjects (145 ± 14 vs 107 ± 7 mins, $p < 0.05$). Normal

values were restored with therapy.

Serum thyroxine and triiodothyronine concentrations did not

correlate within patient or control groups with any parameter of glucose

metabolism assessed. Similarly no correlations were observed for

body weight in the hypothyroid group and their controls where the

latter tended to be younger and lighter than the hypothyroid group

before treatment.

9.4.   DISCUSSION

Turnover estimates made with the use of $[^{14}C]$-1-glucose

are lower due to carbon recycling back to glucose at any level in the

glycolytic-gluconeogenic pathway (Streja et al. 1977; Reichard et al.

1963; Nosadini et al. 1982), although the bulk of this is considered to

occur from lactate and pyruvate (Streja et al. 1963). The difference

between the turnover estimates with these two forms of labelled glucose

is therefore thought to provide an index of the amount of glucose cycled

to 3-carbon intermediates and back.

Total glucose turnover was increased by 48% in hyperthyroidism and decreased by 30% in hypothyroid subjects. Similar findings have been obtained by other workers using isotopic methods in man (Saunders et al. 1980) and animals (Okajima & Ui, 1979). Sixty percent of the increase in glucose turnover noted in this study could be accounted for by the increase in recycling and only 40% by an increase in glucose metabolism by other routes. In contrast to these findings a decrease in hepatic glucose production has recently been observed in hyperthyroid man using catheterisation techniques to obtain estimates of splanchnic glucose exchange (Wahren et al. 1981). The reason for these disparate findings is unclear as the hyperthyroid subjects studied appear similar in age, weight and severity of disease. In other conditions, both techniques may be expected to produce similar estimates of glucose production (Radzuik et al. 1978) and there is no evidence for an increase in renal glucose output in hyperthyroidism. A possible explanation may reside in the dietary preparation before study. A carbohydrate intake in excess of 250 g daily for 48 hours before investigation may have ensured greater glycogen stores in the patients reported in the present study.

Increased glucose turnover may result from an increase in either glycogenolysis or gluconeogenesis. After an overnight fast, hepatic glycogen stores are diminished in hyperthyroidism (Pipher & Paulsen, 1947) and increased gluconeogenesis is considered the major factor (Wahren et al. 1981). Rat liver perfusion experiments have

shown thyroid hormone dependent increases in glucose production

from lactate (Menahan & Weiland, 1969), alanine (Singh & Snyder, 1978)

and glycerol (Sestoft et al. 1977) and the activity of certain gluconeogenic

enzymes (Mennahan & Weiland, 1969; Szepesi & Freedland, 1969)

particularly pyruvate carboxylase (Böttiger et al. 1970), is raised in

the livers of hyperthyroid animals. In the present study, fasting blood

glycerol concentrations were elevated in hyperthyroidism, as found by

other workers (Tibbling, 1969). Glycerol is metabolised largely to

glucose and its uptake is proportional to its concentration in blood

(Bortz et al. 1972). Circulating concentrations of the other

gluconeogenic precursors were normal in the present study but flux

was not measured. Others have, however, reported increased

splanchnic fractional uptake of these substrates in hyperthyroid man

with a return to normal with treatment (Wahren et al. 1981).

Absolute amounts of glucose recycled were increased 3. 6 fold

in hyperthyroidism and percentage recycling also rose (2. 7 fold). This

process is energetically wasteful but represents only a trivial amount of

energy lost: 10. 5 kcal/24 h, assuming ATP equivalent of 7. 5 kcal/mole

and wastage of 4 ATP molecules per molecule of glucose recycled as

the sole energy deficit. However isotopic estimation of recycling may

underestimate total chemical recycling because of interchange between

labelled and unlabelled carbon in substrate pools such as that for

oxaloacetate (Krebs et al. 1966). Furthermore the isotopes used in

the present study will not estimate glucose cycled in "futile" cycles

such as that between glucose and glucose-6-phosphate, activity of which

may be increased by thyroid hormones (Okajima & Ui, 1979). The mechanisms controlling such "futile" cycles are unclear but are independent of catecholamine action in thyroid excess in animals (Okajima & Ui, 1979b).

Increased hepatic glucose production has been attributed to increased glucagon secretion (Kabadi & Eisenstein, 1980). Circulating glucagon levels were however normal in the present and one other study (Wahren et al. 1981). Alternatively increased secretion or increased sensitivity to catecholamines has been postulated (Landsberg & Young, 1978). Catecholamine levels were not measured in the present study but glucose turnover in hyperthyroidism is not restored to normal by β-adrenergic blockade (Saunders et al. 1980).

Similarly, alterations in circulating insulin concentrations can not be implicated being normal in this and other (Sieno et al. 1974), studies although patterns of insulin secretion were not examined. However changes in portal insulin and glucagon concentrations cannot be excluded. It has been suggested that alterations in insulin sensitivity may occur although the evidence is conflicting with enhanced (Elrick et al. , 1961; Elgee & Williams, 1955) normal (Ambrosolini et al. 1956) and diminished (Doar et al. 1969; Holdsworth & Besser, 1968) insulin sensitivity all having been reported in hyperthyroidism.

In the present study peripheral disposal of glucose, assessed by glucose clearance, was diminished in hypothyroidism and elevated in hyperthyroidism with both abnormalities reversed by treatment. However, a major abnormality of insulin action is unlikely in view of

the normal blood glucose concentrations in both hyperthyroid and hypothyroid subjects.

Finally, body weight is a determinant of glucose turnover and glucose turnover may be reduced in the grossly obese (Paul & Bortz, 1969). In the present study, with a smaller range of body weights, no correlation between weight and glucose turnover was seen within the control subjects. Patients were matched by weight to separate control groups and differences in turnover are therefore more likely to be related to thyroid status.

In conclusion, glucose turnover and recycling were markedly enhanced in hyperthyroid subjects. The greater proportion of the increase in turnover was due to the increase in recycling of 3-carbon intermediates. These abnormalities could not be accounted for by alterations in glucose space or changes in the two main glucoregulatory hormones insulin and glucagon. In hypothyroid subjects glucose turnover representing both production and utilisation were decreased. In both groups of subjects values returned to normal with treatment. The precise mechanisms of these changes remains to be elucidated.

10. GLUCONEOGENIC CAPACITY FROM GLYCEROL: EFFECTS OF HYPER AND HYPOTHYROIDISM

10.1. INTRODUCTION

Total glucose production is increased in hyperthyroidism (Chapter 9) (Saunders et al. 1980; Perez et al. 1980) and decreased (Chapter 9) or normal (Saunders et al. 1980) in hypothyroid man and blood glycerol concentrations may be elevated in the former state (Chapter 7) (Tibbling, 1969). The relative contributions glycogenolysis and gluconeogenesis to these changes in glucose production are not, however known.

Animal and in vitro studies have suggested that thyroid hormones may regulate gluconeogenesis from the major gluconeogenic precursors lactate (Menahan & Weiland, 1969; Weinberg & Utter, 1979; Okajima & Ui, 1979) alanine (Ness et al. 1969) and glycerol (Sestoft et al. 1977). In the rat, thyroid hormones cause the induction of l. glycerophosphate dehydrogenase in liver mitochondria (Lee et al. 1959); the activity of this enzyme playing a central role in the regulation of glycerol utilisation by determining the rate of carbon transfer into gluconeogenesis (Schimassek et al. 1963). In normal man and animals glycerol is highly gluconeogenic (Nikkila & Ojala, 1964; Bortz et al. 1972) and is metabolised to glucose in preference to the other gluconeogenic precursors (Steele et al. 1971). In fasting man 80-100% of circulating glycerol is converted to glucose (Bortz et al. 1972; Shaw et al. 1976),

conversion increasing as blood concentrations rise (Winkler et al. 1970).

The liver is responsible for 70-90% of total glycerol uptake (Larsen, 1963;

Gidez & Karnowsky, 1954; Swick & Nakao, 1954; Schambye et al. 1954)

with the remainder metabolised by the kidneys (Borchgrevink & Havel,

1963; Swanson & Thompson, 1969), small intestine and other tissues

(Saunders & Dawson, 1967; Haessler & Isselbacher, 1963; Lin, 1977).

Clearance of glycerol from blood following intravenous glycerol loading

may therefore provide an estimate of the gluconeogenic capacity of

the liver in normal man and in patients with thyroid dysfunction.

(See Chapter 4.4b for discussion of clearance and tolerance tests).

Glycerol clearance has therefore been examined in patients with

thyrotoxicosis, primary thyroid failure and in normal controls using

a bolus intravenous technique with subsequent analysis of decay of

blood glycerol levels.

10.2. MATERIALS AND METHODS

Seven hyperthyroid (6 female, 1 male) and six hypothyroid

(6 F) were compared with separate groups of matched controls (7 F

and 8 F) (Table 10:1). Hyperthyroidism was due to Graves' disease

in all cases (on the basis of clinical features and the absence of thyroid

nodules on ^{99}Tc scanning). Hypothyroidism was due to Hashimotos

thyroiditis in all patients (on the basis of clinical features and the

presence of circulating antibodies to thyroid tissue). No subject had

any evidence of hepatic or renal impairment and none was taking any

drug prior to the study.

Studies were performed on recumbent subjects after an

overnight (10 h) fast. Subjects had abstained from alcohol for at least
48 h and had a daily carbohydrate intake in excess of 250 g for this
period. Bilateral indwelling intravenous cannulae (Venflon, Viggo
Helsinborg) were inserted using 1% lignocaine as local anaesthetic.
Cannula patency was maintained by flushing with 0.154 mol/l saline
after every sample.

Basal samples for hormones and metabolites were withdrawn
25 and 30 minutes after cannulation and assayed as in 6.2. and 6.3.
An intravenous bolus injection of glycerol (0.1 mmol/kg body weight)
diluted with water to isotonicity was then administered over 15 seconds
and further blood samples were taken 1, 2, 3, 4, 5, 6, 8, 10, 12.5,
15, 20, 30, 40, 50 and 60 minutes after injection.

Results are expressed as mean $\pm$ standard error of the mean
(SEM). Statistical analysis was performed using Student's unpaired
t-test. Correlations were sought by the least squares method.
3-Hydroxybutyrate concentrations were log normally distributed and
statistical analyses were performed on log transformed data.

Mathematical Analysis

The decay of blood glycerol following bolus intravenous injection
was closely approximated by a double exponential function of the form
$A e^{-k_1 t} + B e^{-k_2 t}$ where A, B, k_1, and k_2 are constants. Curve fitting
was performed by computer to the points of the blood glycerol decay
curve, after subtraction of basal glycerol concentrations. The area
under the curve was calculated using a polyexponential equation.
Metabolic clearance is equal to the dose of glycerol injected divided

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by the area under the glycerol decay curve

$$M.C.R. \ (1/min) \ = \ \frac{mmol \ injected}{mmol/1/min}$$

Endogenous glycerol production (E. P. R.) is equal to the product of metabolic clearance and basal glycerol concentration

$$EPR \ (mmol/min) \ = \ MCR \ x \ Basal \ [glycerol]$$

It is assumed in these calculations that the endogenous production rate of glycerol remains constant throughout the experimental period. Volume of distribution of glycerol was calculated using the intercepts and exponents for the α and β phases of the glycerol decay curve (Gibaldi, 1969).

10.3. RESULTS

Serum thyroxine and triiodothyronine concentrations were increased in hyperthyroid and decreased in hypothyroid patients when compared to controls (Table 10:1.). Serum thyroid stimulating hormone concentrations were increased in hypothyroidism.

Peak blood glycerol concentrations following glycerol injection ranged between 0.35 and 1.20 mmol/1 and were not significantly different in any group. Glycerol injection had no effect on any of the other metabolite concentrations measured in any group nor on peripheral insulin levels.

Hyperthyroid Subjects

Fasting blood glucose (5.4 ± 0.2 vs 4.6 ± 0.1 mmol/1, $p < 0.01$) (Table 10:2) and blood glycerol (0.12 ± 0.01 vs 0.07 ± 0.01 mmol/1, $p < 0.01$) (Table 10:3) concentrations were increased in hyperthyroid subjects but basal circulating lactate, pyruvate, alanine,

TABLE 10:1.   Clinical details of hyperthyroid, hypothyroid and
                control subjects. Results are mean ± SEM

	Age (yr)	Percent of ideal body weight	Serum thyroxine (nmol/l)	Serum triiodo-thyronine (nmol/l)	Serum thyroid stimulating hormone (mU/l)
Hyperthyroid	34±4	104±3	181±13 ***	4.5±0.3 ***	1.0±0.02
Control	34±3	108±4	97±9	2.0±0.2	1.5±0.2
Hypothyroid	48±1	123±5	28±9 ***	0.9±0.2 **	40.1±7.1 ***
Control	45±2	118±4	89±8	2.0±0.2	1.6±0.5

***   p < 0.001 versus appropriate controls.

**   p < 0.01 versus appropriate controls.

TABLE 10:2. Fasting hormone and metabolite concentrations (mean $\pm$ SEM) in hyperthyroid, hypothyroid and control subjects. For 3-hydroxybutyrate mean (range) is given.

Subjects	Blood Glucose (mmol/l)	Blood Lactate (mmol/l)	Blood Pyruvate (mmol/l)	Blood Alanine (mmol/l)	Blood 3-hydroxybutyrate (mmol/l)	Serum Insulin mu/l
Hyperthyroid	5.4±0.2**	0.94±0.15	0.09±0.01	0.28±0.02	0.08 (0.03-0.18)	7.8 ± 1.0
Control	4.6±0.1	0.88±0.16	0.07±0.01	0.30±0.04	0.08 (0.03-0.18)	8.8 ± 1.5
Hypothyroid	5.1±0.4	1.10±0.07	0.10±0.01	0.31±0.02	0.04 (0.01-0.14)	9.4 ± 1.8
Control	4.9±0.2	0.99±0.15	0.08±0.04	0.34±0.04	0.05 (0.005-0.14)	8.4 ± 1.3

** $p < 0.01$ hyperthyroid subjects vs controls.

TABLE 10:3. Glycerol kinetic data in hyperthyroid and control subjects

Subject	Basal blood glycerol (mmol/l)	Metabolic clearance rate (ml/kg/min)	Volume of distribution (l/kg)	k_1 (min^{-1})	k_2 (min^{-1})	Half disappearance time for 1st component (min)	Half disappearance time for 2nd component (min)	Endogenous production rate (μmol/kg/min)
Hyperthyroid								
1	0.16	41.9	0.43	3.89	0.228	0.2	3.0	6.62
2	0.07	56.8	0.49	6.04	0.136	0.1	5.1	3.69
3	0.13	76.9	0.45	1.52	0.135	0.5	5.1	9.61
4	0.14	44.0	0.80	0.58	0.097	1.2	7.1	6.92
5	0.09	47.2	0.47	1.25	0.211	0.6	3.3	4.29
6	0.12	46.0	0.66	0.51	0.094	1.4	7.0	5.29
7	0.11	27.6	0.50	1.01	0.067	0.7	10.6	3.26
Mean	0.12	48.7	0.54	2.05	0.138	0.7	5.9	5.67
S.E.M.	0.01	5.7	0.06	0.81	0.022	0.2	1.0	0.84
Controls (n = 7)								
Mean	0.07	27.2	0.63	0.83	0.070	1.1	11.9	2.1(2)
S.E.M.	0.01	2.6	0.08	0.02	0.01	0.2	2.3	0.3(4)
p	< 0.01	< 0.005	NS	NS	< 0.05	NS	< 0.05	< 0.01

NS, $p > 0.05$.

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TABLE 10:4. Glycerol kinetic data in hypothyroid and control subjects

Subject	Basal blood glycerol (mmol/l)	Metabolic clearance rate (ml/kg/min)	Volume of distribution (l/kg)	k_1 (min^{-1})	k_1 (min^{-1})	Half disappearance time for 1st component (min)	Half disappearance time for 2nd component (min)	Endogenous production rate (μmol/kg/min)
Hypothyroid								
1	0.04	16.1	0.59	0.41	0.018	1.7	38.3	0.70
2	0.04	16.8	0.68	0.22	0.027	3.2	26.0	0.59
3	0.04	16.0	0.69	0.55	0.016	1.3	43.2	0.63
4	0.07	16.5	0.48	0.82	0.059	0.8	11.8	1.14
5	0.07	20.5	0.65	0.40	0.024	1.7	28.8	1.80
6	0.09	16.6	0.52	0.42	0.036	1.7	19.2	1.49
Mean	0.06	17.1	0.60	0.46	0.030	1.7	27.9	1.05
S.E.	0.01	0.7	0.04	0.08	0.006	0.3	4.7	0.21
Controls (n=8)								
Mean	0.08	25.0	0.52	1.01	0.076	0.92	10.8	2.07
S.E.M.	0.01	1.8	0.09	0.17	0.010	0.22	1.6	0.21
p	NS	< 0.01	NS	< 0.05	< 0.01	< 0.05	< 0.01	< 0.01

NS, $p > 0.05$.

· FIGURE 10:1.   Change in blood glycerol concentration from basal
levels following bolus intravenous glycerol
(0.1 mmol/kg) administration in
a) hyperthyroid (■) and control (□) and
b) hypothyroid (●) and control (○) subjects.
Blood glycerol is shown on a logarithmic scale.

3-hydroxybutyrate and insulin levels were similar to controls (Table 10:2).

The double exponential decay curve for injected glycerol is clearly demonstrated in Figure 10:1 ($r = 0.98 \pm 0.003$). Values for the second exponential constant (k_2) were increased in hyperthyroidism (0.14 ± 0.02 vs 0.07 ± 0.01, $p < 0.05$) and as a result the second component half time was shorter (5.9 ± 1.0 vs 11.9 ± 0.3 mins, $p < 0.05$). Glycerol clearance rate was also increased in hyperthyroidism (48.7 ± 5.7 vs 27.2 ± 2.6 ml/kg/min, $p < 0.005$) as was endogenous glycerol production (5.7 ± 0.8 vs 2.1 ± 0.3 μmol/kg/min, $p < 0.01$) (Table 10:3).

Hypothyroid Subjects

In hypothyroidism, fasting circulating concentrations of glucose, lactate, pyruvate, alanine, glycerol, 3 hydroxybutyrate and insulin were similar to control values (Table 10:2). Both the first ($p < 0.05$) and second component ($p < 0.01$) half times were significantly prolonged in hypothyroidism (Table 10:4). Glycerol clearance rate was decreased (hypothyroid 17.1 ± 0.7 vs control 25.0 ± 1.8 ml/kg/min, $p < 0.01$) and endogenous glycerol production rate was diminished (1.1 ± 0.2 vs 2.1 ± 0.2 μmol/kg/min, $p < 0.01$) in hypothyroid patients.

Correlations

Correlations between glycerol kinetic data, metabolic status and thyroid hormone levels did not reach significance in either patient or control groups.

10.4. DISCUSSION

These studies show that in man hyperthyroidism is associated

with enhanced glycerol clearance after bolus injection of glycerol

which is strongly suggestive of increased gluconeogenic capacity.

No estimate of basal gluconeogenesis is possible from our data but

a 270% increase in endogenous production with only a 70% increase in

fasting levels implies enhanced basal glycerol clearance and hence,

perhaps, increased gluconeogenesis. Converse changes were noted

in hypothyroid subjects.

Fasting blood glucose concentrations were increased in

hyperthyroidism in this, as in other (Chapter 7, Ikejiri

et al. 1978) studies whilst lactate pyruvate and alanine concentrations

were normal. Alone amongst the gluconeogenic precursors,

glycerol levels were increased presumably secondary to enhanced

lipolysis (Chapter 7, Chapter 9, Tibbling, 1969; Brodie et al. 1966;

Rich et al. 1959). In hypothyroidism blood glucose, lactate, pyruvate

and glycerol levels were normal, as has previously been reported

(Chapter 8, Chapter 9).

Hepatic glucose production measured isotopically has been

shown to be increased in hyperthyroidism (Chapter 9) (Saunders et al.

1980; Perez et al. 1980) and diminished (Chapter 9) or normal

(Saunders et al. 1980) in hypothyroidism. Such data does not

distinguish between glycogenolysis and gluconeogenesis nor does it

give information about the capacity of those two processes. In

fasting man, glycerol taken up by the liver enters the gluconeogenic

pathway (Fig. 10:2) and thus clearance of an intravenous glycerol load

provides an estimate of gluconeogenic capacity. Previous studies of

gluconeogenesis in patients with thyroid disease are few. In hyperthyroidism, splanchnic balance studies suggest that hepatic uptake of all the major gluconeogenic precursors is increased (Wahren et al. 1981). For lactate, pyruvate and alanine this is achieved by an increase in fractional extraction whilst for glycerol increased arterial concentrations combined with a 20% increase in hepatic blood flow are responsible. Apart from a single study in congenital hypothyroidism (Hayek, 1979) showing normal gluconeogenesis from alanine, there have been no estimates of gluconeogenesis in hypothyroid man.

The metabolic clearance rate for glycerol is the best index of glycerol disappearance in the kind of study used here as its measurement (amount of glycerol administered divided by $\int_{o}^{\infty}$ delta blood glycerol) is independant of any descriptive compartmental model and its estimate is valid even if glycerol elimination occurs outside the sampling compartment (Wilkinson & Shand, 1971). The amount of glycerol used in this study was chosen to give peak blood glycerol values between 0. 4 and 1. 5 mmol/l. This avoided the very high blood glycerol levels which have been reported to cause renal damage in man and animals (Hagnevick et al. 1974; Anderson et al. 1950). Much higher doses than those used in this study have been used therapeutically in man without adverse effects (Senior & Loridan, 1968).

Glycerol clearance rate was increased by 80% in hyperthyroidism and decreased by 30% in hypothyroidism. The mechanism(s)

underlying these changes are uncertain. Glycerol taken up by the liver (Fig. 10:2) is first phosphorylated by glycerol kinase and enters the gluconeogenic pathway after oxidation by glycerol kinase and enters the gluconeogenic pathway after oxidation by glycerol-3-phosphate dehydrogenase (G3PD). Glycerol kinase is subject to feedback inhibition by glycerol 3 phosphate (Robinson & Newsholme, 1969; Sestoft & Fleron, 1975). In the rat, the activity of mitochondrial G3PD is thyroid hormone dependent (Hoch, 1974) and has long been considered an intracellular marker of thyroid hormone action. Alteration of mitochondrial G3PD activity in our subjects is thus a possible explanation of the observed changes in glycerol metabolic clearance rates. However, Nolte et al. (1972) in an as yet unconfirmed study, failed to demonstrate any increase in mitochondrial G3PD activity in thyrotoxic man.

Altered hepatic blood flow may also contribute to the findings. A 20% increase in hepatic blood has previously been directly demonstrated in hyperthyroid patients (Wahren et al. 1981). Circulating (albeit venous) glycerol concentrations of glycerol were increased by 70% in our thyrotoxic subjects. Assuming a similar 20% increase in hepatic blood flow, this would provide a 100% increase in hepatic glycerol delivery in hyperthyroidism. Fractional glycerol extraction need not therefore be increased to account for increased glycerol clearance rates of 80%. This is compatible with the data of Tibbling (1969) who demonstrated that fractional glycerol turnover was not significantly increased in thyrotoxic humans.

FIGURE 10:2.   Gluconeogenesis from glycerol in the liver

Similarly, in hypothyroidism cardiac output, stroke volume (Graetinger et al. 1958) and flow through many organs is diminished (Scott et al. 1962; Stewart & Evans, 1942; Zondek, 1967) and may contribute to the decreased glycerol clearance rates reported here. In keeping with the changes in glycerol clearance rates, the fractional rate constants (k_1 and k_2) were approximately doubled in hyperthyroidism and halved in hypothyroidism with appropriate reciprocal changes in the calculated first and second half disappearance times. These changes may result from either haemodynamic factors, primary changes in hepatic glycerol uptake or a combination of these two factors.

Calculated endogenous glycerol production rates for normal subjects (2.1 μmol/kg/min) were similar to those quoted in other studies using different methods (2.2 μmol/kg/min; Johnston et al. (In press), 1.6 μmol/kg/min; Bortz et al. (1972)). Endogenous production rates were increased 3 fold in hyperthyroidism and decreased by 50% in hypothyroidism. These results are entirely consistent with previous studies showing lipolysis to be increased in hyperthyroidism (Chapter 7, Chapter 9, Brodie et al. 1966; Rich et al. 1959) and decreased in hypothyroidism (Hamburger et al. 1963; Goodman & Bray, 1966).

Volumes of distribution for glycerol, although variable, were not significantly different in any group suggesting that the changes in glycerol elimination were not secondary to altered distribution space.

Given the findings described above, it is perhaps a little surprising that blood glucose concentrations did not rise following glycerol administration. No definite reasons can be given for this although several factors may have been important. Firstly glycerol normally contributes less than 10% of total glucose output in overnight fasting man thus any changes in blood glucose concentrations may have been small. Secondly, small changes in arterial blood glucose concentrations may not have been reflected in venous sampling and thirdly, although peripheral insulin concentrations were not increased after glycerol administration, changes in portal insulin concentrations cannot be ruled out and may have suppressed hepatic glucose output.

11. INSULIN SENSITIVITY IN HYPERTHYROIDISM

11.1. INTRODUCTION

The blood glucose concentration of hyperthyroid man has been reported to be elevated following the ingestion of glucose (Holdsworth & Besser, 1968; Kreines et al. 1965) and mixed meals (Chapter 7) in most, but not all (Macho, 1936; Gorowski & Wolanska, 1957) studies. The cause of this hyperglycaemia remains uncertain. Insulin sensitivity in hyperthyroidism has been assessed as normal (Ambrosolini et al. 1956), diminished (Doar et al. 1969; Holdsworth & Besser, 1968) or enhanced Elgee & Williams, 1955; Maracek & Feldman 1973; Elrick et al. 1961). This probably reflects the variety of techniques used to measure insulin sensitivity as well as species differences between man (Holdsworth & Besser, 1968; Kreines et al. 1965; Chapter 7) rat (Ambrosolini et al. 1956; Elgee & Williams, 1955) and rabbit (Maracek & Feldman, 1973). Where glucose tolerance is impaired, the finding of normal (Holdsworth & Besser, 1968, Chapter 7) or elevated (Doar et al. 1969) peripheral insulin levels has been taken to indicate insulin resistance, even where the pattern of insulin secretion has not been examined. Measurement of the rate of fall or nadir of blood glucose concentrations after injection of exogenous insulin in man has produced contradictory results, sometimes suggesting enhanced (Elrick et al. 1961) and sometimes decreased (West et al. 1975) sensitivity to insulin. Such studies are however complicated by the counter-regulatory response to hypoglycaemia which is very variable in both time and degree.

The euglycaemic clamp technique allows assessment of insulin sensitivity in the absence of stimulated counter regulatory hormone secretion (Alberti et al. 1979; De Fronzo et al. 1979; Home et al. 1982). The purpose of this study was to investigate with the aid of a blood glucose controlled glucose infusion system (Biostator, Miles) insulin sensitivity in matched normal and hyperthyroid subjects. Insulin sensitivity is defined as the rate of glucose infusion required to maintain the blood glucose level in the face of a constant infusion of insulin. The response of intermediary metabolites to the insulin infusion has also been examined to give a broader assessment of insulin action.

11. 2. MATERIALS AND METHODS

Subjects

Six female patients with hyperthyroidism due to Graves Disease (diffusely enlarged hyperaemic thyroid glands with no nodules on Technicium scanning) were compared with a matched group of seven healthy female controls (Table 11:1). Patients and controls were all within 10% of ideal body weight, and their ages ranged from 22 to 36 years. They were asked to maintain their normal diet for three days before the study and to fast from midnight on the evening beforehand.

No subject was taking oral contraceptive pills or any other drug and none was known to be suffering from any disease other than thyrotoxicosis.

Protocol

On the morning of the study, three PTFE cannulae (Venflon, Viggo,

TABLE 11:1. Clinical details and circulating thyroid hormone levels in hyperthyroid and control subjects

	Age	Sex	% Ideal Body Weight (% IBW)	Serum Thyroxine nmol/l	Serum Triiodothyronine nmol/l
Hyperthyroid (n = 6)	28 ± 2	6 F	100 ± 3	276 ± 33***	8.5 ± 1.6***
Controls (n = 7)	24 ± 1	7 F	100 ± 2	107 ± 4	2.0 ± 0.1

*** $p < 0.001$ Hyperthyroid subjects vs controls.

Helsinborg) were inserted i. v. into each subject. One cannula, in an antecubital fossa, was used for intermittant blood sampling for hormones and metabolites and was flushed with 0.15 mol/1 saline after use. A second cannula, inserted retrogradely and more distally on the same arm was used for continuous sampling of arterialised blood by the biostator. Arterialisation of venous blood was achieved using a heated hand warming system (Abumrad et al. 1981). The third cannula, on the contralateral arm, was used for all infusions. A period of one hour was allowed for equilibration following cannulation, calibration of the machine and to achieve stability of the glucose sensor response.

At the end of calibration (time 0 minutes) highly purified porcine insulin (Actrapid, Novo) was infused i. v. at a rate of 0.05 u/kg body weight. The biostator constants and clamping technique were as described by Home et al. (1982). Blood glucose was clamped 0.28 mmol/1 (5 mg/dl) below fasting levels to avoid stimulation of endogenous insulin during oscillation around the intended clamp value. The insulin infusion continued for 120 minutes and blood samples were taken at -30, 0 and every 15 minutes thereafter for 2 hours. Blood for glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate was assayed as described in 6.2 (Lloyd et al. 1978). Serum insulin was measured by double antibody radioimmunoassay (Soeldner & Slone, 1965) (sensitivity 2 mU/1 : intraassay coefficient of variation (CV) 7%) using human insulin as standard. C-peptide was measured by radioimmunoassay with ethanol

precipitation (Heding, 1975) (sensitivity 0.02 nmol/l, within assay

CV 3%). Serum triiodothyronine (T_3) and serum thyroxine (T_4) were

measured by radioimmunoassay.

Statistics

Results were analysed by standard parametric methods and

expressed as mean $\pm$ standard error unless otherwise indicated. Blood

3-hydroxybutyrate concentrations were log normally distributed and

statistical analysis was performed on log transformed data. Students

independant t-test was employed to detect differences between groups.

Insulin sensitivity was calculated as the amount of glucose infused to

maintain euglycaemia in the second hour of insulin infusion (60-120 min)

when the glucose infusion rate was relatively constant.

11.3. RESULTS

Serum thyroxine (hyperthyroid 276 $\pm$ 33 vs control 107 $\pm$

4 nmol/l, $p < 0.001$) and triiodothyronine (8.5 $\pm$ 1.6 vs 2.0 $\pm$ 0.1

nmol/l, $p < 0.001$) were increased in hyperthyroid subjects (Table 11:1).

Basal values for blood glucose, lactate, pyruvate and alanine

were similar in the two groups (Table 11:2), but glood glycerol

(hyperthyroid 0.11 $\pm$ 0.02 vs control 0.06 $\pm$ 0.01 mmol/l, $p < 0.01$)

and blood 3-hydroxybutyrate (0.28 [0.03 - 0.79] vs 0.09 [0.01 - 0.29]

mmol/l (mean and range), $p < 0.05$) were increased in hyperthyroidism.

Basal serum insulin (hyperthyroid 7 $\pm$ 1 vs control 6 $\pm$ 2 mu/l and

C-peptide (0.32 $\pm$ 0.06 vs 0.33 $\pm$ 0.03 nmol/l) were however identical.

During insulin infusion, plateau serum insulin values were similar in

the two groups (hyperthyroid 44 $\pm$ 2 vs control 44 $\pm$ 1 mU/l) (Fig. 11:1).

TABLE 11:2. Blood intermediary metabolite levels before and after a 2h. infusion of porcine insulin
(0.05 u/kg/h) in hyperthyroid and control subjects

		Glucose mmol/l	Lactate mmol/l	Pyruvate mmol/l	Alanine mmol/l	Glycerol mmol/l	3-Hydroxybutyrate mmol/l
Hyperthyroid (n = 6)	Basal	5.2±0.3	0.63±0.03	0.06±0.005	0.23±0.03	0.11±0.02**	0.28 (0.03-0.79)*
	120min	-	0.96±0.14	0.10±0.01	0.26±0.04	0.05±0.02*	0.01 (0.01-0.03)
	Δ	-	0.33±0.08	0.04±0.01	0.03±0.025	0.06±0.01	0.27 (0.02-0.77)*
Control (n = 7)	Basal	4.9±0.3	0.73±0.07	0.07±0.01	0.28±0.03	0.06±0.01	0.09 (0.01-0.19)
	120min	-	0.99±0.11	0.10±0.02	0.26±0.02	0.02±0.005	0.02 (0.01-0.03)
	Δ	-	0.26±0.09	0.03±0.02	0.02±0.02	0.04±0.01	0.07 (0.01-0.16)

Euglycaemia was maintained by a glucose clamp technique as described in the methods section.
Results are mean ± S.E. except for 3-hydroxybutyrate where mean (range) is given.   Δ =   difference between basal and 120 min values.

* p < 0.05 hyperthyroid vs controls (** p < 0.01).

FIGURE 11.1.   Glucose delivery to maintain blood glucose levels,
                 blood glucose, serum insulin and C-peptide
                 concentrations (mean ± SEM) during infusion of
                 highly purified porcine insulin (0-120 min) in
                 hyperthyroid (●) and control (O) subjects.
                 Values for glucose delivery are expressed as
                 mmol/kg/min and are shown as the mean of each
                 15 min infusion period.

FIGURE 11:2.   Blood lactate, glycerol and 3-hydroxybutyrate
concentrations during insulin infusion with
maintainance of blood glucose by feedback
glucose infusion. For lactate and glycerol mean
± SEM is shown and for 3-hydroxybutyrate mean
value is given. ● = hyperthyroid and
O = control. *P < 0.05 hyperthyroid vs control
(**P < 0.01)

No significant difference was seen in the glucose required to maintain the clamp value at any time.

Blood lactate levels rose to the same extent in both groups (Fig. 11:2, Table 11:2) and blood pyruvate followed a similar pattern. Blood alanine levels were unchanged in both groups during insulin infusion. Blood glycerol concentrations fell in both groups (Fig. 11:2, Table 11:2) although levels in the hyperthyroid patients remained significantly higher than in the controls. Blood 3-hydroxybutyrate levels decreased to similar values in both groups (Fig. 11:2, Table 11:2).

11.4. DISCUSSION

Glucose clamp techniques allow assessment of insulin activity on glucose metabolism without the interference of counter-regulatory responses to hypoglycaemia (De Fronzo et al. 1979; Olefsky & Kolterman 1981). As glucose production will be totally suppressed by the insulin infusion, glucose turnover is equal to the glucose delivered to maintain euglycaemia, which is thus a measure of peripheral insulin activity. For ethical reasons repeated studies to obtain dose response curves were not performed, but the single dose chosen (0.05 u/kg/h) lies on the straight part of the curve in normal subjects (Massi-Benedetti et al. 1981). This infusion rate also gives circulating insulin levels comparable to those seen during glucose tolerance tests (Sestoft & Heding, 1981) and after normal meals (Chapter 7). The highly purified porcine insulin used in this study is known to be identical in activity to human insulin when assessed using the same technique (Home et al. 1982).

In the current study, fasting blood glucose, serum insulin and C-peptide levels were identical in matched hyperthyroid and euthyroid women. No suggestion could be found of any difference in the rate of change or absolute level of glucose turnover during peripheral insulin infusion. Explanations for the glucose intolerance reported by previous studies must therefore be sought elsewhere.

The published evidence for a disturbance of insulin secretion in human and experimental hyperthyroidism is contradictory with enhanced (Doar et al. 1969; Yallow & Berson, 1960) diminished (Daweke et al. 1965; Andreani et al. 1970) or normal (Hales & Hyams, 1964) all being reported. Secretion of biologically inactive insulin in hyperthyroid patients may also be important. As described in 7.4. Sestoft & Heding (1981) have recently demonstrated that hyperthyroid patients secrete a higher proportion of proinsulin to insulin. Although proinsulin cross reacts in the standard insulin radioimmunoassay, it has only 5% of the biological activity of insulin (Sönksen et al. 1973) and hence could cause apparant insulin resistance. Enhanced degradation of insulin has been reported in some (Elgee & Williams, 1955; Maracek & Feldman, 1973) but not all (Wajchenberg et al. 1978) studies, but it is clear from the present study that metabolic clearance rates are unchanged. Increased clearance of insulin cannot in any case explain glucose intolerance as both the action and degradation of insulin are effected via its receptors (Terris & Steiner, 1975).

Basal circulating lactate and pyruvate levels were also similar and rose to the same extent during insulin infusion in patients

and controls. These increases presumably reflect a combination of insulin induced inhibition of hepatic gluconeogenesis and increased hepatic and peripheral glycolysis. Blood alanine concentrations were unchanged by insulin infusion in both groups reflecting a balance of the hepatic and peripheral effects of insulin. 3-Hydroxybutyrate levels were, however, increased in hyperthyroid subjects but suppressed rapidly to control values during insulin infusion. Fasting hyperketonaemia has previously been reported in these patients (Beylot et al. 1980; Bartels et al. 1979) and ketone body levels are known to fall to normal values after mixed meals (Chapter 7) as a result of suppression of lipolysis and ketogenesis. Fasting blood glycerol levels were increased in the hyperthyroid patients but, in contrast to ketone bodies did not suppress to normal values during insulin infusion, perhaps suggesting incomplete suppression of lipolysis. This is consistent with recent studies using adipocytes from hyperthyroid patients which demonstrated impairment of the normal insulin mediated suppression of glycerol release (Wennlund et al. 1981). In accord with this it has already been shown that blood glycerol levels fail to return to normal after mixed meals (Chapter 7).

In summary insulin sensitivity in terms of overall glucose metabolism is normal in hyperthyroidism. Lipolysis is however enhanced at normal fasting insulin levels, and may not be totally suppressed by insulin levels which in normal man profoundly suppresses lipolysis.

12. KETONE BODY KINETICS IN HYPER AND HYPOTHYROIDISM

12.1. INTRODUCTION

Ketone body concentrations in blood are known to rise more
rapidly during fasting in hyperthyroid man than in normal subjects
(Chapter 7, Beylot et al. 1980; Bartels et al. 1979) whilst in
hypothyroidism ketone body concentrations show prolonged suppression
following mixed meals (Chapter 8). Thyroid hormones may influence
ketone body production in the liver either by their effects on
peripheral lipolysis thereby altering NEFA release or by effects on
intrahepatic NEFA metabolism. Both lipolysis (Goodman & Bray, 1966;
Wennlund et al. 1981; Rich et al. 1959) and NEFA turnover (Saunders
et al. 1980) are increased in hyperthyroidism. In hypothyroidism
lipolysis may be diminished (Chapter 8, Arner et al. 1976; Hamburger
et al. 1963) but NEFA turnover is purportedly normal (Saunders et al.
1980).

The effects of thyroid hormone excess and deficiency on ketone
body production and utilisation in vivo are unknown although experiments
using isolated perfused rat liver systems have suggested that hepatic
ketone body production from fixed NEFA concentrations in the
perfusate varies with thyroid status. Isotope dilution studies using
both 3-hydroxybutyrate and acetoacetate as tracers have therefore been
performed in patients with hyper- and hypothyroidism before and
during therapy and in normal subjects.

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5, 6, 8, 10, 12. 5, 15, 20, 30, 40, 50, 60, 75, 90 and 120 mins after tracer

administration. The sampling cannula was flushed with 0.154M saline

between each sample. Subjects remained recumbant during the study

and for at least one hour before.

<u>Preparation of tracer infusate</u>

Ethyl 3-[^{14}C] acetoacetate (250 μCi, specific activity 8.9 mCi/

mmol, Radiochemical Centre, Amersham, U.K.) was hydrolysed to

sodium 3-[^{14}C]-acetoacetate in the presence of freshly redistilled

carrier ethylacetoacetate. Hydrolysis was performed as described

by Nosadini <u>et al.</u> (1980). Radioactive and carrier ethyl acetoacetate

were incubated with 0.5 ml. 2M NaOH at 45°C for 1h. After

neutralisation with 0.5 M HCl and addition of 5 ml distilled sterile H_2O

the solution was washed six times with 15 ml diethyl ether to remove

any impurities and unsaponified ethylacetoacetate then blown with a

stream of nitrogen for 45 mins to remove any radioactive acetone.

Radiochemical purity of sodium 3-[^{14}C]-acetoacetate was ascertained

using the method of Mayes and Felts (1967) by boiling in 4N HCl and

subsequent blowing with a stream of nitrogen. Any radioactivity left

following this treatment is taken to be impurity. The radioactive purity

of sodium 3-^{14}C acetoacetate was determined in each experiment and

was between 90-96%. 250 μCi of 3-[^{14}C]-hydroxybutyrate (specific

activity 56 mCi/mmol, Radiochemical Centre, Amersham, U.K.)

were diluted with 10 ml distilled sterile saline. Both tracer solutions

were sterilised by filtration stored at -20°C in 50 μCi aliquots in

sterile ampoules, and used within 14 days.

Analytical Procedures (Fig.12:1)

Blood for both radioactive and cold acetoacetate and

3-hydroxybutyrate determinations were taken into chilled 20%

perchloric acid and kept on crushed ice until assayed. Samples were

then neutralised with 20% and 5% (w/v) KOH. The radioactive

concentrations of acetoacetate and 3-hydroxybutyrate were determined

as described previously (Nosadini et al. 1982; Bates, 1972)

(Fig.12:1). Duplicate 2 ml aliquots of neutralised supernatant were

boiled for 45 min. in 12 ml of mercuric sulphate/sulphuric acid

solution (6 ml 10% w/v) $HgSO_4$ in $4NH_2SO_4$, 3 ml 50% (w/v) H_2SO_4,

3 ml distilled H_2O) containing 30 µmol carrier lithium acetoacetate

(Sigma Chemical Company). The resulting precipitate (Denige ppt.)

was washed twice with distilled water, dissolved in 0.5 ml 4N HCl,

suspended in 10 ml liquid scintillation fluid (Cocktail T, Hopkins

and Williams) and counted in a liquid scintillation counter

(Intertechnique, Middlesex, England). Efficiency of liquid scintillation

counting was determined using ^{14}C-toluene as an external standard

and each sample count was corrected for quenching. This procedure

gave the counts associated with acetoacetate. Parallel 2 ml aliquots

of neutralised supernatant were incubated for 1 h. at room temperature

in 1 ml 1.5 M tris buffer containing 10 µmol carrier 3-hydroxybutyrate

(Boehringer Corp. Ltd.), 30 µmol NAD (Boehringer Corp. Ltd.) and

37 µl 3-hydroxybutyrate dehydrogenase (specific activity 3 ku/gm;

prepared from Rhodopseudomonas spheroides, Boehringer Corp. Ltd.).

Subsequent boiling in mercuric sulphate/sulphuric acid mixture

FIGURE 12:1.   Pathway for determination of radioactive and
             "cold" ketone body concentrations

NEUTRALISED SAMPLE

Counts associated with 3-hydroxybutyrate = (b) - (a)

assuming steady state blood ketone body concentrations using the

sum of ketone body specific activities (SA) (Keller et al., 1981;

Bates, 1972; Reichard et al. 1974; Ballasse, 1979; Bates et al. 1968).

$$\text{Ketone body SA} = \frac{\text{dpm in Acac} + \text{dpm in BOH}}{[\text{Acac}] + [\text{BOH}]}$$

(For a review of this method see Introduction 4:4d).

The decay in ketone body specific activity approximated

closely to a double exponential function (r = 0.96). The area under

the ketone body SA decay curve was calculated using polyexponential

regression on a mini (Apple II) computer. Kinetic parameters

were calculated for each tracer using the formulae:

Ketone body turnover (KBRT)(μmol /kg/ min) $= \dfrac{\text{Dose of radioactivity administered}}{\int_0^\infty \text{TKSA dt}}$

Mean residence time (MRT)(min) $= \dfrac{\int_0^\infty (t \times \text{TKSA})\, dt}{\int_0^\infty \text{TKSA dt}}$

Ketone Body Pool Size (μmoles) $=$ MRT x RT

Volume of distribution of ketone bodies (V.D.) (% body wt) $= \dfrac{\text{Pool Size} \times 100}{\text{Body wt} \times [\text{TK}]}$

according to Shipley & Clark (1972).

Statistical Analysis

Comparisons between groups were sought using Student's

paired and unpaired t-tests as appropriate. Certain measurements

(insulin, glucagon) were log normally distributed and statistical

analysis was performed on log transformed data. Correlations

were sought using the least squares method or the Spearman

Ranking method as appropriate. Values in the text are given as mean $\pm$ standard error of the mean (SEM) or mean (range).

12.3. RESULTS

Thyroid Hormone Concentrations (Table 12:1)

Serum thyroxine and triiodothyronine concentrations were, as expected, increased in hyperthyroid subjects (T_4 249 $\pm$ 28 vs. 86 $\pm$ 5 nmol/l, $p < 0.001$) and decreased in hypothyroidism (T_4 17 $\pm$ 3 vs. 91 $\pm$ 5 nmol/l, $p < 0.001$). Serum thyroid stimulating hormone (TSH) concentrations were elevated in hypothyroid patients (41.3 $\pm$ 3.1 vs. 1.6 $\pm$ 0.3 mU/l, $p < 0.001$). Normal circulating thyroid hormone and thyroid stimulating hormone concentrations were restored in those patients restudied during therapy (Table 12:1).

Hormone and Metabolite Concentrations (Table 12:2)

Basal hormone and metabolite concentrations are expressed as the mean of the values obtained from the two experiments. Blood glucose, lactate, pyruvate and alanine concentrations were similar in all groups. Blood 3-hydroxybutyrate (0.13 $\pm$ 0.04 vs. 0.05 $\pm$ 0.003 mmol/l, $p < 0.05$) and blood acetoacetate levels (0.11 $\pm$ 0.03 vs. 0.04 $\pm$ 0.01 mmol/l, $p < 0.05$) were increased in hyperthyroid patients as were blood glycerol (0.09 $\pm$ 0.005 vs. 0.05 $\pm$ 0.01 mmol/l, $p < 0.01$) and plasma NEFA concentrations (0.86 $\pm$ 0.07 vs. 0.59 $\pm$ 0.05 mmol/l, $p < 0.01$). Blood glycerol, acetoacetate, 3-hydroxybutyrate and plasma NEFA levels were similar in hypothyroid patients and controls whilst serum insulin and plasma glucagon levels were similar in all groups.

Kinetic Data

Hyperthyroidism : Acetoacetate tracer (Tables 12:3, 12:4)

Total ketone body (KB) concentrations were increased in hyperthyroidism (0.24 ± 0.06 vs 0.09 ± 0.01 mmol/l, $p < 0.05$) and ketone body turnover (KB.RT) was increased (6.3 ± 1.2 vs 1.9 ± 0.3 µmol/kg/min.) when assessed with the acetoacetate tracer ($p < 0.005$). Metabolic clearance rates (MCR) were normal but KB pool size was increased (104 ± 36 vs 28 ± 4 µmol/kg, $p < 0.05$) as was the volume of distribution (KB . VD) (66.2 ± 7.6 vs $30.2 \pm 7.4\%$, $p < 0.01$). Mean residence time (MRT) was similar in both groups. Following treatment KBRT, pool size and KBVD fell to normal whilst MCR and MRT were unchanged.

Hyperthyroidism : 3-hydroxybutyrate tracer (Tables 12:3, 12:4)

Total KB levels were increased (0.25 ± 0.08 vs 0.08 ± 0.01 mmol/l, $p < 0.05$) as were KB.RT (6.8 ± 1.0 vs 1.6 ± 0.2 µmol/kg/min, $p < 0.001$), KB.MCR (36.1 ± 7.4 vs 18.3 ± 1.6 ml/kg/min, $p < 0.05$), KB pool size (81.4 ± 25.7 vs 18.5 ± 2.6 µmol/kg, $p < 0.05$) and KB.VD (53.2 ± 5.2 vs $24.3 \pm 3.3\%$, $p < 0.001$) in hyperthyroidism. KB.MRT was normal. Following antithyroid therapy KB concentrations KB.RT, KB.MCR and KB pool size returned to normal although KB.VD and KB.MRT were unchanged.

Hypothyroidism : acetoacetate tracer (Tables 12.5, 12.6)

Total KB concentrations, KB.RT, KB.MCR, pool size, KB.MRT and KB.VD were similar in hypothyroid and control subjects and were not affected by thyroxine therapy.

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TABLE 12:1. Clinical details of hyperthyroid and hypothyroid subjects before (and during) treatment and of controls. Results for "before treatment" are mean ± SEM. For "during treatment" only mean values are given in brackets.

	Hyperthyroid	Control	Hypothyroid	Control
Age (yrs)	53 ± 3 (55)	48 ± 6	56 ± 2 (54)	54 ± 4
Sex	4F 1M (3F)	3M 3F	4F 2M (2M 2F)	3M 3F
% Ideal body weight	100 ± 3 (105)	103 ± 1	119 ± 5 (116)	117 ± 4
Serum thyroxine (nmol/l)	249 ± 28*** (106)	86 ± 8	17 ± 3*** (107)	91 ± 5
Serum triiodothyronine (nmol/l)	6.5 ± 1.3*** (1.7)	1.5 ± 0.1	0.7 ± 0.1*** (1.9)	1.8 ± 0.1
Serum thyroid stimulating hormone (mU/l)	< 1 (5.8)	1.9 ± 0.5	41.3 ± 3.1*** (2.4)	1.6 ± 0.3

*** $p < 0.001$ patients vs. appropriate controls.

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TABLE 12:2. Basal Hormone and intermediary metabolite concentrations in hyperthyroid and hypothyroid patients before (and during) treatment and in controls. Results are mean ± SEM for pretreatment values and mean only for during treatment. For insulin and glucagon mean (range) is given.

Blood	Hyperthyroid	Control	Hypothyroid	Control
Glucose mmol/l	4.8 ± 0.1 (4.7)	4.8 ± 0.1	4.9 ± 0.2 (5.2)	5.1 ± 0.2
Lactate mmol/l	0.65 ± 0.16 (0.74)	0.74 ± 0.14	0.65 ± 0.05 (0.74)	0.79 ± 0.10
Pyruvate mmol/l	0.07 ± 0.01 (0.07)	0.07 ± 0.01	0.07 ± 0.01 (0.08)	0.98 ± 0.01
Alanine mmol/l	0.24 ± 0.03 (0.27)	0.30 ± 0.02	0.26 ± 0.02 (0.27)	0.30 ± 0.03
Glycerol mmol/l	0.09 ± 0.005** (0.04)	0.05 ± 0.01	0.04 ± 0.01 (0.05)	0.05 ± 0.01
NEFA mmol/l	0.86 ± 0.07** (0.67)	0.59 ± 0.05	0.62 ± 0.05 (0.07)	0.66 ± 0.02
3-hydroxybutyrate mmol/l	0.13 ± 0.04* (0.05)	0.05 ± 0.003	0.09 ± 0.04 (0.08)	0.05 ± 0.01
Acetoacetate mmol/l	0.11 ± 0.03* (0.05)	0.04 ± 0.01	0.07 ± 0.01 (0.08)	0.05 ± 0.01
Serum Insulin mU/l	6.0 ± (4.9-7.3)(6.3)	7.9 (5.7-11.1)	3.9 (1.0-7.2)(5.1)	7.1 (1.2-12.2)
Plasma Glucagon pg/ml	51 (35-80)(46)	44 (8-72)	65 (0-150)(55)	50 (11-85)

* $p < 0.05$ patients vs. appropriate controls.   ** $p < 0.01$ patients vs. appropriate controls.

TABLE 12:3. Blood ketone body concentrations, ketone body turnover and metabolic clearance rates in hyperthyroid subjects before (and during) therapy and in normal controls following both acetoacetate and 3-hydroxybutyrate tracer injections

Patients	ACETOACETATE TRACER			3-HYDROXYBUTYRATE TRACER		
	Total ketone body concentrations (mmol/l)	Ketone body turnover (μmol/kg/min)	Metabolic clearance rate (ml/kg/min)	Total ketone body concentrations (mmol/l)	Ketone body turnover (μmol/kg/min)	Metabolic clearance rate (ml/kg/min)
Hyperthyroid 1	0.46(0.08)	10.3 (1.3)	18.1 (16.3)	0.55 (0.08)	10.4 (1.3)	19.0 (16.4)
2	0.25(0.15)	6.3 (2.1)	25.2 (13.9)	0.24 (0.11)	6.8 (1.8)	34.0 (16.7)
3	0.20(0.11)	7.0 (2.8)	36.9 (25.0)	0.13 (0.16)	6.5 (2.7)	50.0 (16.6)
4	0.19	5.0	26.5	0.21	4.6	21.8
5	0.08	3.0	37.6	0.11	5.9	55.9
Mean	0.24 (0.11)	6.3 (2.0)	28.9 (18.4)	0.25 (0.12)	6.8 (1.9)	36.1 (16.6)
SEM	0.06	1.2	3.7	0.08	0.4	3.3
Controls 1	0.04	1.0	23.9	0.07	1.4	20.2
2	0.09	2.8	30.1	0.06	1.5	24.5
3	0.07	1.6	24.5	0.10	2.3	17.6
4	0.12	1.9	16.2	0.09	1.5	15.9
5	0.11	2.1	10.1	0.10	1.3	13.2
6	0.10	2.0	19.1	0.08	1.4	18.2
Mean	0.09	1.9	20.7	0.08	1.6	18.3
SEM	0.01	0.3	3.1	0.01	0.2	1.6
Significance (Patients vs controls)	< 0.05	< 0.005	NS	< 0.05	< 0.001	< 0.05

TABLE 12:4. Total ketone body pool size, mean residence time and volume of distribution following both acetoacetate and 3-hydroxybutyrate tracer administration in hyperthyroid subjects before (and during) therapy and in controls

Patients	ACETOACETATE TRACER			3-HYDROXYBUTYRATE TRACER		
	Pool Size (μmol/kg)	Mean Residence Time (min.)	Volume of Distribution (% body wt.)	Pool Size (μmol/kg)	Mean Residence Time (min.)	Volume of Distribution (% body wt.)
Hyperthyroid 1	228 (18)	22 (14)	76 (43)	181 (24)	17 (18)	57 (56)
2	145 (44)	23 (21)	89 (53)	64 (29)	9 (16)	44 (48)
3	70 (50)	10 (18)	64 (82)	72 (42)	11 (16)	72 (48)
4	47	9	45	57	12	46
5	30	10	57	33	21	47
Mean	104 (37)	15 (18)	66 (59)	81 (32)	14 (17)	53 (50)
SEM	36	3	8	26	2	5
Controls 1	38	32	20	29	10	35
2	40	12	60	12	8	24
3	10	9	23	22	10	18
4	20	11	18	19	14	20
5	29	14	15	12	9	16
6	31	15	45	17	12	33
Mean	28	16	30	19	11	24
SEM	4	3	7	3	1	3
Significance (p)	< 0.05	NS	< 0.01	< 0.005	NS	< 0.001

TABLE 12:5. Blood ketone body concentrations, ketone body turnover and metabolic clearance rates in hypothyroid subjects before (and during) therapy and in controls following both acetoacetate and 3-hydroxybutyrate tracer administration

Patients	ACETOACETATE TRACER			3-HYDROXYBUTYRATE TRACER		
	Total ketone body concentration (mmol/l)	Ketone body turnover (µmol/kg/min)	Metabolic clearance rate (ml/kg/min)	Total ketone body concentration (mmol/l)	Ketone body turnover (µmol/kg/min)	Metabolic clearance rate (ml/kg/min)
Hypothyroid						
1	0.09	1.0	11.5	0.15	1.8	12.4
2	0.07 (0.11)	1.7 (1.9)	23.3 (17.3)	0.11 (0.07)	2.0 (1.5)	18.8 (21.4)
3	0.37	3.5	9.4	0.41	4.0	9.7
4	0.18 (0.23)	2.9 (2.0)	15.2 (8.7)	0.18 (0.24)	3.3 (3.8)	17.3 (15.8)
5	0.09 (0.09)	2.4 (1.7)	26.6 (18.8)	0.12 (0.08)	2.1 (1.6)	17.8 (26.8)
6	0.17 (0.23)	1.9 (1.9)	11.2 (8.3)	0.10 (0.32)	1.4 (2.6)	9.7 (8.2)
Mean	0.16 (0.17)	2.2 (1.9)	16.2 (13.3)	0.18 (0.18)	2.4 (2.4)	14.3 (18.1)
SEM	0.04	0.4	2.9	0.05	0.4	1.7
Controls						
1	0.08	2.5	31.0	0.08	1.5	18.2
2	0.07	1.6	24.5	0.13	2.3	17.8
3	0.06	1.3	22.4	0.06	1.0	17.4
4	0.13	2.2	16.8	0.10	1.3	13.9
5	0.11	1.5	13.5	0.12	1.8	15.4
6	0.11	1.8	15.5	0.09	2.1	23.1
Mean	0.09	1.8	20.6	0.10	1.7	17.6
SEM	0.01	0.2	2.7	0.01	0.2	1.3
Significance (p)	NS	NS	NS	NS	NS	NS

TABLE 12:6. Total ketone body pool size, mean residence time and volume of distribution following both acetoacetate and 3-hydroxybutyrate tracer administration in hypothyroid patients before (and during) therapy and in controls

Subject	ACETOACETATE TRACER			3-HYDROXYBUTYRATE TRACER		
	Pool Size (μmol/kg)	Mean Residence Time (min.)	Volume of Distribution (% body wt.)	Pool Size (μmol/kg)	Mean Residence Time (min.)	Volume of Distribution (% body wt.)
Hypothyroid						
1	17	17	31	30	17	34
2	27 (33)	16 (17)	60 (45)	26 (33)	13 (22)	37 (56)
3	96	28	47	59	15	26
4	76 (38)	27 (19)	56 (22)	103 (84)	31 (22)	76 (47)
5	34 (26)	14 (15)	50 (42)	32 (26)	15 (16)	36 (48)
6	60 (55)	31 (29)	44 (30)	23 (65)	16 (25)	30 (26)
Mean	52 (38)	22 (20)	46 (35)	46 (52)	18 (22)	40 (44)
SEM	13	3	3	13	2	7
Control						
1	17	9	20	29	19	35
2	10	9	22	23	10	18
3	34	26	64	33	32	61
4	62	28	46	33	25	30
5	20	14	18	18	10	16
6	26	15	32	37	18	55
Mean	28	17	34	29	19	36
SEM	8	3	7	3	4	8
Significance	NS	NS	NS	NS	NS	NS

TABLE 12:7. Correlations between kinetic parameters and metabolic indices in hyperthyroid subjects before treatment. Correlation coefficients are shown above and significance below.

A refers to values obtained during acetoacetate tracer administration and B to those using 3-hydroxybutyrate.

	[TK]A	[TK]B	MCRA	MCRB	RTA	RTB	PoolA	PoolB	MRTA	MRTB	VDA	VDB	T3	T4	%IBW	NEFA
[KB] A	-															
[KB] B	0.96 <0.01	-														
MCR A	NS	-	-													
MCR B	-	NS	0.93 <0.05	-												
RT A	0.96 <0.01	-	NS	-	-											
RT B	-	0.89 <0.05	-	NS	0.94 <0.05	-										
POOL A	0.91 <0.05	-	NS	-	0.81 <0.05	-	-									
POOL B	-	0.95 <0.02	-	NS	-	0.92 <0.05	0.90 <0.05	-								
MRT A	NS	-	NS	-	NS	-	0.89 <0.05	-	-							
MRT B	-	NS	-	NS	-	NS	-	NS	NS	-						
VD A	NS	-	NS	-	NS	-	NS	-	0.91 <0.05	-	-					
VD B	-	NS	-	NS	-	NS	-	NS	-	NS	NS	-				
T3	NS	0.94 <0.05	NS	NS	NS	NS	NS	NS	0.93 <0.05	NS	0.90 <0.05	NS	-			
T4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.93 <0.05	NS	0.91 <0.05	-		
% IBW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	
NEFA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

[KB] = total ketone body concentration; MCR = metabolic clearance rate for ketone bodies; RT = turnover;
MRT = mean residence time; VD = volume of distribution; T_3 = serum triiodothyronine; T_4 = serum thyroxine;
%IBW = percent of ideal body weight. A refers to values obtained during acetoacetate and B during
3-hydroxybutyrate tracer administration. NEFA = plasma non-esterified fatty acid concentration.

TABLE 12:8. Correlations between kinetic parameters and metabolic indices in hypothyroid subjects before treatment. (For symbols see Table 12.7.).

		[TK]A	[TK]B	MCRA	MCRB	RTA	RTB	Pool A	Pool B	MRTA	MRTB	VDA	VDB	T₃	T₄	%IBW	NEFA
[KB]	A	-															
[KB]	B	0.91 <0.01	-														
MCR	A	NS	-	-													
MCR	B	-	NS	0.86 <0.05	-												
RT	A	0.86 <0.05	-	NS	-	-											
RT	B	-	0.88 <0.05	-	NS	0.84 <0.05	-										
POOL	A	NS	-	NS	-	NS	-	-									
POOL	B	-	NS	-	NS	-	NS	NS	-								
MRT	A	NS	-	NS	-	NS	-	0.88 <0.05	NS	-							
MRT	B	-	NS	-	NS	-	NS	NS	0,79 <0.05	NS	-						
VD	A	NS	-	NS	-	NS	-	NS	NS	NS	-	-					
VD	B	-	NS	-	NS	-	NS	-	0.81 <0.05	-	NS	NS	-				
T₃		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-			
T₄		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-		
%IBW		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	
NEFA		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

TABLE 12:9. Correlations between kinetic parameters and metabolic indices in control subjects. (For symbols see Table 12:7)

	[TK]A	[TK]B	MCRA	MCRB	RTA	RTB	Pool A	Pool B	MRTA	MRTB	VDA	VDB	T3	T4	%IBW	NEFA
[KB] A	-															
[KB] B	NS	-														
MCR A	NS	-	-													
MCR B	-	NS	NS	-												
RT A	NS	-	NS	-	-											
RT B	-	0.63 <0.05	-	NS	NS	-										
POOL A	NS	-	NS	-	NS	-	-									
POOL B	-	NS	-	NS	-	NS	NS	-								
MRT A	NS	-	NS	-	NS	-	0.75 <0.01	-	-							
MRT B	-	NS	-	NS	-	NS	-	0.73 <0.01	NS	-						
VD A	NS	-	NS	-	NS	-	0.57 =0.05	-	NS	-	-					
VD B	-	NS	-	NS	-	NS	-	0.75 <0.005	-	0.74 <0.01	0.54 =0.06	-				
T3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-			
T4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.61 <0.05	-		
% IBW	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	
NEFA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-

TABLE 12:10. Correlations between kinetic parameters and metabolic indices in all subjects (hyperthyroid, hypothyroid and controls). For symbols see Table 12:7.

	TKBA	TKBB	MCRA	MCRB	RTA	RTB	PA	PB	MRTA	MRTB	VDA	VDB	T_3	T_4	IBW	NE
[KB] A	-															
[KB] B	0.93 <0.001	-														
MCR A	NS	-	-													
MCR B	-	NS	0.78 <0.001	-												
RT A	0.83 <0.001	-	NS	-	-											
RT B	-	0.77 <0.001	-	0.52 <0.01	0.93 <0.001	-										
POOL A	0.92 <0.001	-	NS	-	0.86 <0.001	-	-									
POOL B	-	0.83 <0.001	-	NS	-	0.83 <0.001	0.87 <0.001	-								
MRT A	NS	-	0.48 <0.05	-	NS	-	0.42 <0.05	-	-							
MRT B	-	NS	-	NS	-	NS	-	NS	0.41 <0.05	-						
VD A	0.53 <0.01	-	NS	-	0.67 <0.001	-	0.71 <0.001	-	NS	-	-					
VD B	-	NS	-	0.48 <0.05	-	0.50 =0.01	-	0.63 <0.001	-	0.56 <0.005	0.62 <0.005	-				
T_3	0.59 <0.01	0.54 <0.01	NS	0.50 <0.05	0.87 <0.001	0.85 <0.001	0.75 <0.001	0.64 <0.01	NS	NS	0.63 <0.01	0.47 <0.01	-			
T_4	0.42 <0.05	NS	0.45 <0.05	0.64 <0.01	0.78 <0.001	0.77 <0.001	0.58 <0.001	0.47 <0.05	NS	NS	0.53 <0.01	0.40 <0.05	0.95 <0.001	-		
% IBW	NS	NS	NS	0.48 <0.05	0.46 <0.05	0.43 <0.05	NS	NS	NS	NS	NS	NS	0.42 <0.05	0.51 <0.01	-	
NEFA	0.46 <0.05	0.41 <0.05	NS	NS	0.42 <0.05	0.47 <0.05	NS	NS	NS	NS	NS	NS	0.50 <0.01	0.52 <0.01	-	-

Hypothyroidism : 3-hydroxybutyrate tracer (Tables 12:5, 12:6)

Kinetic parameters, as above, were similar in patients and controls and were unaffected by treatment of hypothyroidism.

Correlations (see Tables 12:7, 12:8, 12:9 and 12:10)

Considering data from both experiments significant correlations emerged between KB concentrations ($r = 0.83$, $p < 0.001$ for acetoacetate and $r = 0.77$, $p < 0.001$ for 3-hydroxybutyrate), KB.RT ($r = 0.93$, $p < 0.001$), pool size ($r = 0.83$, $p < 0.001$), KB.VD ($r = 0.62$, $p < 0.005$) and KB.MRT ($r = 0.41$, $p < 0.05$). For hyperthyroid, hypothyroid and control subjects together circulating thyroid hormone levels in blood correlated with KB.RT ($p < 0.001$ all comparisons) and these correlations held within the hyperthyroid group but not within either control group or in hypothyroid patients.

12:4.   DISCUSSION

In the post absorptive state, ketone bodies play a minor role in supplying the energy needs in mammals (Robinson & Williamson, 1980) but in other metabolic conditions ketone bodies are major metabolic substrates (Owen et al. 1969). Isotopic techniques provide relatively non invasive methods for measuring rates of production and utilisation of ketone bodies in vivo (Bates et al. 1968) and infusion of either $3-[^{14}C]$-acetoacetate or $3-[^{14}C]$-hydroxybutyrate has been widely used in animals and humans (Ballase, 1979; Barton, 1973; Bates et al. 1968; Keller et al. 1978; Keller et al. 1981). However, this approach is not without potential problems. Acetoacetate and 3-hydroxybutyrate are interconverted too rapidly to be considered independent substrates

but too slowly for their specific activities in blood to become equal
(Barton, 1980). Most investigators therefore attempt to circumvent
this problem by using the combined specific activities of acetoacetate
and 3-hydroxybutyrate to quantify total ketone body turnover although
this compromise may not be valid (Barton, 1980; Cobelli et al. 1982).

It was therefore decided to investigate ketone body kinetics
in overnight fasted subjects using both $3-[^{14}C]$-acetoacetate and
$3-[^{14}C]$-hydroxybutyrate as tracers on separate occasions in random
order. This approach allowed a direct comparison of kinetic data
obtained with the two tracers and calculations have been performed
by conventional means. The estimations obtained have also provided
the basis of a new modelling approach to ketone body kinetics
(Cobelli et al. 1982). These modelling studies of the data have been
performed entirely by others and the results will not therefore be used
in this thesis .

In the normal subjects mean KB.RT was similar by either
tracer although individual differences of up to 80% were apparant. These
differences were not consistent in direction and the most likely
explanation is that KB.RT varies from day to day. Further studies
using the same isotope repeatedly in the same subject are required
but should await the development of stable isotope techniques.
Similar explanations pertain for the variability in KB.MCR, pool,
KB.MRT and KB.VD despite the close agreement in their mean values.
Mean KB.RT for normals reported here are similar to those found
in previous studies where fasting KB concentrations were comparable

(1.2 μmol/kg/min, Bradley et al. 1981; 1.8 μmol/kg/min,

Miles et al. 1980).

In hyperthyroidism, circulating KB concentrations were

increased as in previous studies (Chapter 7; Beylot et al. 1980;

Bartels et al. 1979), whilst ketone body turnover rates were 3-4 fold

increased. Circulating glycerol and NEFA levels were increased

consistent with increased lipolysis noted in several other studies

(Chapter 7; Rich et al. 1959; Tibbling, 1969). Since, in addition,

hepatic blood flow may be 20-35% increased in hyperthyroidism (Wahren

et al. 1981), hepatic NEFA supply is likely to be 3-4 fold increased.

This derived figure is in accord with the data of Hagenfeldt et al.

(1981) who showed a 3-fold increase in hepatic NEFA uptake in fasting

hyperthyroid patients during splanchnic balance studies. These

authors also showed a 400% increase in KB production, similar to the

findings reported in the present study. Thus a potent effect of thyroid

hormones to increase hepatic NEFA supply is likely to be a major

determinant of the increased KB production demonstrated. Perfused

rat liver studies have also suggested that thyroid hormones may also

alter the ketogenic "set" of the liver, diverting fatty acids

preferentially away from the triglyceride synthetic pathway and

towards β oxidation and ketone body formation (Keyes & Heimberg,

1979; Laker & Mayes, 1981). Precise mechanisms at a cellular level

remain to be elucidated.

The 3-4 fold increase in ketone body production demonstrated

here compares with only a 50% increase in glucose production

(Chapter 9; Saunders et al. 1980; Perez et al. 1980). This increase in glucose production and utilisation can largely be accounted for by increased Cori cycle activity with no significant increase in irreversible glucose disposal (Chapter 9). These findings indicate that a significant portion of the increased energy demands of hyperthyroidism are met by increased lipid metabolism.

Peripheral disposal of ketone bodies (KB. MCR) was increased in hyperthyroidism (although this was statistically significant only when 3-hydroxybutyrate was used as tracer) suggesting that thyroid hormones stimulate the ability of peripheral tissues to extract ketone bodies from blood.

Following antithyroid therapy KB concentrations, KBRT, KBMCR, pool size and KBVD returned towards normal.

In hypothyroidism, fasting KB concentrations and all kinetic parameters were normal irrespective of the isotope used. No previous studies concerning in vivo ketone body production in hypothyroidism have appeared in the literature although normal fasting ketone body concentrations (Saunders et al. 1980; Chapter 8) normal NEFA levels (Chapter 8; Saunders et al. 1980) and NEFA turnover (Saunders et al. 1980) have all been described. Perfused hypothyroid rat liver studies have produced contradictory results with normal (Laker & Mayes, 1981) and decreased (Keyes & Heimberg, 1979) KB production being reported.

Treatment with L-thyroxine had no effect on any parameter of ketone body metabolism measured in this study.

Ketone body turnover rates correlated positively with

circulating ketone body levels in this as in previous studies in man

(Bates et al. 1968; Nelson et al. 1941). Thyroid hormone levels did

not correlate with KBRT within individual groups although when all

subjects were considered together a highly significant correlation emerged

even on non parametric testing. Surprisingly there was no correlation

between NEFA and KBRT overall or within individual groups.

Thus ketone body turnover was significantly increased in

hyperthyroidism. This may be the result of increased hepatic NEFA

supply due to increased lipolysis. A direct effect of thyroid hormones

on intrahepatic metabolism may also be important but further

experiments are required to investigate this point. The data also

suggests that thyroid hormones are unnecessary for ketogenesis to

progress normally in man.

13.   KETONE BODY PRODUCTION BY ISOLATED RAT
        HEPATOCYTES: EFFECTS OF EXPERIMENTAL
        HYPERTHYROIDISM

13.1.   INTRODUCTION

Previous studies in thyrotoxic man have demonstrated

elevated blood ketone body concentrations during fasting (Chapters 11, 12;

Beylot et al. 1980; Bartels et al. 1980) with increased hepatic ketone

body production (Hagenfeldt, 1981; Chapter 12).   Enhanced ketogenesis

may be due either to an increase in the supply of precursor fatty

acids to the liver, to alteration of hepatic ketogenic capacity or to a

combination of these two processes.   Lipolysis is increased in

hyperthyroidism (Chapter 7; Arner et al. 1981; Goodman & Bray, 1966;

Tibbling, 1969) with increases in both blood concentrations

(Chapter 7; Goodman & Bray, 1966; Tibbling, 1969) and turnover

(Saunders, Hall & Sonksen, 1980) of non-esterified fatty acids.

Hepatic blood flow is also increased in thyrotoxic man (Wahren et al.

1981) further contributing to increased hepatic fatty acid supply.

Whether thyroid hormones have any direct effect on intrahepatic fatty

acid metabolism is however uncertain.   Isolated rat hepatocyte

preparations allow the effects of hormones on hepatic metabolism to

be studied in a controlled and manipulable environment.   Using this

technique effects of insulin (Barker, 1980), glucagon (Christiansen,

1977; McGarry et al. 1978) and catecholamines (Burrin et al. 1982)

on intrahepatic fatty acid metabolism have been examined by others

but the effects of thyroid hormones are unknown.   Acute effects of

thyroid hormones on ketogenesis have therefore been examined by

incubating hepatocytes from fed and fasted normal rats with

triiodothyronine in a concentration $(10^{-8}M)$ exceeding normal free

circulating levels in the rat. Chronic effects of thyroid hormones

on ketogenesis have been examined by measuring ketone body

production in the presence and absence of added $(1mM\ U^{-14}C$

palmitate) fatty acid substrate by hepatocytes from fed and fasting

normal rats and from rats pretreated with triiodothyronine for 7 days.

Circulating hormone and metabolite concentrations have also been

determined in rats prepared in the same way (Table 13:1).

13:2.   MATERIALS AND METHODS

1.   Preparation of solutions

Stock (conc.) Krebs Hensleight Buffer

NaCl	34.7g	)
KCl	1.77g	)
KH_2PO_4	0.81g	) Dissolved in distilled water
$MgSO_4:7H_2O$	1.46g	) and made up to 1 Litre
$CaCl_2:2H_2O$	1.87g	)
$NaHCO_3$	12.94g	)

Fatty acid free albumin

100g Fraction V bovine albumin (BSA) (Sigma, Poole, Dorset)

were dissolved in 500ml distilled water at 4°C. The solution was

dialysed against distilled water for 4h and then for four 1h periods.

The solution was removed from the dialysis tubing and brought to

pH 3.5. Activated charcoal (20g/l) was added and the solution stirred

for 1h at 4°C and centrifuged at 7000 rpm for 30 mins to remove

larger particles. The supernatant was separated and filtered through as artorius filter. The albumin was brought to pH 7.4 and the concentration determined gravimetrically. The solution was adjusted to give a final concentration of 10% (w/v) and stored at -20°C in 20 ml aliquots.

Solution A (10 x conc. Hepes [N_2 hydroxy ethyl piperazine-N-2-ethane sulphuric acid] solution)

NaCl	83 g	)
KCl	5 g	)
Hepes	24 g	) Made up to 1 litre at pH 7.6.
Tricine	18 g	) Kept at 4°C.
2M NaOH	55 ml	)

Solution B

EGTA	120 mg	)
Soln. A	50 ml	) pH 7.6. Made up on the day of experiment. Oxygenated at 37°C
Distilled H_2O	450 ml	)

Solution C

Soln. A	50 ml	) pH 7.6. Made up on the day of
Distilled H_2O	450 ml	) experiment. Oxygenated at 37°C.

Solution C

Soln. A	50 ml	) pH 7.6. Made up on the day of
Distilled H_2O	450 ml	) experiment. Oxygenated at 37°C.

Solution D

Soln. A	10 ml	)
Collagenase (Worthington Type II; 135 units/mg)	50 mg	) Kept in deep freeze (-20°C) until) day of use. Oxygenated, pH 7.6) at 37°C
Ca.Cl_2:$2H_2O$	73.5 mg	)
Distilled H_2O	90 ml	)

Solution E

Conc. Krebs solution	20 ml	) Made up to 100ml with
		) distilled water. Stored at
1.3% NaHCO$_3$	16 ml	) -20°C until day of use.
		) Oxygenated for 30 min
3.4% Hepes	16 ml	) before adjusting to pH
		) 7.4 at 37°C.
10% fatty acid-free		)
bovine serum albumin		)
(BSA)	10 ml	)

Solution F

Conc. Krebs solution	20 ml	)
		)
1.3% NaHCO$_3$	16 ml	) Made up to 100ml with
		) distilled water. Stored at
3.4% Hepes	16 ml	) -20°C until day of use.
		) Oxygenated for 30 min at
10% BSA	20 ml	) 37°C and adjusted to
		) pH 7.6.
Bacitracin	100 mg	)
		)
Trasylol	100,000 kiu	)

L-Carnitine

L-carnitine (Sigma Chemicals) 6.5 mg was added to 40 ml.

hepatocyte suspension before preincubation to yield a final

concentration of 1 mmol/l.

Trypan blue

67 mg Trypan blue	) dissolved in 25 ml distilled water,
	) filtered through a 0.2 millipore
120 mg NaCl	) filter. Solution was stored until use
	at -20°C in 3.0 ml aliquots.

Radioactive Palmitic Acid (Radioactive Substrate)

104 mg Palmitic acid (sodium salt; Sigma Chemical Co. Ltd.)

were dissolved in 25ml distilled water at 50°C to give a 15 mM

solution. 90 µl uniformly labelled palmitic acid (403 mCi/

mmol, Radiochemical Centre, Amersham, Bucks.).

was added to 9 ml 15 mM palmitic acid and 7.24 ml of this solution '

was added to 70 ml Soln. F. 1.5 ml of this final solution was added

to each culture flask to which 0.5 ml hepatocyte suspension was

subsequently added. This yielded a final concentration of 1 mM

palmitic acid.

2.   Preparation of rats

Male Wistar rats (200-220 g) were used in all experiments.

Previous studies in this department have shown that rats of this weight

eat 20 g diet pellets/day (small animal diet No. 1., Special Diet Services,

Essex). To induce hyperthyroidism 1.6 mg of L-triiodothyronine

were dissolved in distilled water made slightly alkaline by addition of 1-2

drops of 1mM NaOH and sprayed evenly over 1 kg of diet pellets.

20 g diet prepared in this way were fed daily. The dose of

L-triiodothyronine administered was therefore 32 μg/day (Laker &

Mayes, 1981). Control rats were fed with 20 g of standard pellets

daily. In 48 h starved rats, 32 μg T_3 were administered

intraperitoneally for the last 2 days of preparation. Circulating

insulin and metabolite concentrations were determined in separate groups

of fed and fasting normal and thyrotoxic rats prepared as above

(n = 6 all groups). Ketone body production from endogenous and

exogenous substrate was assessed in fed normal (n = 6), fed

thyrotoxic (n = 6), fasted normal (n = 5) and fasted thyrotoxic rats

(n = 5). Acute effects of thyroid hormones were studied by incubating

hepatocytes from fed normal (n = 4) fasted normal (n = 4) fed

thyrotoxic (n = 4) and fasted normal rats (n = 4) with 10^{-8}M

triiodothyronine (Table 13:1).

Rats were anaesthetised using pentabarbitone (55mg/kg)
intraperitoneally. The abdomen was incised and the viscera displaced
to expose the portal vein. Sutures were placed round the portal
vein which was cannulated using a 20G Medicut cannula. Sutures were
tightened to hold the cannula in place. The inferior vena cava (i. v. c.)
was cut at the level of the renal veins and solution B was perfused
through the liver at a rate of 40 ml/min after also cutting the i. v. c.
above the liver. The rate was reduced to 30 ml/min after 1 min and
perfusion with solution B continued for a further 3 minutes. Perfusion
was changed to solution C at 40 mls/min for 1 minute and 30 ml/min
for 3 minutes. Solution D (collagenase) was then perfused and
recirculated at 40 ml/min for 1 minute and 30 ml/min for 9 minutes.
The perfusion was then stopped and the liver placed in a petri-dish
containing solution E at 4°C. The liver was gently teased apart with
forceps and dispersed in the solution which was subsequently filtered
through nylon mesh (pore size 100 μm and made up to 40 ml with
solution E.

Preincubation of hepatocytes

The crude cell suspension was oxygenated and incubated with
1 mM carnitine at 37°C for 20 minutes in an airtight culture flask in a
water bath with an automatic shaker. Previous studies in the laboratory
had suggested that this concentration of carnitine was optimal for
ketone body production.

Purification of hepatocytes

The cells were centrifuged at 200 r. p. m. for two minutes

at 4°C and the supernatant discarded. The cells were washed twice

with solution E and resuspended in a known volume of solution F.

Viability of hepatocytes

100 μl of cells suspended in F were diluted in 300 μl

trypan blue. The solution was mixed by inversion and introduced into

an improved Neubauer counting chamber (McFarlane Robson Ltd.).

The total number of cells present was calculated by counting

(25 squares = 1/10,000 ml) and the number of dead cells (those

taking up trypan blue) subtracted to yield the number of live cells.

Viability was expressed as the percentage of live cells present, and

was determined in the fresh preparation and after 60 minutes incubation.

Incubation of hepatocytes

0.5 ml hepatocyte suspension were incubated at 37°C in

airtight culture flasks as follows

a)   with 1.5 ml solution F + 1 mM radioactive palmitate

b)   with 1.5 ml solution F.

In those experiments where thyroid hormone was added directly to

the incubation medium 0.34 μg T_3 was added to 40 ml solution F

(with or without radioactive palmitate) to a final concentration of

10^{-8}M. Ketone body production was assessed at 60 minutes only.

4.   Preparation of hepatocyte samples for metabolic estimations

Samples (0.5 ml) were taken from incubation flasks at times

0 (in quadruplicate) 30 mins (triplicate) and 60 mins (triplicate).

Samples were added to 2.0 ml 3% perchloric acid (w/v) at 4°C.

The P.C.A. extract was centrifuged for 20 min at 2000 r.p.m. at

4°C and the supernatant transferred to preweighed tubes. The acid

weight was recorded and the extract neutralised with 20% and 2% KOH.

Extracts were centrifuged again and the neutral weight recorded.

The sample extracts were then assayed for 3-hydroxybutyrate and

acetoacetate concentrations as shown below.

Acetoacetate assay

Acetoacetate assays were performed spectrophotometrically

on a Zeiss Spectrophotometer. Cuvettes for the assay were prepared

as below

Cuvette	Distilled water ml	Cocktail ml	Sample ml
Blank	1.5	0.5	-
Control	1.0	1.0	-
Sample	0.5	1.0	0.5

The cocktail consisted of the following;

Potassium phosphate buffer (0.1 M) pH 7.0

$(K_2HPO_4$ 10.3 g/l   20 ml) Sufficient

$K_2H_2PO_4$ 5.3 g/l)   ) for

NADH   6 mg) 20 samples

Cuvettes were agitated and the optical density (OD)

read at 340 nm. 10 μl 3-hydroxybutyrate dehydrogenase

were added to each cuvette (except the blank) and mixed. The OD

was again determined at 340 nm after 30 minutes and reading repeated

until a steady value was obtained.

3-Hydroxybutyrate assay

Cuvettes were prepared as below:

Cuvette	Distilled Water ml	Cocktail ml	Sample ml
Blank	1.0	1.0	0
Control	1.0	1.0	0
Samples	0.5	1.0	0.5

In this case the cocktail consisted of

0.1 M Tris-HCl buffer	10 ml	)
EDTA	12.5 mg	)
Hydrazine hydrate	0.5 ml	) ph 8.5
NAD	15 mg	)

Cuvettes were agitated and the OD read at 340 nm. 10 µl of Hydroxybutyrate dehydrogenase (HBDH) was added to each cuvette except the blank and the OD read again at 340 nm after 60 minutes.

Calculation of results

The 3-hydroxybutyrate and acetoacetate assays depend on the change in absorption of light at 340 nm due to the conversion of NAD^+ to NADH or vice versa. Results can therefore be calculated using the same general formula.

$$\frac{OD_{340}\ sample - OD_{340}\ control}{6.22\ (molar\ extinction\ coefficient\ for\ NAD/NADH)} \times \frac{vol.\ PCA\ extract}{vol.\ sample}$$

$$\times \frac{reaction\ vol.}{sample\ vol.} \times \frac{neutral\ wt.}{acid\ wt.}$$

$$= \frac{OD\ sample - OD\ control}{6.22} \times \frac{2.5}{0.5} \times \frac{2.0}{0.5} \times \frac{neutral\ wt.}{acid\ wt.}$$

This gives the concentration of the metabolite in µmol/ml (or mM).

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5.   Measurement of ^{14}C ketone body production from U-C^{14}
      palmitic acid

Radioactively lab el led 1 mmol/l palmitic acid was

prepared as described above, and incubations carried out as before.

Radioactivity in ketone bodies was determined by a modification of

the method of Bates, Krebs and Williamson (1965). 0.25 ml of neutral

PCA extract was boiled for 45 min in 12 ml mercuric sulphate/

sulphuric acid/distilled water mixture as described previously

(Chapter 12). Radioactivity in palmitic acid was calculated by

counting aliquots of Solution F to which radioactive substrate had

been added (total palmitate d.p.m.).

Counts were corrected for background and quenching and

recoveries of radioactive acetoacetate and 3-hydroxybutyrate were

calculated by passing known amounts of each through the assay

procedure. Radioactivity associated with acetoacetate and total

ketone bodies was calculated as shown below.

$$c.p.m. \times \frac{PCA\ vol.}{Sample\ vol.} \times \frac{Cuvette\ vol.}{Sample\ vol.} \times \frac{Neutral\ wt.}{Acid\ wt.} \times \frac{100}{\%\ recovery}$$

$$= \quad x\ dpm/ml.$$

$$= \frac{x}{(total\ palmitate\ dpm/4)} = y\ \mu mol/ml$$

$$y/cells/ml = \mu mol/10^{6}\ cells.$$

Radioactivity associated with 3-hydroxybutyrate was determined by

subtraction of the counts for acetoacetate from those of total ketone

bodies. It was assumed in these calculations that each mole of

palmitate gave rise to 4 moles of ketone bodies.

6.   Calculation of circulating insulin and intermediary
        metabolite concentrations

        Circulating insulin and intermediary metabolite

concentrations were measured in normal and thyrotoxic fed and

fasted rats (n = 6 for each group). For glucose, lactate, pyruvate,

alanine, glycerol and 3-hydroxybutyrate 0.2 ml of blood was added

to 2 ml 3% (w/v) perchloric acid and measured by automated

fluorimetric methods (Lloyd et al. 1978). Acetoacetate from the

same extract was measured as in 13.2 (3).   Serum insulin was

measured by double antibody radioimmunoassay using rat serum as

standard (Soeldner & Stone, 1965).

7.   Statistics

        Comparisons between groups were made using Student's

paired and unpaired t-tests as appropriate.

Precision

        Within rat coefficient of variation of labelled ketone body

production production was 6.3% whilst between rat variation was 27.6%.

Recovery

        The recovery of added ^{14}C-labelled acetoacetate in the assay

of ^{14}C-acetoacetate and total ketone body production was 81 $\pm$ 8%

whilst the recovery of added ^{14}C-3-hydroxybutyrate in the assay for

total ketone body production was 73 $\pm$ 9%.

Cell Viability (Trypan blue exclusion)

        Mean $\pm$ SEM viability of hepatocytes from all groups was

94 $\pm$ 3% at 0 time and 89 $\pm$ 4% at 60 minutes.

TABLE 13:1.   Outline of Experiments Performed

A.   Chronically Thyrotoxic Animals (32 μg T$_3$ daily for 7 days)

    1.   Ketone Body Production in the Absence of added
         Fatty Acid Substrate

        (a)   Thyrotoxic Fed Rats (n = 6)

        (b)   Normal Fed Rats (n = 6)

        (c)   Thyrotoxic Starved Rats (n = 5)

        (d)   Normal Starved Rats (n = 5)

    2.   Ketone Body Production from 1mM Palmitate

        (a)   Thyrotoxic Fed Rats (n = 6)

        (b)   Normal Fed Rats (n = 6)

        (c)   Thyrotoxic Starved Rats (n = 5)

        (d)   Normal Starved Rats (n = 5)

    3.   Circulating Hormone and Metabolite Concentrations

        (a)   Thyrotoxic Fed Rats (n = 6)

        (b)   Normal Fed Rats (n = 6)

        (c)   Thyrotoxic Starved Rats (n = 6)

        (d)   Normal Starved Rats (n = 6)

B.   Acute Effects of Triiodothyronine (10^{-8}M) on Ketone Body
        Production by Hepatocytes from Normal Rats

        (a)   Fed Rats (n = 4)

        (b)   Starved Rats (n = 4)

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13.3.   RESULTS

Circulating Insulin and Metabolite Concentrations (Table 13:2)

Peripheral insulin concentrations were significantly diminished in fed thyrotoxic rats compared to controls (16.2 ± 3.5 vs 33.0 ± 5.2 mU/L p < 0.05.. Insulin concentrations fell with starvation in both groups and were not significantly different. Blood glucose concentrations were similar in fed and 48 h fasted normal and thyrotoxic rats as were blood alanine concentrations. Blood lactate was increased in thyrotoxic rats in both the fed (thyrotoxic 2.29 ± 0.13 vs normal 1.64 ± 0.13 mmol/1, p < 0.01) and fasted state (2.18 ± 0.15 vs 1.87 ± 0.27 mmol/1, p < 0.01) whilst blood pyruvate was increased in fed thyrotoxic rats only (0.15 ± 0.01 vs 0.07 ± 0.01 mmol/1, p < 0.01).

Blood glycerol was as expected, increased in normal fasted rats compared with fed normal rats (0.06 ± 0.01 vs 0.50 ± 0.03 mmol/1, p < 0.001) and was increased further in thyrotoxic rats compared to normals (fed 0.42 ± 0.04 mmol/1, p < 0.001; fasted 0.84 ± 0.08 mmol/1, p < 0.001). A similar pattern was observed for blood acetoacetate concentrations (fed normal 0.11 ± 0.04 vs fasted normal 0.91 ± 0.21 mmol/1, p < 0.001; fed thyrotoxic 0.63 ± 0.19 mmol/1, p < 0.001; fasted thyrotoxic 1.39 ± 0.27 mmol/1, p < 0.05). Blood 3-hydroxybutyrate concentrations were increased in fed thyrotoxic rats (1.64 ± 0.17 vs control 0.26 ± 0.01 ml/1, p < 0.001) but were similar to control values during fasting. Blood ratios of 3-hydroxybutyrate to acetoacetate were similar in fed normal and thyrotoxic rats but this ratio was diminished in fasted thyrotoxic

rats (fasted normal 2. 11 $\pm$ 0. 42 vs fasted toxic 1.18 $\pm$ 0. 49, p $<$ 0. 05).

Ketone body production by isolated rat hepatocytes in the absence of added substrate (endogenous production) (Table 13:3)

Basal. Total ketone body production was similar in fed

normal and thyrotoxic rats (normal 0. 028 $\pm$ 0. 004 vs thyrotoxic

0. 027 $\pm$ 0. 010 μmol/10^6 cells) and increased significantly in fasted

normal (p $<$ 0. 01) but not in fasted thyrotoxic rats (p $<$ 0. 05 v

fasted normals). The ratio of 3-OHB:Acac produced was decreased

in thyrotoxic rats both during feeding (normal 0. 85 $\pm$ 0. 07 vs thyrotoxic

0.35 $\pm$ 0. 07 μmol/10^6 cells, p $<$ 0. 001) and fasting (normal 0. 30 $\pm$ 0. 05

vs 0. 07 $\pm$ 0. 02 μmol/10^6 cells, p $<$ 0. 001).

30 mins. Endogenous total ketone production was diminished

in thyrotoxic rats both when fed (normal 0. 099 $\pm$ 0. 004 vs thyrotoxic

0. 055 $\pm$ 0. 014 μmol/10^6 cells, p $<$ 0. 01) and fasted (0. 269 $\pm$ 0. 025 vs

0. 039 $\pm$ 0. 009 μmol/10^6 cells, p $<$ 0. 001) and this was associated with

a decrease in both acetoacetate and 3-hydroxybutyrate production.

The 3-OHB:Acac ratio was decreased in fed (p $<$ 0. 001) and fasted

(p $<$ 0. 001) thyrotoxic rat hepatocytes. Acetoacetate and 3-hydroxybutyrate

production were increased in fed and starved normal rats compared to

0 minute values but no increases were seen in hepatocytes from

thyrotoxic rats.

60 mins. Total ketone body production was again diminished

in fed (normal 0. 150 $\pm$ 0. 001 vs thyrotoxic 0. 089 $\pm$ 0. 028 μmol/10^6

cells; p $<$ 0. 05) and fasted (0. 407 $\pm$ 0. 034 v 0. 059 $\pm$ 0. 011 μmol/10^6

p $<$ 0. 001) thyrotoxic rats and the 3-OHB:Acac ratio was also decreased

(fed and fasted, p $<$ 0. 001). Ketone body production was also increased

in both fed and fasted normal rats compared with the 0 min and

30 min values but no increase was seen in thyrotoxic rats.

Ketone body production from 1 mmol U-^{14}C-palmitate (Table 13:4)

30 mins. Total ketone body production was increased

in hepatocytes from fed thyrotoxic rats compared to fed controls

(0.532 $\pm$ 0.079 v. 0.294 $\pm$ 0.048 μmol/10^6 cells, p< 0.05). No

further increase was seen with starvation in hyperthyroidism. The

3-OHB Acac ratio was decreased in both fed (p <0.001) and fasted

(p < 0.001) thyrotoxic rats. A significant increase in ketone body

production was apparent in starved normal rats compared to fed

normals (0.435 $\pm$ 0.059 μmol/10^6 cells, p < 0.05).

60 mins. Hyperthyroidism was again associated with

increased ketone body production in the fed state (0.940 $\pm$ 0.139 vs

0.471 $\pm$ 0.050 μmol/10^6 cells, p < 0.05), no further increase

occurring with fasting. Ketone body production was however

increased in starved normal rats (0.783 $\pm$ 0.090 μmoles/10^6 cells,

p< 0.01 vs fed normals) to levels similar to those found in fasted

thyrotoxic rat. The 3-OHB Acac ratio was significantly diminished in

both fed (p < 0.001) and fasted (p < 0.001) thyrotoxic rats compared

to controls.

Ketone body production by normal rat hepatocytes : Effects of
incubation with 10^{-8}M triiodothyronine (Table 13:5)

No effect of 10^{-8}M triiodothyronine incubated with normal

fed and fasted rat hepatocytes on acetoacetate, 3-hydroxybutyrate or

on the ratio of their production was apparent in this study.

TABLE 13:2.   Blood intermediary metabolite and insulin levels in
              fed and 48h starved normal and thyrotoxic rats
              (Mean $\pm$ SEM)

	FED NORMAL (n = 6)	FED THYROTOXIC (n = 6)	STARVED NORMAL (n = 6)	STARVED THYROTOXIC (n = 6)
Blood glucose mmol/l	4.0±0.4	3.9±0.13**	3.9±0.3	3.8±0.2
Blood lactate mmol/l	1.64±0.13	2.29±0.13**	1.87±0.27	2.18±0.15**
Blood pyruvate mmol/l	0.07±0.01	0.15±0.01**	0.07±0.02	0.08±0.01••
Blood alanine mmol/l	0.25±0.04	0.31±0.04	0.26±0.05	0.34±0.05
Blood glycerol mmol/l	0.06±0.01	0.42±0.04***	0.50±0.03•••	0.84±0.08**••
Blood acetoacetate mmol/l	0.11±0.04	0.91±0.21***	0.63±0.19•••	1.39±0.27•**
Blood 3-hydroxy-butyrate mmol/l	0.26±0.01	1.64±0.17***	1.31±0.13•••	1.64±0.26
3-hydroxy-butyrate : acetoacetate ratio	2.36±0.42	1.80±0.38	2.11±0.42	1.18±0.49*
Total ketone bodies mmol/l	0.37±0.04	2.55±0.21***	1.94±0.28•••	3.03±0.41*
Serum insulin mu/l	33.0±5.2	16.2±3.5*	6.7±1.0•••	5.2±0.2•••

* $p < 0.05$ thyrotoxic vs control (** $p < 0.01$, *** $p < 0.001$)

• $p < 0.05$ starved vs fed (•• $p < 0.01$, ••• $p < 0.001$)

TABLE 13:3. Ketone body production in the absence of added substrate by hepatocytes from fed and starved normal and thyrotoxic rats (Mean ± SEM)

		FED NORMAL (n = 6)	FED THYROTOXIC (n = 5)	48h STARVED NORMAL (n = 6)	48h STARVED THYROTOXIC (n = 5)
Acetoacetate	0'	0.015±0.003	0.020±0.005*	0.039±0.003●●	0.027±0.010
μmol/10^6 cells	30'	0.068±0.003	0.046±0.008	0.192±0.042●●●	0.035±0.008***
	60'	0.094±0.01	0.069±0.012*	0.289±0.034●●●	0.056±0.001***
3Hydroxybutyrate	0'	0.013±0.002	0.007±0.003	0.012±0.003	0.002±0.0005
μmol/10^6 cells	30'	0.031±0.002	0.009±0.007*	0.077±0.028●●	0.004±0.001***
	60'	0.056±0.001	0.019±0.014*	0.118±0.045●●	0.004±0.002***
Total ketone	0'	0.028±0.004	0.027±0.010	0.051±0.006●●	0.029±0.008*
bodies	30'	0.099±0.004	0.055±0.014**	0.269±0.025●●●	0.039±0.009●●●***
μmol/10^6 cells	60'	0.150±0.01	0.089±0.028*	0.407±0.031●●●	0.059±0.011***
3Hydroxybutyrate	0'	0.85±0.07	0.35±0.07***	0.30±0.05●●	0.07±0.02***●●
: acetoacetate	30'	0.46±0.04	0.15±0.05***	0.56±0.27	0.09±0.03***
ratio	60'	0.62±0.07	0.19±0.07***	0.48±0.23	0.07±0.03●●

* p < 0.05 Normal v. thyrotoxic (** p < 0.01, *** p < 0.001)

● p < 0.05 Fed v fasting (●● p < 0.01, ●●● p < 0.001)

TABLE 13:4. Ketone body production from 1mM U ^{14}C palmitate by hepatocytes from fed and starved normal and thyrotoxic rats (Mean $\pm$ SEM)

		FED NORMAL (n = 6)	FED THYROTOXIC (n = 5)	STARVED NORMAL (n = 6)	STARVED THYROTOXIC (n = 5)
Acetoacetate μmol/10^6 cells	30'	0.104±0.021	0.341±0.045***	0.127±0.019	0.318±0.031**
	60'	0.197±0.031	0.642±0.064***	0.284±0.057●	0.684±0.068***
3-hydroxy-butyrate μmol/10^6 cells	30'	0.190±0.035	0.196±0.053	0.308±0.047●	0.144±0.043**
	60'	0.290±0.035	0.289±0.093	0.499±0.065●	0.203±0.052*
Total ketone bodies μmol/10^6 cells	30'	0.294±0.048	0.532±0.079*	0.435±0.059●	0.436±0.050
	60'	0.471±0.050	0.940±0.139*	0.783±0.090●●	0.899±0.108
3-hydroxy-butyrate : acetoacetate ratio	30'	2.18±0.49	0.60±0.16***	2.45±0.27	0.392±0.057***
	60'	1.70±0.40	0.45±0.12***	2.09±0.39	0.342±0.055***

* $p < 0.05$ (** $p < 0.01$, *** $p < 0.001$) normal vs. thyrotoxic

● $p < 0.05$ (●● $p < 0.01$, ●●● $p < 0.001$) fed vs. starved.

TABLE 13:5. Ketone body production from 1 mM U ^{14}C palmitate by isolated normal rat hepatocytes : effects of addition of 10^{-8}M triiodothyronine to incubation medium (60 min.) (mean $\pm$ SEM)

	FED (control) (n = 4)	FED (+ T_3) (n = 4)	STARVED (control) (n = 4)	STARVED (+ T_3) (n = 4)
Acetoacetate μmol/10^6 cells	0.24$\pm$0.09	0.31$\pm$0.10	0.37$\pm$0.05	0.43$\pm$0.04
3-Hydroxy-butyrate μmol/10^6 cells	0.21$\pm$0.03	0.23$\pm$0.02	0.68$\pm$0.25	0.72$\pm$0.30
Total ketone bodies μmol/10^6 cells	0.46$\pm$0.13	0.52$\pm$0.11	1.05$\pm$0.27	1.14$\pm$0.28
3.OHB:AcAc ratio	1.19$\pm$0.42	0.90$\pm$0.17	1.88$\pm$0.63	1.19$\pm$0.42

All comparisons control vs T_3 addition NS.

13.4. DISCUSSION

Blood glycerol concentrations in fed and fasted thyrotoxic rats were elevated compared to controls. These findings are consistent with previous studies showing an effect of thyroid hormones to increase lipolysis in the rat (Deykin & Vaughan, 1963; Vaughan, 1967) and to impair insulin mediated suppression of glycerol release from isolated rat adipocytes (Fisher & Ball, 1967). Blood ketone body concentrations were also increased in fed thyrotoxic rats with a less striking increase after 48 h starvation compared to controls.

Circulating insulin levels were markedly decreased in fed thyrotoxic rats compared to controls in the absence of any change in blood glucose levels. Insulin secretion has previously been reported to be diminished (Levy et al. 1969; Andreani et al. 1970), normal (Hales & Hyans, 1964; Holdsworth & Besser, 1968) or increased (Doar et al. 1969; Aranda et al. 1972) using a variety of species and experimental techniques even though the pattern of insulin secretion has not been examined. In hyperthyroid man true insulin hyposecretion may indeed also occur but is marked by concommitant hypersecretion of proinsulin (Sestoft & Heding, 1981) which cross-reacts with insulin on standard double antibody radioimmunoassay.

Ketone body formation by hepatocytes from fed thyrotoxic rats in the absence of added palmitate was diminished at both 30 and 60 min. and unlike the situation in normal rat hepatocytes, failed to increase with subsequent starvation. Decreased hepatic triglyceride stores in thyrotoxic rats could explain such a situation and evidence

in favour of this hypothesis is provided by isolated perfused rat

liver studies (Keyes & Heimberg, 1979; Laker & Mayes, 1981).

Ketone body production from added palmitate substrate

was increased in fed thyrotoxic rat hepatocytes at both 30 and 60

min. compared to fed controls demonstrating that ketogenesis is

already primed in these animals. In control rats, ketone body

production increased by 70% with starvation whilst in thyrotoxic rats

no such increase was apparant and production rates during starvation

were similar in both groups. The reasons for this are uncertain.

It is possible that production rates from 1 mM palmitate using the

system described were already maximal in fed thyrotoxic animals

(60 min. value $\sim 1.0 \, \mu M/10^6$ cells). Previous studies in this

laboratory have shown similar rates of ketone body production at

60 mins in starved diabetic rats where extremely high rates of

ketogenesis would be expected (J. M. Burrin, personal communication).

Further studies using varying concentrations of substrate and

varying durations of incubation will be necessary to resolve this

question.

Increased ketone body production from fixed substrate

concentrations in hyperthyroidism have previously been demonstrated

in isolated perfused rat liver preparations (Keyes & Heimberg, 1979;

Laker & Mayes, 1981; Bartels & Sestoft, 1981) but the mechanisms

by which these changes occur are uncertain. Involvement of hepatic

cyclic nucleotides appears likely. Cyclic AMP (cAMP) and

dibutyryl cAMP both diminish output of triglycerides and VLDL

and increase ketogenesis in perfused livers from normal fed rats. ·
(Heimberg et al. 1969; Klausner et al. 1978) whilst T_3 treatment is
known to increase hepatic cAMP (Singh & Snyder, 1978) and hepatic
protein kinase activity (Rogues et al. 1977). Precise knowledge of
the effect of thyroid hormones on other known regulators of intra-
hepatic fatty acid metabolism (malonyl CoA and the acyl carnitine
transferase system) remains to be determined. Ketogenesis from
octanoic acid (which does not require the acyl carnitine transferase
system to enter mitochondria) is unaffected by thyroid status suggesting
that thyroid hormones may act at this level (Bartels & Sestoft, 1981).

An alternative explanation to a direct hepatic effect of thyroid
hormones lies in the fact that peripheral insulin levels were
significantly diminished in fed thyrotoxic rats who demonstrated
enhanced lipolytic activity, increased circulating ketone body
concentrations and increased ketone body production. Insulin
deficiency, however mediated, may prime the liver for ketogenesis
(McGarry et al. 1978) before removal from the intact animal.
Previous studies have neglected to consider this aspect.

Thus during feeding lipolytic activity is increased in
hyperthyroid rats as witnessed by increased blood glycerol levels.
Ketone body production by the liver is also increased and as a result
ketone body concentrations in blood are elevated. During fasting,
blood glycerol levels increase by a further 100% but ketone body
production from fixed substrate is not increased. Ketone body
concentrations in blood rise only modestly. These findings suggest

that in fasting hyperthyroid rats the pathways for ketone body production may be saturated and this is supported by the production rates by hepatocytes. However, in the intact animal substrate supply may vary considerably allowing for higher rates than predicted from the fixed substrate studies. Under these circumstances, increased peripheral ketone body utilisation could explain the changes in circulating metabolites.

The situation in the rat is however not directly comparable to that in thyrotoxic man in whom ketone body concentrations during feeding are normal. The degree of circulating hypoinsulinaemia may be an important aspect in this respect. During fasting however, ketone body concentrations rise and ketone body production increases as previously described (Chapter 12). Splanchnic balance studies have suggested that the bulk of this increase can be accounted for by increased presentation of NEFA to the liver but a direct effect of thyroid hormones on intrahepatic NEFA metabolism could not be excluded (Hagenfeldt et al. 1981). Evidence that a β adrenergic mechanism could underlie such a change was suggested by Beylot et al. (1980) who demonstrated that ketone body concentrations in blood decreased in hyperthyroid subjects treated with propranolol in the absence of any significant changes in blood glycerol or plasma NEFA concentrations. Relative hypoinsulinaemia (Sestoft & Heding, 1981) together with normal (Chapter 7) or increased (Kabadi & Eisenstein, 1980) plasma glucagon levels may also be important factors.

A striking decrease in the ratio of 3-hydroxybutyrate :

acetoacetate (3OHB:Acac .) production by hyperthyroid rat

hepatocytes was demonstrated in this as in one previous study

(Bartels & Sestoft, 1981). This suggests a more oxidised cytosolic

and mitochondrial redox state. Consistent with this an effect of

thyroid hormones to increase the rate of transfer of reducing

equivalents from the cytosol to the mitochondria in hepatocytes has

previously been described (Hoch, 1974; Sestoft, 1980). However,

a decrease in the ratio of 3OHB to AcAc produced has not been a

universal finding (Keyes & Heimberg, 1979; Laker & Mayes, 1981)

although Laker and Mayes (1981) did demonstrate a decreased

lactate : pyruvate production again suggestive of a more oxidised

redox potential in thyrotoxic rat livers.

Direct addition of supraphysiological concentrations of

triiodothyronine to incubated normal rat hepatocytes had no effect

on ketone body production in the fed or starved state. This is

consistent with observations suggesting that thyroid hormones act on

nuclear receptors to induce protein, probably enzyme synthesis

with subsequent expression of thyroid hormone action (see Chapter 4).

Such effects would not be expected to be manifest before 60 minutes,

the earliest detectable changes being reported after 2 hours

(Hoch, 1974). Longer incubations are required to investigate this

aspect of thyroid hormone effects on ketogenesis and would clearly be

decisive in determining a 'direct' role for thyroid hormones on

intrahepatic fatty acid metabolism.

14.   DISCUSSION

14.1.   CARBOHYDRATE METABOLISM

     i)   Post absorptive man

        As in many previous studies, hyperthyroidism was

characterised by an elevation of the fasting blood glucose

concentration (Chapters 7, 9, 10, 11) (for review, see Chapter 4).

This represents a balance between glucose production and glucose

utilisation.   Further studies were required to determine whether

over-production or under utilisation of glucose was responsible.

Isotope dilution studies were therefore performed in overnight fasted

thyrotoxic and control subjects (Chapter 9).   Two isotopes of glucose

were used as tracers and were administered by bolus injection.

$[^3H]$-3-glucose behaves as an "irreversible" tracer in that the tritium

label is lost as tritiated water at the triose phosphate level of the

glycolytic pathway and recycling of label back to glucose does not

occur.   Turnover estimates using this isotope represent the amount

of glucose metabolised to this point and beyond.   In normal man this

isotope provides the best estimate of "absolute" glucose turnover in

the post-absorptive state (Saunders et al. 1979).   A theoretical criticism

does arise in thyrotoxicosis in that this isotope will not take account

of potential substrate cycle activity at the fructose-6-phosphate :

fructose-1-6-diphosphate level which has been shown to be increased

in thyrotoxic rats (Okajima & Ui, 1979).   Similarly cycling at the

glucose:glucose-6-phosphate level will not have been recognised so that

absolute glucose turnover may have been overestimated.

Simultaneous administration of $[^{14}C]$-1. glucose allowed assessment of glucose recycling from more distant sites. Glycolytic metabolites of this isotope (3-carbon derivatives) have the potential to recycle to form new radioactive glucose molecules thereby lowering the apparant glucose turnover rates. The difference between the two derived turnover rates is due to such recycling. The bulk of this recycling is considered to occur from lactate (Streja et al. 1963).

Absolute glucose turnover, assessed as above, was increased by 50% in hyperthyroid patients. This finding is in agreement with other studies using the same isotope of glucose in man (Perez et al. 1980; Saunders et al. 1979) and rats (Okajima & Ui, 1979). Contradictory data has recently been produced from splanchnic balance studies demonstrating a decrease in hepatic glucose output in hyperthyroid subjects of similar age and severity of disease. (Wahren et al. 1981). The reason for this discrepancy is unclear. In normal man, after an overnight fast, over 90% of glucose production comes from the liver. Increased glucose production from the kidney, well recognised in prolonged fasting in normal subjects, could occur more rapidly in hyperthyroidism. An increase in total glucose production, assessed isotopically, could thus be compatible with a decrease in hepatic glucose output. Increased renal glucose production has not however been demonstrated in hyperthyroidism. Alternatively, dietary preparation may be an important factor. The subjects reported in this thesis had a daily carbohydrate intake of at least 250g/day whereas in the splanchnic balance study details of

dietary preparation are not given.

Glucose recycling was increased 3. 6 fold in hyperthyroid patients. In normal man, such cycling serves to preserve glucose not required in a particular tissue for utilisation elsewhere. However, attention has recently been directed towards substrate and Cori cycles as potential sites of energy consumption or heat production in hyperthyroidism (Okajima & Ui, 1979, Sestoft, 1980). The finding of increased glucose-lactate (Cori) cycling reported here is consistant with data from hyperthyroid rats (Okajima & Ui, 1979) and is the first direct assessment of recycling in hyperthyroid man. The excess energy consumption in this process is however minimal although the concept is important.

Assuming a wastage of 4ATP molecules per molecule of glucose recycled as the sole energy deficit, the amount of energy involved would be only 10. 5 kcal per 24h. However, as will be discussed later, fuel economy in hyperthyroidism may be more heavily based on lipid metabolism. Given also that the body has greater stores of fat than carbohydrate and that substrate cycles may operate within the adipocyte (NEFA:triglyceride), the potential for energy consumption in adipose tissue may be considerably greater. The demonstration that lipolysis and lipogenesis both proceed at accelerated rates in isolated rat adipocytes (Sestoft, 1980) lends weight to this hypothesis. Energy derived from the hydrolysis of ATP during operation of such cycles cannot be harnessed to any metabolically useful purpose, and is dissipated as heat. Many of the

clinical features of hyperthyroidism (such as heat intolerance, sweating

and tachycardia.) could be explained on this basis.

Turnover rates determined using $[^{14}C]$-1-glucose represent

'irreversible' glucose disposal and were not significantly increased

in hyperthyroidism. The greater part of the increase in total glucose

turnover could thus be accounted for by increased recycling. This is in

contrast to the findings in hyperthyroid rats where irreversible

glucose disposal was also increased (Okajima & Ui, 1979).

Isotopic studies therefore suggest that glucose production

is increased in hyperthyroid but they do not differentiate between

changes in glycogenolysis and gluconeogenesis nor do they indicate

the total capacity of either process.

An attempt was therefore made to investigate gluconeogenesis

in vivo in man. Blood concentrations of the gluconeogenic precursors

lactate, pyruvate and alanine were similar in hyperthyroid and control

subjects whilst blood glycerol concentrations were increased in

hyperthyroidism. Circulating levels of gluconeogenic precursors do

not however give sufficient information to extrapolate results to changes

in gluconeogenesis.

Administration of a radioactively labelled gluconeogenic

precursor and measurement of its appearance in glucose is an

attractive means for increasing gluconeogenesis. However, fairly

large amounts of radioactivity may have to be given to achieve satisfactory

increases in glucose specific activity for subsequent analysis. This

would be particularly true should isotopes of lactate or pyruvate be

used because of the numerous and diverse metabolic pathways in

which there intermediates participate (Okajima et al. 1981; Katz et al.

1981). Exchange of isotope between these various metabolites also

hampers interpretation of the results. In addition the majority of

patients suitable for study were young and female to whom

radioisotopes could not be administered ethically.

Alternatively, splanchic balance studies would have permitted

estimation of uptake of gluconeogenic precursors by the liver. Such

studies have been performed (Wahren et al. 1981) and have shown

that uptake of all the major gluconeogenic precursors by the liver is

increased in hyperthyroidism. For lactate, pyruvate and alanine this

is achieved by increased fractional splanchnic extraction whilst for

glycerol increased arterial concentrations leading to increased hepatic

supply is responsible. These data are consistent with isolated

perfused rat liver studies which have also demonstrated increased

hepatic uptake of all the major gluconeogenic precursors

(see Chapter 4:3). Splanchnic balance studies are however invasive

and involve catheterisation of the hepatic vein and a peripheral

artery and thus pose serious ethical problems. Also, the assumption

from these studies is that hepatic uptake of a gluconeogenic precursor,

during fasting is equivalent to its incorporation into glucose, which

may not be true in normal or diseased man (Cherrington, 1981).

The other principal method involves administration of a

substrate load and measurement of its clearance from blood. For

lactate, pyruvate and alanine the assumption that clearance from blood

could be equivalent to incorporation into glucose is untenable because
of their diverse metabolic fates (see above). However, in the fasting
state, glycerol has no major metabolic fate other than hepatic uptake
for gluconeogenesis (Shaw et al. , 1976) and indeed is metabolised
to glucose in preference to other precursors (Sestoft et al. 1977).
The underlying principal of the study undertaken was therefore to
"flood" the metabolic pathway for glycerol clearance with a bolus i. v.
glycerol injection so that the subsequent clearance rate of glycerol
could be equated to the capacity for gluconeogenesis (see Chapter 4:4).

Using this technique glycerol clearance was increased by
80% in hyperthyroidism compared to controls of similar age and weight
suggesting a considerable increase in gluconeogenic capacity.
Assuming that glycerol clearance is independent of its concentration
within the range employed, the finding of a 3 fold rise in endogenous
glycerol production with only a 70% increase in fasting blood glycerol
levels suggests that basal rates of gluconeogenesis from glycerol
are also increased in hyperthyroidism. It should however be stressed
again that the assumption that clearance can be equated directly with
gluconeogenesis remains to be firmly established.

The contribution of glycogenolysis to hepatic glucose
production has not been assessed in this thesis but previous studies
have shown that hepatic glycogen stores are diminished in overnight
fasted thyrotoxic man (Pipher and Paulsen, 1947) and animals
(Battarbee, 1974). In addition splanchnic balance studies have shown
that glycogen breakdown contributed less to hepatic glucose production
in thyrotoxic subjects compared to normals after an overnight fast

(Wahren et al. 1981).

Thus in hyperthyroidism blood glucose concentrations are increased after an overnight fast as a result of increased hepatic glucose production. The bulk of this increase is probably the result of enhanced gluconeogenesis. Irreversible glucose disposal is normal but recycling of glucose derived 3-carbon intermediates is greatly increased.

In hypothyroidism fasting blood glucose concentrations were normal. This is in agreement with previous reports (Saunders et al. 1980) although some studies have shown a small decrease (Lamberg 1965; Levy et al. 1970). Despite these findings a small, but highly significant decrease in glucose turnover was apparent together with a significant reduction in irreversible glucose disposal. Whilst similar results have been demonstrated in hypothyroid rats (Okajima & Ui, 1979) Saunders et al. (1980) found glucose turnover values in hypothyroid man to be within the normal range although a significant increase was demonstrable following thyroxine replacement therapy. Glucose recycling, not previously investigated in hypothyroid man, was normal in contrast to rat studies where recycling was diminished (Okajima & Ui, 1979).

Circulating concentrations of lactate, pyruvate and alanine were normal in fasted hypothyroid subjects whilst glycerol concentrations were diminished (Chapter 8) or normal (Chapters 9, 10, 11). The reason for the variable fasting blood glycerol concentrations is uncertain because dietary preparation was similar in all studies.

These results are comparable with those of Saunders et al. (1980)
but other studies have shown diminished lactate (McDaniel et al.
1977), and alanine (Bondy, P.K.1949; Ness et al. 1969)
concentrations.

Diminished gluconeogenesis from all the major precursors
has previously been reported from studies using hypothyroid
perfused rat liver preparations (Chapter 4:4). Evidence concerning
gluconeogenesis in hypothyroid man is however scanty. The finding
of normal glucose recycling suggests that gluconeogenesis from lactate
and pyruvate is normal. Glycerol clearance rates were however
decreased by 33% indicated that gluconeogenic capacity from this
substrate is diminished in hypothyroidism. In addition a 50%
decrease in endogenous glycerol production in the absence of any
decrease in basal glycerol concentrations in the subjects studies
suggests that basal glycerol clearance (and hence basal gluconeogenesis)
is also diminished. Gluconeogenesis from alanine has previously been
investigated only in infants with congenital hypothyroidism (Hayek, 1979)
and was reported to be normal.

No estimates of glycogenolysis in hypothyroidism has been
made here but previous reports have shown that whilst hepatic glycogen
stores are normal in hypothyroid rats, glycogenolysis is impaired
(Battarbee, 1974).

Thus in hypothyroidism fasting blood glucose concentrations
are normal but hepatic glucose production is diminished as is
irreversible glucose disposal. Glucose recycling is normal but

gluconeogenesis from glycerol may be diminished.

ii)   <u>Fed man</u>

Impaired tolerance to oral glucose has frequently been described in hyperthyroidism (Hales & Hyams, 1964; Holdsworth & Besser, 1968; Marks <u>et al.</u>, 1960), but the mechanisms of its production remain uncertain. Rapid absorption of glucose from the gastrointestinal tract may be a factor (Holdsworth & Besser, 1968) but the glucose tolerance test is to some extent an unphysiological one in that a meal consisting of 50-100 g of pure glucose is rarely, if ever, encountered in everyday life. For this reason the blood glucose and intermediary metabolite response to mixed meals was examined (Chapter 7).

Post-prandial hyperglycaemia was readily demonstrable and in addition blood glucose concentrations remained elevated throughout a 12h period of normal meals and activity. Peak post-prandial blood glucose concentrations occurred at the same time in patients and controls suggesting that absorption from the gut was not the major factor. Peripheral insulin concentrations, measured by standard double antibody radioimmunoassay were similar in hyperthyroid and control subjects despite prevailing hyperglycaemia in the patient group. A defect in insulin secretion and/or action was therefore apparent (for review see Chapter 4:4).

Previous studies of insulin sensitivity in hyperthyroid man and animals have yielded conflicting results largely due to the variety of experimental techniques employed. Thus increased, (Elgee &

Williams, 1955; Maracek et al. 1973), decreased (Ikejiri et al. 1977)

or normal (Elrick et al. 1961) insulin sensitivity, have all been

described.   In these studies the index of insulin sensitivity has been

the rate of fall of blood glucose concentrations following oral glucose

or intravenous insulin administration.   Factors other than true

insulin sensitivity may therefore be involved particularly if

hypoglycaemia is induced and counter-regulatory hormone secretion

is stimulated.   The euglycaemic hyperinsulinaemic clamp technique

allows assessment of insulin sensitivity at physiological (usually fasting)

blood glucose concentrations and in the absence of stimulated

counterregulatory hormone secretion (De Fronzo et al. 1979)

(see Chapter 11).

No difference in insulin sensitivity could be found between

hyperthyroid and control subjects.   Also, very similar steady state

circulating insulin concentrations were achieved suggesting that insulin

metabolic clearance rates were identical in the two groups.

Explanations for hyperglycaemia after mixed meals or oral glucose

in hyperthyroidism must therefore be sought elsewhere.

Diminished insulin secretion by isolated rat pancreatic tissue

(Milcu et al. 1975) and, more recently, hypersecretion of proinsulin

in response to oral and intravenous glucose in man (Sestoft & Heding,

1981) have suggested that hyperthyroidism may be associated with

impairment of normal pancreatic insulin secretory mechanisms.

Thus, in man high circulating proinsulin levels may cross react with

insulin when measured by standard double antibody radioimmunoassay

and mask true "hypoinsulinaemia" in thyrotoxicosis.   This hypothesis

could be investigated further using the hyperglycaemic clamp
technique in which insulin secretory pattern and capacity can be
determined in response to a glucose infusion designed to maintain
blood glucose concentrations at a desired hyperglycaemic value
(usually 10 mmol/l) (Ponchner et al. 1982).

Hyperlactataemia and hyperpyruvicaemia were apparant in
hyperthyroid subjects after mixed meals. Since neither production
nor utilisation of these metabolites were measured changes in either
could have been responsible. Evidence suggests that increased
production is the major factor as after oral glucose loading there is
an increase in both peripheral glucose utilisation (Elgee & Williams,
1955; Maracek & Feldman, 1973) and in lactate production measured
directly by forearm balance studies (Araki et al. 1968).
Hyperglycaemia per se may also be important as recent evidence
suggests that the circulating glucose level may be critical in the
regulation of blood lactate concentrations (Cherrington, 1981).
These studies have shown that in the presence of peripheral insulin
levels similar to those reported here after meals, lactate released
by insulin sensitive tissues varies positively with ambient blood
glucose concentrations (Blackshear et al., 1974; Dietz, 1978).

During the hyperinsulinaemic euglycaemic clamp experiments
(which mimic a pure glucose meal) incremental rises in lactate and
pyruvate were identical in thyrotoxic and control subjects. The
reason for the difference between this finding and the response to mixed
meals despite similar peripheral insulin concentrations is uncertain.

Several factors may be involved. The total amount of glucose

infused during clamping was 28-30g (whereas a mean oral

carbohydrate load with mixed meals was 45-50g) thereby limiting

the potential for lactate production. Also, blood glucose concentrations

were maintained at fasting levels during clamping and this may

directly affect peripheral lactate production (vide supra).

In hypothyroid subjects blood glucose concentrations were

mildly elevated after breakfast and the peak blood glucose level was

delayed. Meal tolerance thereafter was normal. Impaired tolerance

to oral glucose has previously been reported (Gutman et al. 1972)

and attributed to impaired absorption of glucose from the gastro-

intestinal tract (Holdsworth & Besser, 1968; Althausen & Stockholm,

1980). Whilst this may explain a delay in peak post prandial blood

glucose levels it would be expected to produce a "flattening" of the blood

glucose profile. The exaggerated blood glucose response reported

here suggests impaired peripheral glucose utilisation in

hypothyroidism. Sensitivity to exogenous insulin was not assessed in

hypothyroid subjects, nor was the pattern of insulin secretion. Thyroid

hormones are however required for normal insulin secretion by

isolated rat pancreatic cells (Milcu et al. 1975).

Hyperlactataemia and hyperpyruvicaemia were apparent after

meals in hypothyroidism (as in hyperthyroidism) and the bulk of the

available evidence suggests that this is due to diminished clearance

(Shamer et al. 1971; McDaniel et al. 1977). An effect of ambient

hyperglycaemia (Cherrington et al. 1981) may also be important.

iii)   Effects of Therapy

Antithyroid therapy for 6 months (Chapter 7) had no effect on fasting blood glucose concentrations or on the response of blood glucose, lactate and pyruvate to mixed meals. Similarly Kreines et al. (1965) and Cavagnini et al. (1974) reported persisting intolerance to oral glucose in subjects whose hyperthyroidism had apparently been successfully treated. The reasons for persistance of these abnormalities are uncertain particularly because some aspects of glucose metabolism (e.g. glucose turnover and recycling) do return to normal within this period.

Serum insulin levels were unaltered by antithyroid treatment. In animals, thyroid hormone excess is associated with damage to pancreatic beta cells (Holst, 1921). In dogs, although short term exposure to thyroid hormone excess produces reversible damage, permanent pancreatic damage may be caused by more prolonged exposure (Houssay, 1946), resulting in persisting insulin hyposecretion. The presence of hyperglycaemia with normal serum insulin levels in our patients suggests that insulin hyposecretion persisted despite treatment. The pattern of insulin secretion with regard to insulin : proinsulin ratio in hyperthyroid patients following treatment is unknown.

Persisting hyperlactataemia and hyperpyruvicaemia after meals is difficult to explain but may again relate to ambient blood glucose concentrations (Cherrington, 1981). Further studies are required to determine whether glucose metabolism ever returns entirely to normal in treated thyrotoxic patients.

In hypothyroidism, thyroxine therapy over a six month period had no effect on fasting or post-prandial blood glucose, lactate or pyruvate concentrations. Circulating insulin levels increased significantly and were higher than in controls. Untreated hypothyroidism may thus be associated with both insulin hyposecretion and insulin resistance. Thyroxine therapy may restore insulin secretory capacity but have no effect on insulin resistance. No ready explanation for the failure of post-prandial hyperlactataemia to respond to treatment can be proposed, particularly as glucose turnover and recycling return to normal within 6 months (Chapter 9; Saunders et al. 1980; Okajima & Ui, 1979).

14.2.   LIPID METABOLISM

i)   Post-absorptive man

The finding of increased levels of NEFA and glycerol in hyperthyroid subjects both after an overnight fast and immediately prior to each meal suggests increased lipolysis. This has previously been demonstrated in hyperthyroidism both in vivo (Rich et al. 1959; Harlan et al. 1963) and in isolated adipose tissue (Arner et al. 1979; Wennlund et al. 1981). These latter authors have suggested that the increase in lipolysis may be due to increased β adrenergic sensitivity perhaps coupled with diminished responsiveness of the adipocyte to circulating insulin. A direct effect of thyroid hormones cannot be confirmed or excluded on available data. The turnover of both glycerol (Tibbling, 1969) and NEFA (Saunders et al. 1980) is also increased in hyperthyroid man confirming that both production and utilisation of

these substrates are enhanced. Data from the glycerol clearance
study reported here showing enhancement of glycerol clearance and
production (Chapter 10) is in agreement with these findings.

Blood ketone body concentrations were also increased in
hyperthyroid patients (Chapter 7, 10, 11, 12) and, as in normal
subjects, this was related to an increase in ketone body production.
The 3-fold increase in ketone body production reported in
hyperthyroidism agrees well with the splanchnic balance studies of
Hagenfeldt et al. (1981). Because of this increased production,
several authors (Hagenfeldt et al. 1981; Bartels et al. 1979) have
postulated that hyperthyroidism is characterised by a state of
"accelerated starvation". Contrary to this view studies reported
here have demonstrated fasting hyperglycaemia with increased glucose
production,features not associated with prolonged starvation. The
increase in glucose production was however modest (50%) and most
was used for recycling. Compared to the increase in ketone body
production this suggests that whilst overall energy demands are
increased in hyperthyroidism a significant proportion of this increase
is met by increased fat metabolism.

The reason for the increased ketone body production is
uncertain. Increased supply of substrate NEFA due to increased
lipolysis and increased hepatic blood flow are clearly important
factors but whether thyroid hormones alter the metabolic "set" of the
liver such that fatty acid oxidation and ketone body production are
favoured is unclear. Hagenfeld et al. (1981) believed that the

major part of the increase in ketone body production in hyperthyroidism could be accounted for by the increase in precursor supply although an effect on hepatic metabolism could not be excluded by their data.

Effects on intrahepatic NEFA metabolism in hyperthyroidism have previously been investigated using isolated perfused livers from hyperthyroid rats (Keyes & Heimberg, 1979; Laker & Mayes, 1981; Bartels & Sestoft, 1980). These studies have universally demonstrated that hyperthyroidism is associated with increased ketone body production from exogenous substrate. Ketone body production rates in the absence of added substrate (endogenous production) had not previously been examined nor had the hormonal environment prevailing in the donor rats.

Using isolated hepatocytes from recently fed rats (Chapter 13) thyroid hormone pretreatment was associated with a significant increase in ketone body production from 1 mM palmitate. After 48h starvation however there was no apparent difference in ketone body production by hepatocytes from hyperthyroid compared to control or indeed fed hyperthyroid rats. These observations have previously been described in studies using perfused rat livers (Bartels & Sestoft, 1980) and may be related to experimental design. Possibly ketone body production from 1 mM palmitate was already maximal in fed thyrotoxic rat hepatocyte preparations with no possibility for an increase with subsequent starvation. This maximal production rate could also be achieved by normal rat hepatocytes after 48 h starvation. Further studies using different substrate

concentrations and varying fasting periods are required to resolve the question.

The intrahepatic events which regulate these processes are largely unknown but it is likely that the acyl-carnitine transport (ACT) system is an important regulatory step. Bartels and Sestoft (1981) have shown that whilst ketone body production from long chain fatty acids (which enter mitochondria via the ACT system) is increased in hepatocytes from recently fed thyrotoxic rats, production rates from octanoic acid (a medium chain fatty acid which does not use the ACT system to enter mitochondria) are similar to control values. This is also consistent with data from Van Tol (1971) showing an effect of thyroid hormones to increase the activity of the carnitine shuttle.

Of the postulated regulators of the ACT system activity, malonyl CoA has received most attention (McGarry et al. 1977 & 1978). This compound, the first committed intermediate of the fatty acid synthesis sequence is a potent inhibitor of the ACT system. Malonyl CoA levels in the liver are increased in the fed state when conversion of glucose to fat is enhanced resulting in inhibition of the ACT system and hence fatty acid oxidation. Conversely, during fasting, little glucose is available for conversion to fat, malonyl CoA levels are low, ACT is activated and ketone body production accelerated. Insulin is a potent inhibitor of ketogenesis in the intact animal firstly by its action to suppress lipolysis but secondly by its capacity to greatly increase the activity of acetyl CoA carboxylase (which catalyses

the reaction acetyl CoA→ Malonyl CoA) (Lee et al. 1973, Klain et al.

1974). Glucagon on the other hand has been shown to markedly and

rapidly decrease hepatic fatty acid synthesis and to simultaneously

diminish by 70% the activity of acetyl CoA carboxylase (Klain &

Weber, 1973; McGarry et al. 1978). These observations lead

McGarry and Foster to hypothesize a bihormonal control of the ACT

system. The finding of decreased peripheral insulin concentrations

in fed thyrotoxic rats may therefore be of crucial importance in

that the livers may have been primed for ketogenesis before

removal. The findings reported here would be compatible with an

effect of hyperthyroidism to inhibit pancreatic insulin secretion in the

rat without necessarily implicating a direct effect of thyroid hormones

on rat liver metabolism.

In the absence of added palmitic acid substrate ketone body

production was diminished in hepatocytes from hyperthyroid rats

implying that endogenous substrate stores were also diminished.

Intrahepatic triglyceride levels were not determined here but

previous studies (Bartels & Sestoft, 1981; Laker & Mayes, 1981;

Keyes & Heimberg, 1979) have shown them to be decreased.

Metabolic clearance rates for ketone bodies, measured

isotopically were increased in hyperthyroid man suggesting

adaptation of peripheral tissues to ketone body utilisation as a source

of calories. The reason for this is uncertain but the oxidation of

ketone bodies by peripheral tissues produces an immediate sparing

of glucose (for cerebral use) by inhibiting glycolysis (as in heart

and kidney) and/or pyruvate oxidation (in all tissues) (Fenselau, 1981). The products of the limited degradation of glucose, pyruvate and lactate, can be returned to the liver for gluconeogenesis. This sequence of events may explain why irreversible glucose is normal in hyperthyroidism whilst Cori cycle activity and total glucose turnover are increased. As the brain adapts to ketone body utilisation (mechanism unknown) the obligatory requirement of nervous tissue for glucose will fall, the requirement for gluconeogenesis will be diminished so that protein can be conserved. Ketone bodies also have a direct inhibitory effect on proteolysis (Felig, 1973) in skeletal muscle thus further reducing the demand on protein stores/ muscle bulk which would otherwise become severely depleted in hyperthyroidism.

Initial studies in fasting hypothyroid men reported here suggested, on the basis of diminished blood glycerol concentrations, that lipolysis was decreased (Chapter 8). In subsequent studies (Chapters 9, 11, 12) hypothyroidism was associated with normal fasting blood glycerol concentrations. The reason for these discrepant findings is not readily apparant as dietary preparation was identical. Normal fasting NEFA concentrations were also found and NEFA turnover has been shown by others (Saunders et al. 1980) to be normal in hypothyroid man. Previous studies have however suggested diminished lipolysis in hypothyroidism both in vitro (Arner et al. 1981) and in vivo (Goodman & Bray, 1966) with diminished responsiveness to circulating catecholamines as the

likely mechanism (Arner et al. 1981). The finding of decreased

endogenous glycerol production rates (Chapter 10) is again suggestive

of diminished lipolysis in hypothyroidism despite the presence of

normal fasting glycerol levels.

Fasting blood ketone body concentrations were normal as

were ketone body production and clearance rates suggesting that

not only is hepatic substrate supply normal but intrahepatic NEFA

metabolism is also normal. Studies using isolated perfused rat

liver preparations have yielded conflicting results with both normal

(Laker & Mayes, 1981) and diminished (Keyes & Heimberg, 1979)

ketone body production from fixed amounts of substrate being reported.

The balance of the evidence reported in this thesis therefore

suggests that thyroid hormone deficiency is associated with diminished

lipolysis with normal blood glycerol levels the result of diminished

glycerol clearance. Ketone body metabolism is however normal.

ii)   Fed man

Although the normal diurnal patternsfor plasma NEFA

and blood glycerol concentrations were maintained in hyperthyroid

subjects (rising before and falling after meals), post prandial

concentrations of both remained elevated compared to controls.

These findings suggest that meal induced suppression of lipolysis

may be impaired in hyperthyroidism. Circulating levels of both these

metabolites also fail to suppress following oral glucose administration

in hyperthyroidism (Ortigosa et al. 1976) whilst studies using isolated

fat tissue have shown impaired insulin mediated suppression of glycerol

release (Wennlund et al. 1981). These findings suggest that

resistance to insulin action may be present in thyrotoxic human

adipose tissue. Further evidence in support of this view comes

from the hyperinsulinaemic euglycaemic clamp studies (Chapter 11).

These showed that blood glycerol concentrations in hyperthyroid

subjects failed to suppress to control values during insulin infusion.

The mechanism(s) underlying these changes are uncertain but

alteration of insulin receptor number or affinity has been postulated

(Wennlund et al. 1981). Further studies are underway to investigate

these possibilities in more detail.

In contrast to glycerol and NEFA levels, circulating ketone

body concentrations returned to normal following mixed meals and

during insulin infusion (Chapter 7, 11). This suggests that

suppression of ketogenesis is normal in hyperthyroidism despite

increased precursor supply from the periphery.

In hypothyroidism the normal diurnal profiles for blood

glycerol, plasma NEFA and blood ketone body concentrations were

lost, concentrations neither increasing before nor falling after

meals. Since blood ketone body production is normal in hypothyroidism

(Chapter 12) loss of the diurnal profile may simply reflect changes in

blood glycerol and plasma NEFA. Plasma NEFA levels reflect the

balance between lipolysis and reesterification with the adipocyte as

well as hepatic uptake and utilisation by peripheral tissues. Following

oral glucose hypothyroid subjects show impaired suppression of plasma

NEFA concentrations (Gutman et al. 1972). The reason for this is

unclear but in the presence of diminished peripheral glucose utilisation

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reesterification of NEFA by adipocytes may be impaired
(Chapters 8, 9; Okajima & Ui, 1979). Although there is discrepant
data concerning fasting concentrations, the loss of normal diurnal
blood glycerol concentrations does suggest that there may be prolonged
post-meal suppression of lipolysis compared to the situation in
normal subjects.

iii) Effects of therapy

In hyperthyroid subjects fasting blood glycerol, plasma
NEFA and blood ketone body concentrations returned to control values
following 6 months of antithyroid therapy (Chapters 7, 12) suggesting
that normal rates of lipolysis and ketogenesis were restored.
Normal responsiveness of isolated human adipocytes to both
catecholamines (Arner et al. 1979) and insulin (Wennlund et al. 1981)
is also restored by adequate treatment of hyperthyroidism. Ketone
body production and clearance (Chapter 12) are also normal after
treatment as are glycerol (Tibbling, 1969) and NEFA (Saunders et al.
1980) turnover. Normal ketone body production may reflect normal
hepatic substrate NEFA but splanchnic balance studies (Hagenfeldt
et al. 1981) have shown that the ratio of ketone body production to
hepatic fatty acid uptake is also decreased suggesting an additional
effect on intrahepatic fatty acid metabolism.

Diurnal profiles of glycerol, NEFA and ketone body
concentrations retained a normal pattern in treated hyperthyroid
subjects and were similar to or even lower than values found in
controls following mixed meals (Chapter 7). These results are in

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contrast to the profiles for glucose and the gluconeogenic precursors which were unaltered by treatment. The reasons for these discrepant results are uncertain (vide supra).

In hypothyroidism the initially decreased fasting blood glycerol concentrations observed in the metabolic profile experiments (Chapter 8) were increased towards normal by thyroxine therapy. In other studies (Chapters 11, 12) normal fasting glycerol concentrations were unaltered by treatment. Plasma NEFA and blood ketone body concentrations were normal after overnight fasting before and after replacement therapy as were ketone body production and metabolic clearance. Diurnal profiles for blood glycerol, ketone bodies and plasma NEFA were restored towards a more normal pattern.

14:3.   EFFECTS OF THYROID HORMONES ON CYTOSOLIC AND
MITOCHONDRIAL REDOX STATE AND HYDROGEN
SHUTTLE ACTIVITY

Although none of the studies reported here were specifically, designed to look at this question, several observations suggest that thyroid hormones may have important effects on the intracellular redox state. Thyroid hormones not only determine the amount of pyridine nucleotides (NAD^+, $NADP^+$, NADH, NADPH) in the tissues of experimental animals but also the balance between the oxidised and reduced forms (Hoch, 1974). In the cytosol and mitochondria hyperthyroidism favours the oxidised form and hypothyroidism the reduced. Changes in mitochondrial and cytosolic redox state may be reflected in blood as alterations in the ratio of lactate to pyruvate (L:P)

or in the ratio of 3 hydroxybutyrate to acetoacetate (3-OHB:Acac).
The finding therefore of a paradoxical increase in L:P ratio
following meals in hypothyroid subjects suggests a more reduced
cytosolic redox state implying a failure of transfer of reducing
equivalents from the cytosol to the mitochondria.

Thyroid hormones may exert their influence on redox
states partly by their effects on H-shuttle activity. The activity of
mitochondrial α-glycerophosphate dehydrogenase (α GPD) has special
metabolic significance. The enzyme converts α-glycerophosphate
(α GP) to dihydroxyacetone phosphate (DHAP) with generation of the
reducing equivalent, FADH. DHAP is transferred to the cytoplasm
where it is converted to α GP by a cytosolic α-glycerophosphate
dehydrogenase. The latter reaction converts NADH to NAD^{+} and
regenerates α GP for mitochondrial use. Since the mitochondria are
impermeable to NADH the system provides a mechanism for the
transfer of reducing equivalents from the cytosol to the mitochondrial
with the regeneration of NAD^{+}. Malic enzyme adopts a similar role
in another H-shuttle mechanism and the activity of this enzyme and
α GPD are known to be dependant on thyroid hormones in the rat both
by increasing synthesis of the enzymes themselves (Lee & Lardy,
1965; Lee et al. 1959; Lardy et al. 1960) and also the coenzyme FAD
(Wolf & Rivlin, 1970) (see Figure 10:2).

The rather surprising finding of a marked decrease in the
ratio of production of 3-hydroxybutyrate to acetoacetate by isolated
hepatocytes from hyperthyroid rats (Chapter 13) could also be

explained on the basis of changes in the cellular redox potential if

the balance within the mitochondria was towards a more oxidised

state as shown by Hoch (1974). Decreased 3-hydroxybutyrate to

acetoacetate production has been described by some (Bartels &

Sestoft, 1981) but not all (Laker & Mayes, 1981) workers using

isolated perfused hyperthyroid rat liver preparations. In hyperthyroid

man (3-OHB : Acac) production was normal as judged by splanchnic

balance studies although effects due to recycling of ketone bodies from

the periphery to the liver cannot be excluded. The observation that

a decreased 3-hydroxybutyrate to acetoacetate production by

hyperthyroid rat hepatocytes was not reflected by a decreased 3-OHB :

Acac ratio in blood suggests either preferential peripheral utilisation

of acetoacetate or substantial interconversion of the two ketone bodies

peripherally. Freeze-clamp studies of rat peripheral tissues are

planned to investigate this in more detail.

Further evidence suggesting an important role for

mitochondrial α GPD in the metabolic sequelae of thyroid over and

underactivity comes from the glycerol infusion data described in

Chapter 10 showing thyroid hormone dependent changes in glycerol

clearance in man. Glycerol taken up by the liver is first

phosphorylated. In the rat, the activity of glycerol kinase is

unaffected by thyroid state but gluconeogenesis from glycerol in

isolated perfused livers is greatly increased in hyperthyroidism as

feedback inhibition by glycerol-3-phosphate on glycerol kinase is

lacking (Sestoft et al. 1977). Accumulation of glycerol-3-phosphate

does not occur because of the extraordinarily large induction of mitochondrial α GPD by thyroid hormones (Lee et al. 1959). The activity of mitochondrial α GPD could play a role in the calorigenic action of thyroid hormones by at least two different mechanisms (1) by allowing a higher rate of glycerol phosphorylation (Sestoft et al. 1977) and of glycerol conversion to glucose (Schimasseck et al. 1963; Werner & Berry, 1944; Sestoft et al. 1977; Lin, 1977) and (2) by reducing the phosphorylation : oxidation ratio for oxidation of cytosolic reducing equivalents from three to two (NAD linked shuttles versus FAD linked shuttles respectively). It is therefore of interest that Nolte et al. (1972) did not find any increase in the activity of mitochondrial α GPD in the liver of hyperthyroid man. Although this observation remains to be confirmed it is indirectly supported by the finding that fractional glycerol turnover is not increased in hyperthyroid man (Tibbling, 1969). Haemodynamic factors may thus be as or more important in determining glycerol clearance in man than changes in mitochondrial α GPD activity.

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DIURNAL HORMONE–METABOLITE PROFILES IN HYPOTHYROIDISM

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SUMMARY

To investigate the influence of thyroid hormones on intermediary metabolism in man, hormone and metabolite profiles were obtained over a 12-h period of normal meals and activity in eight hypothyroid subjects before and during thyroxine replacement therapy, and in sixteen matched controls.

The fasting blood glucose concentration and the mean 12-h blood glucose concentration were normal in hypothyroid subjects but the blood glucose response to breakfast was exaggerated. Fasting blood lactate and pyruvate levels were normal but post-prandial hyperlactataemia and hyperpyruvicaemia were found and mean 12 h values for lactate (hypothyroid $1·08 \pm 0·06$ v. control $0·77 \pm 0·03$ mmol/l, $P < 0·01$) and pyruvate ($0·10 \pm 0·01$ v. $0·08 \pm 0·003$ mmol/l, $P < 0·01$) were elevated. Blood alanine concentrations were elevated only in the evening. Although plasma non-esterified fatty acid levels were normal, fasting blood glycerol levels were decreased ($0·06 \pm 0·01$ v. $0·08 \pm 0·01$ mmol/l, $P < 0·001$) and this decrease persisted throughout the 12-h period. Blood total ketone body concentrations did not differ from controls, but, as for plasma NEFA and blood glycerol, the normal preprandial rise in concentration was absent. Serum insulin, glucagon and growth hormone concentrations did not differ from control values at any time.

Six months of thyroxine (T4) treatment produced a rise in blood glycerol concentrations (mean 12 h value during T4 therapy, $0·06 \pm 0·01$; before T4 therapy, $0·04 \pm 0·005$ mmol/l; $P < 0·01$) but not to control values ($0·08 \pm 0·01$ mmol/l). Concentrations of glucose and other gluconeogenic precursors were unaltered by therapy but the insulin response to meals and the mean 12 h serum insulin concentration were increased.

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Hypothyroidism is therefore associated with mild postprandial hyperglycaemia which is not normalized by thyroxine therapy, despite an increased insulin response to food. The elevated concentrations of gluconeogenic precursors could result from diminished hepatic uptake. Lipolysis in hypothyroidism is diminished and only partially normalized by conventional replacement therapy.

The clinical features of thyroid hormone deficiency are well recognized and many abnormalities of carbohydrate, lipid and protein metabolism in both animals and man have been described.

Animal studies have shown that hypothyroidism is associated with decreases in gluconeogenesis (Freedland & Krebs, 1967), glycogenolysis (Battarbee, 1974), peripheral glucose utilization (Scow & Cornfield, 1954), lipolysis (Bray & Goodman, 1968), lipogenesis (Bates *et al.*, 1955; Bray & Goodman, 1968) and protein turnover (Hoberman & Graff, 1951).

In man fasting hypoglycaemia (Lamberg, 1965), impaired tolerance to oral glucose (Crawford, 1940) with normal (Holdsworth & Besser, 1968) or impaired (Shah & Cerchio, 1973) peripheral insulin responses have all been reported. Decreased lipolysis, both *in vivo* (Hamburger *et al.*, 1963) and in isolated human adipocytes (Rosenqvist, 1972) has been demonstrated, but little is known about the effects of hypothyroidism on ketone body metabolism.

The effects of these changes on circulating hormone and metabolite concentrations in hypothyroid man under normal circumstances are unknown and the effects of subsequent thyroid hormone replacement have not previously been described. We have therefore examined hormone and metabolite profiles during normal meals and activity in hypothyroid subjects before and during thyroxine replacement therapy and in euthyroid controls.

PATIENTS AND METHODS

Eight (six female and two male) patients with hypothyroidism were compared with sixteen control subjects of similar age, sex and ideal body weights (Table 1). No subject had a personal or family history of diabetes and none were on any medication at the time of initial study. Liver function tests were normal. Hypothyroidism was secondary to Hashimoto's thyroiditis in six subjects (on the basis of spontaneous hypothyroidism occurring in subjects with circulating antibodies to thyroid tissue), partial thyroidectomy for colloid goitre in one subject and radioiodine therapy in one subject.

An intravenous teflon cannula was inserted into an antecubital vein at 0800 h after an overnight (10 h) fast, and subjects remained recumbent until 0830 h. The cannula was maintained patent with 0·15 mmol/l saline. Blood samples for hormones and metabolites were withdrawn at 0825 h and 0830 h. Breakfast was eaten at 0830 h and further blood samples were withdrawn at half-hour intervals until 2000 h. Meals were standard hospital meals containing approximately 2000 calories with 45% carbohydrate, 40% fat and 15% protein by weight. Lunch was eaten at 12 noon and the evening meal at 1800 h. All subjects consumed a dietary intake of 300 g of carbohydrate for at least 48 h prior to the study. After breakfast subjects were mobile and encouraged to take gentle exercise throughout the test although remaining at rest for at least 10 min before each sample time. Hypothyroid subjects were restudied when clinically euthyroid and conventional thyroid

Table 1. Clinical details of patients before and during therapy and of controls. For controls, mean + range is quoted and for serum thyroid stimulating hormone (TSH) the normal range is quoted

Patient no.	Age	Sex	Before therapy				During therapy				Interval between studies (months)
			Serum triiodothyronine (nmol/l)	Serum thyroxine (nmol/l)	Serum TSH (mu/l)	% ideal* body weight	Serum triiodothyronine (nmol/l)	Serum thyroxine (nmol/l)	Serum TSH (mu/l)	% ideal body weight	
1	18	F	1·3	30	29	104	2·1	98	4·6	103	6
2	57	F	0·4	13	30	109	2·6	121	4·6	106	5
3	66	F	0·8	42	21	110	1·2	81	2·8	111	7
4	45	F	0·8	28	20	108	1·5	122	4·0	103	5
5	48	F	0·8	30	40	105	1·5	120	6·6	93	4
6	49	F	0·4	40	40	139	1·7	121	3·3	128	8
7	56	M	1·0	46	18	99	1·5	131	6·4	100	6
8	39	M	1·1	52	28	110	1·4	60	3·8	102	6
Mean ±SEM	46±5	6F 2M	0·8±0·1	34±8	28±3	111±4	1·7±2	106±9	4·5±0·5	106±4	5·9±0·5
Controls	41	14F 2M	1·6	98	<7	109					
	(18–66)		(1·3–2·3)	(77–112)		(94–122)					

* Assessed from Metropolitan Life Assurance Tables.

function tests were normal. Treatment consisted of L-thyroxine 100–200 μg daily to maintain normal circulating serum thyroxine and serum thyroid stimulating hormone concentrations. The mean interval between studies was 5·9 months.

Blood for glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate was taken in 5% (V/V) chilled perchloric acid and assayed by automated fluorimetric enzymatic methods (Lloyd *et al.*, 1978). Acetoacetate was assayed by a kinetic spectrophotometric method (Price *et al.*, 1977). All metabolite samples were separated immediately, frozen at −20°C and assayed within 24 h. Total ketone bodies refers to the sum of acetoacetate and 3-hydroxybutyrate concentrations. Serum insulin (Soeldner & Slone, 1965) thyroid stimulating hormone (Hall *et al.*, 1971), triiodothyronine (Hesch & Evered, 1973) and growth hormone (Hartog *et al.*, 1964) were assayed by double antibody radioimmunoassay and serum thyroxine by radioimmunoassay (Malinkrodt, RIA-MAT® T4). Samples for glucagon were taken into chilled tubes containing 1 mmol EDTA in 1250 Kiu trasylol and assayed by wick chromatography (Ørskov *et al.*, 1978). Plasma non-esterified fatty acids NEFA were assayed by a radiochemical method (Ho & Meng, 1969).

Statistical analyses were performed using Student's paired and unpaired *t* tests as appropriate. Certain measurements (ketone bodies, glucagon and insulin) were log-normally distributed and statistical analysis was performed on log-transformed data.

Correlations were sought by the method of least squares or the Spearman ranking method where appropriate. Values in the text are given as the mean ± the standard error of the mean or as mean + range.

Informed consent was obtained from all subjects and the study was approved by the Ethical Committee of Newcastle Area Health Authority (Teaching).

RESULTS

Thyroid function tests

Results for serum thyroxine (T4) serum triiodothyronine (T3) serum thyroid stimulating hormone (TSH) and percent ideal body weight (% IBW) are shown in Table 1. Serum T4 and T3 concentrations were lower in hypothyroidism ($P < 0·001$) than in controls and increased to normal with thyroid hormone replacement. Serum TSH levels were higher in hypothyroidism ($P < 0·001$) and fell, with treatment, to normal values. Although there was a significant fall with thyroxine therapy ($P < 0·05$), percentage of ideal body weight in hypothyroid subjects before and after therapy was similar to controls.

Fasting metabolites and hormones

Metabolite concentrations Fasting concentrations of blood glucose and the gluconeogenic precursors, lactate, pyruvate, and alanine, blood ketone bodies and plasma NEFA were similar in hypothyroid patients and controls (Table 2). Blood glycerol concentrations were however decreased in hypothyroidism (patients, 0·06 ± 0·01; controls, 0·08 ± 0·01 mmol/l; $P < 0·001$).

Hormone concentrations There were no significant differences between fasting serum insulin, growth hormone and plasma glucagon concentrations in hypothyroid and control subjects.

Effect of thyroxine therapy Fasting blood concentrations of glucose, lactate, pyruvate, alanine and 3-hydroxybutyrate were unchanged by thyroxine replacement therapy. Blood

Table 2. Fasting and 12 h mean hormone and metabolite concentrations in control subjects and hypothyroid patients before and after replacement thyroxine therapy

Hormone or metabolite	Fasting values						Mean 12 h values					
	Hypothyroid	Hypothyroid during therapy	Control	P_1	P_2	P_3	Hypothyroid	Hypothyroid during therapy	Control	P_1	P_2	P_3
Glucose (mmol/l)	4·5±0·5	4·8±0·1	4·8±0·1	ns	ns	ns	5·6±0·3	5·8±0·2	5·5±0·1	ns	ns	ns
Lactate (mmol/l)	0·88±0·11	0·78±0·14	0·69±0·06	ns	ns	ns	1·08±0·06	1·0±0·1	0·77±0·03	ns	<0·001	<0·01
Pyruvate (mmol/l)	0·08±0·01	0·08±0·01	0·07±0·05	ns	ns	ns	0·10±0·01	0·10±0·01	0·08±0·003	ns	<0·01	<0·01
Lactate:pyruvate	10·3±1·0	10·1±1·0	10·2±1·3	ns	ns	ns	10·8±0·3	10·6±0·3	10·3±0·2	ns	ns	ns
Alanine (mmol/l)	0·30±0·02	0·29±0·04	0·29±0·02	ns	ns	ns	0·40±0·02	0·38±0·05	0·33±0·02	ns	<0·05	ns
3-hydroxybutyrate (mmol/l)	0·06(0·01-0·14)	0·07(0·04-0·18)	0·06(0·01-0·12)	ns	ns	ns	0·04(0·02-0·05)	0·03(0·01-0·05)	0·04 (0·02-0·08)	ns	ns	ns
Acetoacetate (mmol/l)	0·04(0·02-0·07)	0·08(0·03-0·16)	0·04(0·01-0·21)	<0·05	ns	<0·05	0·04(0·03-0·06)	0·06(0·01-0·09)	0·04 (0·01-0·08)	ns	ns	<0·05
Total ketone bodies (mmol/l)	0·10(0·04-0·18)	0·15(0·09-0·28)	0·10(0·02-0·25)	ns	ns	ns	0·08(0·06-0·10)	0·09(0·08-0·11)	0·09 (0·04-0·20)	ns	ns	ns
Glycerol (mmol/l)	0·06±0·01	0·08±0·01	0·08±0·01	<0·05	<0·001	ns	0·04±0·005	0·06±0·01	0·08±0·01	<0·01	<0·001	<0·05
NEFA (mmol/l)	0·73±0·04	0·64±0·06	0·58±0·03	ns	ns	ns	0·52±0·05	0·48±0·04	0·53±0·04	<0·05	ns	ns
Insulin (mu/l)	4(1-14)	6(1-12)	6(1-13)	ns	ns	ns	14(3-33)	33(12-54)	18(8-29)	ns	ns	ns
Glucagon (ng/l)	20(0-40)	31(0-55)	26(5-50)	ns	ns	ns	23(4-44)	22(1-35)	22(10-40)	ns	ns	ns
Growth hormone (miu/l)	9·53(0·9-31·0)	4·8(0·5-9·8)	7·4(0·4-19·1)	ns	ns	ns	2·4(0·5-3·0)	2·2(0·7-5·0)	2·8(0·8-4·6)	ns	ns	ns

Values are mean ± standard error of the mean except for 3-hydroxybutyrate, acetoacetate, total ketone bodies, insulin and glucagon where mean (range) is given.

P_1 = significance on Student's paired t test between untreated and treated hypothyroid subjects.

P_2 = significance on Student's unpaired t test between untreated hypothyroid subjects and controls.

P_3 = significance on Student's unpaired t test between treated hypothyroid subjects and controls.

t tests were performed on straight or log transformed data as in the text.

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acetoacetate levels rose significantly [during treatment 0·08 (0·03–0·16), untreated 0·04 (0·02–0·07) mmol/l; $P < 0·05$]. Blood glycerol levels also rose (during treatment $0·08 \pm 0·01$, untreated $0·06 \pm 0·01$ mmol/l; $P < 0·05$). Serum insulin, growth hormone and plasma glucagon levels were unchanged by thyroxine replacement.

Diurnal profiles

Metabolite concentrations Mild hyperglycaemia was apparent after breakfast in hypothyroid subjects (Fig. 1) although the glycaemic response to lunch and evening meal was normal. As a result the mean 12-h blood glucose value was unaltered by hypothyroidism. Blood lactate and pyruvate responses to meals were exaggerated and mean 12 h values for blood lactate (hypothyroid $1·08 \pm 0·08$; control $0·77 \pm 0·03$ mmol/l; $P < 0·001$) and blood pyruvate (hypothyroid $0·10 \pm 0·01$; control $0·08 \pm 0·003$ mmol/l; $P < 0·01$) were elevated in hypothyroidism (Fig. 2) (Table 2). The ratio of blood lactate to blood pyruvate in control subjects fell after each main meal (Table 3). In hypothyroid subjects, lactate:pyruvate ratios rose with meals and were significantly higher than in control subjects.
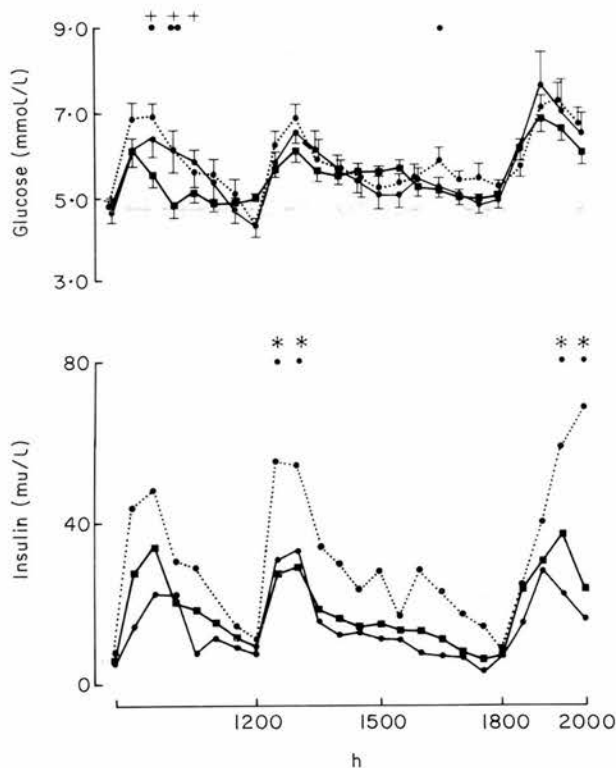

Fig. 1. Blood glucose and serum insulin profiles in hypothyroid subjects before (●——●) and during (●-----●) thyroxine therapy and in controls (■——■). The first sample was taken after an overnight fast. Thereafter meals were taken at 0830, 1200 and 1800 h with snacks at 1030 and 1530 h. * $P < 0·05$ hypothyroid subjects before therapy v. hypothyroid subjects during therapy. (** $P < 0·01$). $+P < 0·05$ hypothyroid subjects before therapy v. controls ($++ = P < 0·01$). ● $P < 0·05$ treated hypothyroid subjects v. controls (●● $= P < 0·01$).
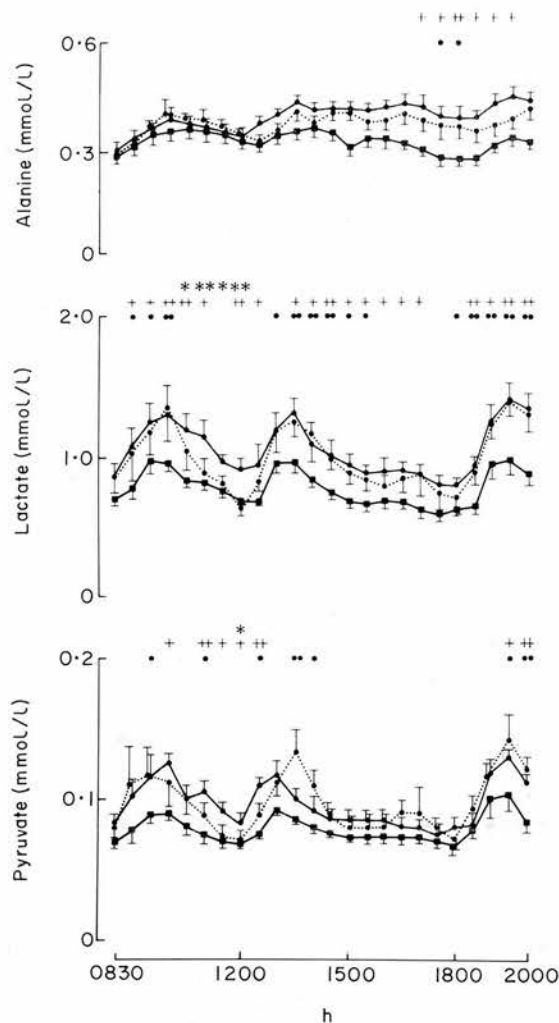
Fig. 2. Blood alanine, lactate and pyruvate profiles in hypothyroid subjects before and during thyroxine therapy and in controls. See legend to Fig. 1 for key and conditions.

Table 3. Blood lactate:pyruvate ratios before and 2 h after the three main meals in hypothyroid subjects before and during therapy and in controls. (For symbols and conditions see legend to Table 2)

	Hypothyroid before therapy	Hypothyroid during thyroxine therapy	Controls	P_1	P_2	P_3
Before breakfast	10·3 ± 0·7	10·1 ± 0·6	10·2 ± 0·3	ns	ns	ns
2 h after breakfast	11·7 ± 0·6	10·6 ± 0·4	9·7 ± 0·4	ns	< 0·05	ns
Before lunch	10·3 ± 0·4	10·2 ± 0·6	10·5 ± 0·4	ns	ns	ns
2 h after lunch	11·5 ± 0·5	10·9 ± 0·6	10·2 ± 0·4	ns	< 0·05	ns
Before supper	10·6 ± 0·3	10·5 ± 0·7	11·2 ± 0·3	ns	ns	ns
2 h after supper	12·1 ± 0·8	11·6 ± 0·3	10·1 ± 0·4	ns	< 0·05	< 0·05

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Blood alanine concentrations were similar in hypothyroid and control subjects in the morning and early afternoon but were significantly higher in hypothyroidism over the last 4 h of the study (Fig. 2). As a result mean 12 h blood alanine levels (Table 2) were significantly higher in hypothyroidism (0.40 ± 0.02 v. 0.33 ± 0.02 mmol/l, $P < 0.05$). In control subjects, blood total ketone body, glycerol and plasma non-esterified fatty acid (NEFA) showed a characteristic diurnal pattern with rises in circulating levels of each metabolite before meals (Fig. 3). In hypothyroidism, this diurnal pattern for blood glycerol and blood ketone bodies was lost. Blood glycerol levels were decreased throughout the study period (0.04 ± 0.01 v. 0.08 ± 0.01 mmol/l, $P < 0.001$). A similar, though less striking decrease was also evident for blood ketone bodies before the evening meal. The rise in ketone body concentrations between lunch and the evening meal, assessed as the area under the ketone body curve, was decreased in hypothyroidism ($P < 0.05$). Plasma NEFA levels were comparable in patients and controls.
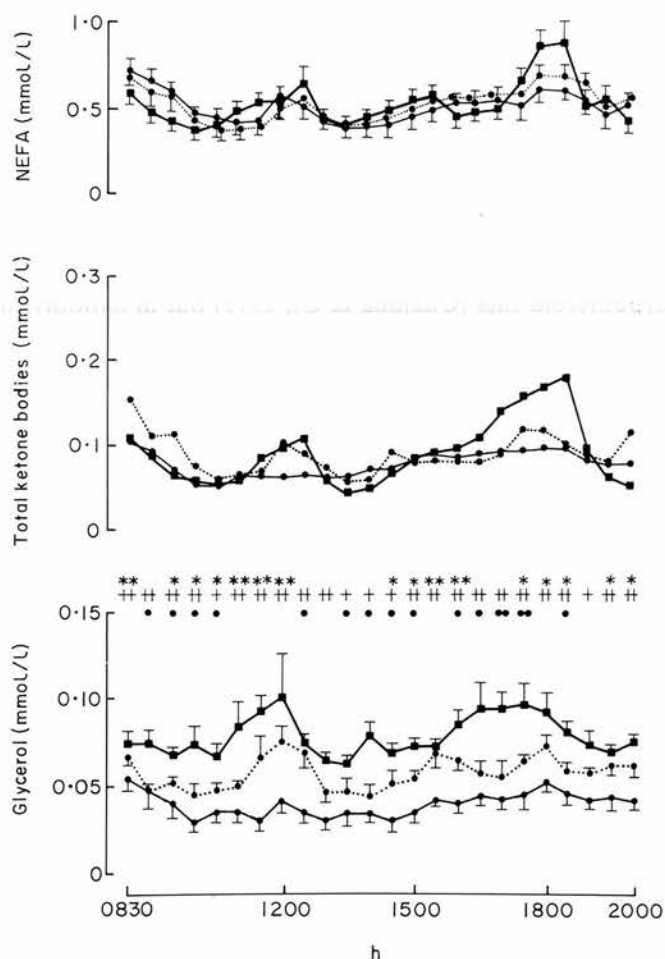

Fig. 3. Diurnal profiles for plasma non-esterified fatty acids, blood ketone bodies and blood glycerol in hypothyroid subjects before and during therapy and in controls. For conditions and symbols see Fig. 1.

Hormone concentrations Serum insulin, growth hormone and plasma glucagon levels did not differ in hypothyroid and control subjects at any time.

Effects of thyroxine therapy Thyroxine therapy did not restore the blood glucose response to breakfast to normal but serum insulin levels after meals were increased particularly in the evening, and the mean 12-h serum insulin concentration was elevated (Table 2). Plasma glucagon concentrations were not affected.

Mean 12 h values for blood lactate, pyruvate or alanine concentrations (Table 2) were also unaltered by thyroxine therapy, but blood glycerol concentration rose significantly (untreated 0.04 ± 0.01, during treatment 0.06 ± 0.01 mmol/l, $P < 0.01$) although they remained lower than in controls (0.08 ± 0.01 mmol/l, $P < 0.05$). Total ketone body concentrations were unaltered by replacement therapy although a normal diurnal pattern was restored.

Serum T4, T3 and TSH concentrations did not correlate with blood levels of any of the hormones or metabolites measured in hypothyroid subjects before or during therapy or in controls.

DISCUSSION

Previous studies in hypothyroid subjects have shown either a normal (Saunders *et al.*, 1980) or decreased (Lamberg, 1965; Levy *et al.*, 1970; Shah *et al.*, 1972) fasting blood glucose concentration. The decrease in blood glucose has been attributed to both impaired hepatic glycogenolysis (Battarbee, 1974) and impaired gluconeogenesis (Bondy, 1949; Freedland & Krebs, 1967; Menahan & Weiland, 1969; Sestoft *et al.*, 1977). In the present study, fasting blood glucose concentration was normal. Glucose turnover is decreased in hypothyroid rats (Okajima & Ui, 1979) but in hypothyroid man, glucose turnover may be decreased (McCulloch *et al.*, 1980) or normal (Saunders *et al.*, 1980).

The blood glucose response to breakfast was mildly elevated and the peak blood glucose value delayed in hypothyroidism. Absorption of glucose from the gastrointestinal tract is delayed both in man (Holdsworth & Besser, 1968) and experimental animals (Althausen & Stockholm, 1980). This may explain the delay in blood glucose peak but does not explain the exaggerated glycaemic response. This suggests impaired peripheral utilization of glucose. A similar intolerance to oral glucose has previously been noted (Gutman *et al.*, 1978).

Fasting blood concentrations of the gluconeogenic precursors, lactate, pyruvate and alanine were normal in the present and previous (Saunders *et al.*, 1980) but not all (McDaniel *et al.*, 1977) studies. Peripheral release of lactate (McDaniel *et al.*, 1977) and alanine (Bondy, 1949; Ness *et al.*, 1969) may be reduced in hypothyroidism but the normal circulating levels suggest that any impairment of gluconeogenesis is not secondary to a reduction in precursor supply. The total amount of 3-carbon substrates of glucose origin recycled to glucose in hypothyroid man is decreased although the proportion recycled is normal (McCulloch *et al.*, 1980). This suggests that lactate, pyruvate and alanine uptake by the liver is decreased in hypothyroidism in proportion to the decrease in hepatic glucose output.

The exaggerated lactate and pyruvate responses to meals may be secondary to increased peripheral production or diminished clearance. Most studies in hypothyroidism suggest diminished peripheral glucose utilization and diminished lactate (Shamer *et al.*, 1971; Okajima & Ui, 1979) and pyruvate output (McDaniel *et al.*, 1977) from peripheral tissues. Increased production of lactate and pyruvate after meals is therefore unlikely as

the cause of the hyperlactataemia and hyperpyruvicaemia, making a defect in hepatic uptake more likely.

The ratio of blood lactate to blood pyruvate was elevated after meals. This ratio may reflect the prevailing cytosolic redox state which is in turn dependent on the transfer of reducing equivalents across the mitochondrial membrane (Krebs *et al.*, 1975; Alberti & Nattrass, 1977). Thyroid hormones are important in this transfer process (Müllhoffer & Loy, 1974; Rognstadt, 1977; Sestoft, 1980), which may therefore be deficient in hypothyroidism.

Fasting blood alanine concentrations were normal in hypothyroidism and were only significantly greater than control values during the last 4 h of the study. Hyperalaninae-mia may be due either to diminished alanine uptake for gluconeogenesis or alternatively to a loss of the normal protein sparing effects of ketone bodies. Ketone bodies are known to inhibit protein degradation and peripheral alanine output (Sherwin *et al.*, 1976). The lower ketone body concentrations, particularly during the afternoon period, could possibly be responsible for the elevated blood alanine concentrations observed.

Alone among the gluconeogenic precursors, circulating blood glycerol levels were diminished in hypothyroidism. This finding, and the findings of other studies (Goodman & Bray, 1966; Fisher & Ball, 1967) suggests decreased lipolysis. Thyroid hormones have lipolytic actions *in vitro* (Vaughan, 1967), and a permissive effect for the lipolytic actions of other hormones, particularly the catecholamines both *in vitro* (Arner *et al.*, 1979; Debons & Schwartz, 1961) and *in vivo* (Hamburger *et al.*, 1963). In hypothyroidism plasma catecholamine levels may be increased (Christensen, 1972) and sensitivity to their peripheral effects reduced (Goodman & Bray, 1966; Rosenqvist, 1972; Reckless *et al.*, 1976). These changes may be mediated by altered phosphodiesterase activity (Armstrong *et al.*, 1974). The presence of normal rather than low plasma non-esterified fatty acid levels is not readily explained but has previously been reported (Saunders *et al.*, 1980). It is possible that despite diminished lipolysis, decreased NEFA uptake by the hypothyroid liver balances the diminished release.

The hypoketonaemia observed may be secondary to diminished lipolysis although substrate supply as judged by plasma NEFA measurements appears adequate. Recent studies have suggested that intrahepatic fatty acid oxidation and ketone body production may be thyroid hormone dependent (Keyes & Heimberg, 1979; Bartels & Sestoft, 1980).

Serum insulin levels in hypothyroidism have been reported to be decreased (Okajima & Ui, 1979; Jolin *et al.*, 1970; Shah & Cerchio, 1973), normal (Holdsworth & Besser, 1968), or increased (Andreani *et al.*, 1968; Renauld *et al.*, 1979), with a marked species variation. Thyroxine is necessary for normal insulin secretion by isolated pancreatic cells *in vitro* (Milcu *et al.*, 1975). In the present study serum insulin concentrations were similar to control values in the presence of elevated ambient blood glucose concentrations suggesting insulin hyposecretion. The mechanism of this is uncertain. Elevated blood glucose levels with insulin concentrations similar to controls also suggest insulin resistance in hypothyroidism. Alternatively the insulin measured by radioimmunoassay may be inactive. In human hyperthyroidism a high proportion of circulating 'insulin' measured by radioimmunoassay may be proinsulin (Sestoft & Heding, 1980) which is biologically inactive. This finding may account for the apparent insulin resistance in hyperthyroidism. Sensitivity to exogenous insulin at physiological blood glucose concentrations has previously been reported to be either normal (Iwatsubo *et al.*, 1967; Brauman & Corvilain, 1968), or reduced (West *et al.*, 1975) in hypothyroid man.

Some of the abnormalities described in hypothyroidism, in particular the mild elevation in blood glucose and gluconeogenic precursor concentrations have previously been described in obese subjects (Doar & Wynn, 1970). The patients outlined in the present study were within 10% ideal body weight with one exception. Abnormalities in intermediary metabolite concentrations are not noted in patients with such mild obesity (Johnston *et al.*, 1981). Similarly weight change with thyroxine therapy was small and could not explain any metabolic changes noted.

Thyroxine therapy had no effect on circulating blood glucose concentrations but did cause a significant elevation in the serum insulin response to meals. This may reflect a permissive effect of thyroid hormones on pancreatic insulin secretion. Blood lactate and pyruvate responses to meals were not normalized by thyroxine therapy and it is difficult to explain the persistent abnormality of glucose or gluconeogenic precursor concentrations with treatment.

Thyroxine therapy did cause a significant elevation in blood glycerol concentrations although levels remained lower than those obtained in control subjects. This change presumably reflects a thyroid-hormone-induced effect on peripheral lipolysis.

Plasma glucagon levels were unaltered in hypothyroidism and no effect of thyroid hormone therapy was seen. Plasma glucagon response to protein meals has been shown to be impaired in hyperthyroid subjects but no previous information on plasma glucagon levels in hypothyroidism has been published. Growth hormone deficiency has previously been demonstrated in hypothyroidism (Eisenberg *et al.*, 1972; Peake *et al.*, 1973) but in the present study growth hormone levels were normal. Thyroxine therapy had no effect on circulating growth hormone levels.

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Diurnal metabolic profiles in hyperthyroidism

M.D., 1984.

E.M.L.

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Abstract. Hormone and metabolite profiles during a 12 h period of normal meals and activity were examined in nine hyperthyroid subjects with Graves' disease and sixteen matched controls. Six hyperthyroid subjects were restudied when euthyroid on carbimazole and thyroxine. Thyrotoxic patients had mild fasting hyperglycaemia (mean ± SEM blood glucose, $5·5 \pm 0·2$ v. $4·8 \pm 0·1$ mmol/l, $P < 0·01$), elevated blood glycerol ($0·15 \pm 0·02$ v. $0·08 \pm 0·01$ mmol/l, $P < 0·001$) and elevated plasma non-esterified fatty acid (NEFA) concentrations ($0·91 \pm 0·06$ v. $0·58 \pm 0·03$ mmol/l, $P < 0·001$) when compared to controls. Fasting blood concentrations of the gluconeogenic precursors lactate, pyruvate and alanine, blood ketone body concentrations and circulating insulin and growth hormone levels were similar in hyperthyroid and control subjects. Blood glucose responses to meals were exaggerated and the mean 12 h blood glucose was increased ($6·1 \pm 0·1$ v. $5·5 \pm 0·1$ mmol/l, $P < 0·01$) in hyperthyroidism. Similarly, hyperlactataemia and hyperpyruvicaemia were observed after meals. Blood ketone body, blood glycerol and plasma NEFA levels showed exaggerated pre-prandial peaks and the mean 12 h values for blood glycerol ($0·12 \pm 0·01$ v. $0·08 \pm 0·01$ mmol/l, $P < 0·01$) and plasma NEFA ($0·71 \pm 0·03$ v. $0·53 \pm 0·04$ mmol/l, $P < 0·01$) were increased. Concentrations of insulin and growth hormone remained similar to control values throughout the study period. Blocking therapy with carbimazole and thyroid hormone replacement with thyroxine for 5–10 months suppressed blood glycerol, plasma NEFA and blood ketone body levels to normal or subnormal values but had no effect on the elevated blood glucose, blood lactate or blood pyruvate profiles.

Graves' disease with hyperthyroidism is thus associated with abnormalities of carbohydrate metabolism which are not restored to normal by 5–10 months oral antithyroid therapy. The changes in lipid metabolism in hyperthyroidism are normalized by this treatment.

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Key words. Hyperthyroidism, thyroid hormones, thyroxine, triiodothyronine, intermediary metabolism, insulin growth hormone, glucose, lactate, pyruvate, alanine, ketone bodies.

Introduction

Although the clinical features of hyperthyroidism have been recognized for many years, mechanisms of their production remain uncertain. In addition numerous, and often conflicting, metabolic abnormalities have been described in hyperthyroid man. Fasting blood glucose concentrations have been reported to be normal [1, 2] or elevated [3, 4] with normal [5] or abnormal tolerance to oral [6, 7] or intravenous glucose [3]. Peripheral insulin responses to oral glucose have also been described as normal [8], enhanced [3] or impaired [9]. Plasma nonesterified fatty acid (NEFA) concentrations [3, 10] and turnover [2] may be increased with enhanced ketosis during starvation [11]. Data concerning the response of these abnormalities to restoration of euthyroidism is also conflicting and poorly documented.

The purpose of the present study was to investigate hormone and intermediary metabolite concentrations both after an overnight fast and during normal meals and activity in hyperthyroid subjects before and after treatment and in euthyroid controls.

Subjects and Methods

Subjects

Nine (eight female, one male) patients with hyperthyroidism were compared with sixteen (thirteen female, three male) normal controls (Table 1). No subject had a personal or family history of diabetes and none was on any medication at the time of the initial study. Patients had Graves' disease on the basis of clinical features, such as ophthalmopathy, and an absence of thyroid nodules on ^{99}Tc scanning.

Hyperthyroid subjects were restudied when clinically euthyroid and when thyroid function tests had been normal for at least 2 months (Table 1). Treatment

Table 1. Clinical details of patients before and during therapy and of controls

			Before therapy				During therapy				
Patient	Age	Sex	Serum thyroxine (nmol/l)	Serum triiodo-thyronine (nmol/l)	Serum TSH (mU/l)	% ideal body weight	Serum thyroxine (nmol/l)	Serum triiodo-thyronine (nmol/l)	Serum TSH (mU/l)	% ideal body weight	Interval between studies (months)
1	38	F	283	11·2	0·1	114	94	1·4	1·6	119	5
2	63	F	347	12·3	0·1	110	84	1·4	2·8	115	10
3	61	F	189	3·9	0·1	138	89	1·2	3·1	136	6
4	33	F	327	12·1	0·1	105	87	1·7	3·2	110	5
5	57	F	222	3·8	1·8	121	106	1·4	8·0	122	9
6	30	F	312	12·4	1·2	100	95	2·1	4·2	105	5
7	58	F	172	5·3	1·4	98					
8	43	M	225	6·6	1·6	102					
9	43	F	164	3·0	1·4	110					
Controls	41 (23–61)	13F 3M	98 (77–112)	1·6 (1·2–2·3)	(<7)	109 (94–122)					

For controls mean (range) is given and for serum thyroid stimulating hormone (TSH) the normal range is quoted.

consisted of carbimazole 30–45 mg daily with subsequent addition of thyroxine to maintain a normal circulating serum thyroxine and serum thyroid stimulating hormone concentration. Antithyroid therapy was taken on the day of repeat study. The mean interval between studies was 7 months (range 5–10).

Protocol

Intravenous teflon cannulae were inserted at 08.00 hours after an overnight (10 h) fast and subjects remained recumbent until 08.30 hours. Basal samples for hormone and metabolite estimations were withdrawn at 08.25 and 08.30 hours when breakfast was taken. Lunch was at 12.00 hours and dinner at 18.00 hours with snacks at 10.00 and 15.30 hours. Blood samples were withdrawn at 30 min intervals until 20.00 hours. Meals were standard hospital meals containing approximately 2500 calories with 45% carbohydrate 40% fat and 15% protein by weight. All subjects consumed a daily intake of 300 g of carbohydrate for at least 48 h before study. After breakfast, subjects were mobile and encouraged to take gentle exercise throughout the test but remained at rest for at least 10 min before each sample time. All subjects gave informed consent before the study which was approved by the Newcastle Hospitals Ethical Committee.

Methods

Blood for glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate was taken into 5% (v/v) chilled perchloric acid and assayed by automated fluorimetric enzymatic methods [12]. Acetoacetate was assayed by a kinetic spectrophotometric method [13] on perchlorate samples. Possible interference in metabolite assays by carbimazole was excluded by addition of this drug in a concentration of 10 mg/l, far in excess

of expected plasma values, to paired blood samples. Serum insulin [14], thyroid stimulating hormone [15], triiodothyronine [16] and growth hormone [17] were assayed by double antibody radioimmunoassay and serum thyroxine by radioimmunoassay (Mallinckrodt, RIA-MAT® T$_4$). Plasma non-esterified fatty acids (NEFA) were assayed by a radiochemical method [18].

Statistical analyses were performed using Student's paired and unpaired *t*-tests as appropriate. Certain measurements (ketone bodies, insulin and growth hormone) were log-normally distributed and statistical analysis was performed on log transformed data. Correlations were sought by the least squares method or the Spearman ranking method where appropriate. Values in the text are given as the mean ± standard error of the mean (SEM) or mean (range).

Results

1. *Fasting metabolite and hormone levels in untreated hyperthyroidism*

(a) *Metabolite concentrations.* Fasting blood glucose concentrations were elevated in untreated hyperthyroidism ($5·5 \pm 0·1$ mmol/l, hyperthyroid subjects; $4·8 \pm 0·1$ mmol/l, controls; $P < 0·01$). Concentrations of the gluconeogenic precursors, lactate, pyruvate and alanine were similar in hyperthyroid and control subjects (Table 2).

Fasting blood glycerol concentrations were elevated in hyperthyroidism ($0·15 \pm 0·02$ *v.* $0·08 \pm 0·01$ mmol/l, $P < 0·001$) as were plasma non-esterified fatty acid levels ($0·91 \pm 0·06$ *v.* $0·58 \pm 0·03$ mmol/l, $P < 0·001$). Blood ketone body concentrations were variable and did not differ between hyperthyroid and control subjects.

(b) *Hormone concentrations.* Fasting serum insulin and growth hormone concentrations were similar in hyperthyroid subjects and controls (Table 2).

Table 2. Fasting and mean 12 h values for hormones and metabolites in hyperthyroid subjects before and during treatment and in controls

	Fasting values						12 h values					
	Hyperthyroid	Hyperthyroid during therapy	Controls	P_1	P_2	P_3	Hyperthyroid	Hyperthyroid during therapy	Controls	P_1	P_2	P_3
Glucose (mmol/l)	5·5±0·1	5·4±0·5	4·8±0·1	ns	<0·01	ns	6·1±0·1	6·0±0·1	5·5±0·1	ns	<0·01	<0·05
Lactate (mmol/l)	0·87±0·12	0·75±0·16	0·69±0·06	ns	ns	ns	0·98±0·07	1·01±0·1	0·77±0·03	ns	<0·05	<0·05
Pyruvate (mmol/l)	0·08±0·01	0·08±0·01	0·07±0·01	ns	ns	ns	0·09±0·01	0·09±0·01	0·07±0·003	ns	<0·05	<0·05
Lactate: pyruvate	11·4±0·6	10·4±0·4	10·2±0·3	ns	ns	ns	10·4±0·1	10·9±0·4	10·3±0·2	ns	ns	ns
Alanine (mmol/l)	0·31±0·04	0·36±0·04	0·29±0·02	ns	ns	ns	0·35±0·01	0·39±0·01	0·33±0·02	ns	ns	ns
3-Hydroxy butyrate (mmol/l)	0·10 (0·03–0·37)	0·06 (0·02–0·1)	0·06 (0·02–0·12)	ns	ns	ns	0·09 (0·04–0·21)	0·04 (0·01–0·06)	0·04 (0·02–0·08)	ns	ns	ns
Acetoacetate (mmol/l)	0·10 (0·01–0·22)	0·03 (0·02–0·05)	0·04 (0·01–0·09)	ns	ns	ns	0·05 (0·03–0·09)	0·04 (0·03–0·045)	0·04 (0·01–0·08)	<0·05	ns	ns
Total ketone bodies (mm/l)	0·20 (0·03–0·06)	0·09 (0·06–0·15)	0·10 (0·02–0·25)	ns	ns	ns	0·14 (0·06–0·3)	0·07 (0·06–0·08)	0·09 (0·04–0·2)	ns	ns	ns
Glycerol (mmol/l)	0·15±0·02	0·06±0·01	0·08±0·01	<0·001	<0·001	ns	0·12±0·01	0·05±0·004	0·08±0·01	<0·01	<0·01	<0·01
NEFA (mmol/l)	0·91±0·06	0·64±0·07	0·58±0·03	<0·001	<0·001	ns	0·71±0·03	0·44±0·08	0·53±0·04	<0·001	<0·01	<0·05
Insulin (mU/l)	6 (1–10)	2 (1–6)	6 (1–13)	ns	ns	ns	18 (11–33)	19 (13–34)	18 (9·7–29)	ns	ns	ns
Growth hormone (miu/l)	2·2 (0·4–4·6)	10·5 (0·2–41·0)	7·4 (0·4–19·1)	ns	ns	ns	2·1 (0·5–6·1)	4·3 (0·9–5·9)	2·8 (0·6–7·4)	ns	ns	ns

Values are mean ± standard error of the mean except for 3-hydroxybutyrate, acetoacetate, total ketone bodies, insulin and growth hormone where mean (range) is given. P_1 = significance on Student's paired t-test between untreated and treated hyperthyroid subjects. P_2 = significance on Student's unpaired t-test between untreated hyperthyroid subjects and controls. P_3 = significance on Student's unpaired t-test between treated hyperthyroid subjects and controls. t-tests were performed on straight or log transformed data as in the text.
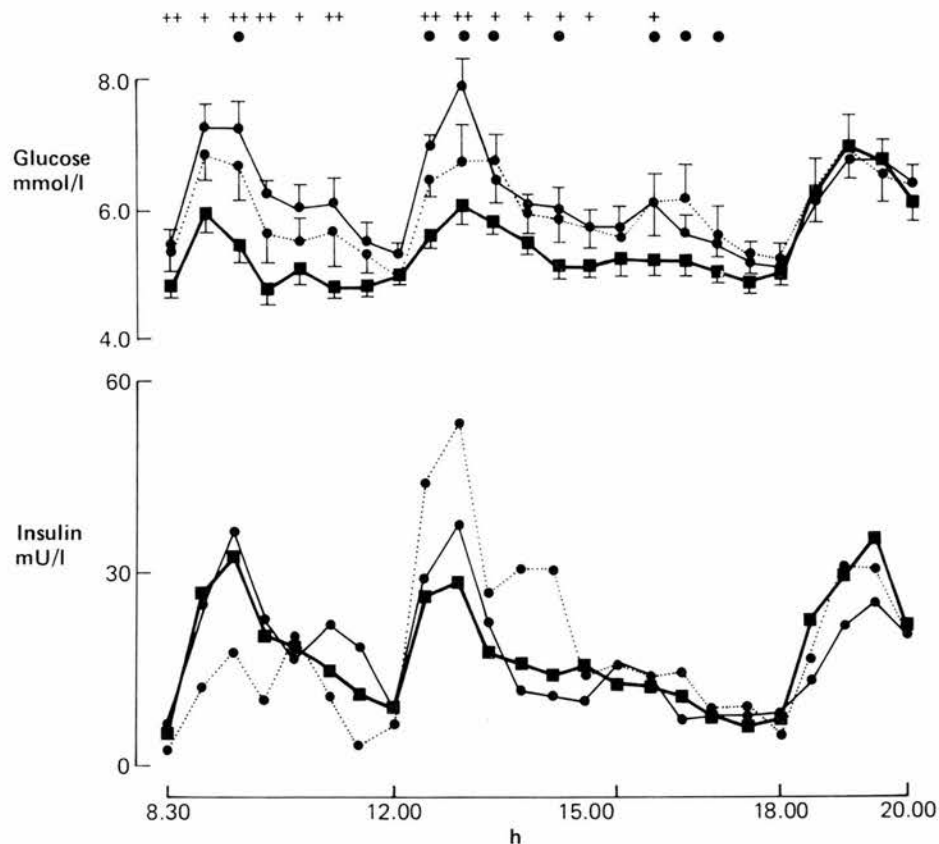
Figure 1. Blood glucose and serum insulin profiles in hyperthyroid subjects before (●—●) and during (●·····●) therapy and in controls (■—■) The first sample was taken after an overnight fast. Thereafter meals were taken at 08.30, 12.00 and 18.00 hours with snacks at 10.00 and 15.30 hours. *P < 0·05 hyperthyroid subjects before therapy *v.* hyperthyroid subjects during therapy (**P < 0·01). + P < 0·05 hyperthyroid subjects before therapy *v.* controls (+ + P < 0·01). ●P < 0·05 hyperthyroid subjects during therapy *v.* controls (●●P < 0·01).

2. *Diurnal hormone and metabolite profiles in untreated hyperthyroidism*

(a) *Metabolite concentrations.* Blood glucose profiles over 12 h are shown in Fig. 1. The peak blood glucose responses to breakfast (7·3 ± 0·4 *v.* 6·0 ± 0·2 mmol/l, P < 0·01) and lunch (8·0 ± 0·4 *v.* 6·1 ± 0·3 mmol/l, P < 0·001) although synchronous in both groups were exaggerated in hyperthyroidism. The mean blood glucose concentration over the 12 h was also increased (Table 2). Similarly, blood lactate and pyruvate concentrations after breakfast and lunch were increased in untreated hyperthyroidism (Fig. 2) as were the 12 h mean values for lactate and pyruvate (Table 2). Blood alanine concentrations were similar in hyperthyroid subjects and controls (Fig. 2, Table 2).

In control subjects, blood total ketone body, plasma NEFA and blood glycerol concentrations fell with feeding and rose with fasting before meals (Fig. 3). This diurnal pattern was preserved in hyperthyroidism but the premeal peaks for blood ketone body, plasma NEFA and blood glycerol levels were exaggerated. As a result, the 12 h mean values for blood glycerol and plasma NEFA were elevated in hyperthyroidism (Table 2).

(b) *Hormone concentrations.* Serum insulin and

growth hormone concentrations were similar in hyperthyroid and control subjects over the 12 h (Fig. 1 and Table 2).

3. *Effects of antithyroid therapy*

(a) *Fasting hormones and metabolites.* Blocking-replacement treatment had no effect on fasting blood glucose concentrations (Table 2). Blood glycerol and plasma NEFA levels decreased to normal, however, with therapy. Concentrations of other metabolites, insulin and growth hormone were not influenced by treatment.

(b) *Diurnal hormone and metabolite profiles.* Blood glucose concentrations remained elevated in hyperthyroid subjects after treatment (Fig. 1), with 12 h mean values higher than in controls (6·0 ± 0·1 *v.* 5·5 ± 0·2 mmol/l, P < 0·05) (Table 2). Similarly, hyperlactataemia and hyperpyruvicaemia persisted during antithyroid therapy (Fig. 2 and Table 2). Treatment also resulted in a significant increase in blood alanine concentrations, not evident in the untreated state (Fig. 2).

Circulating ketone body, NEFA and glycerol concentrations decreased with antithyroid therapy (Fig. 3) although the meal related fluctuations were preserved. Plasma NEFA and blood glycerol levels were sup-
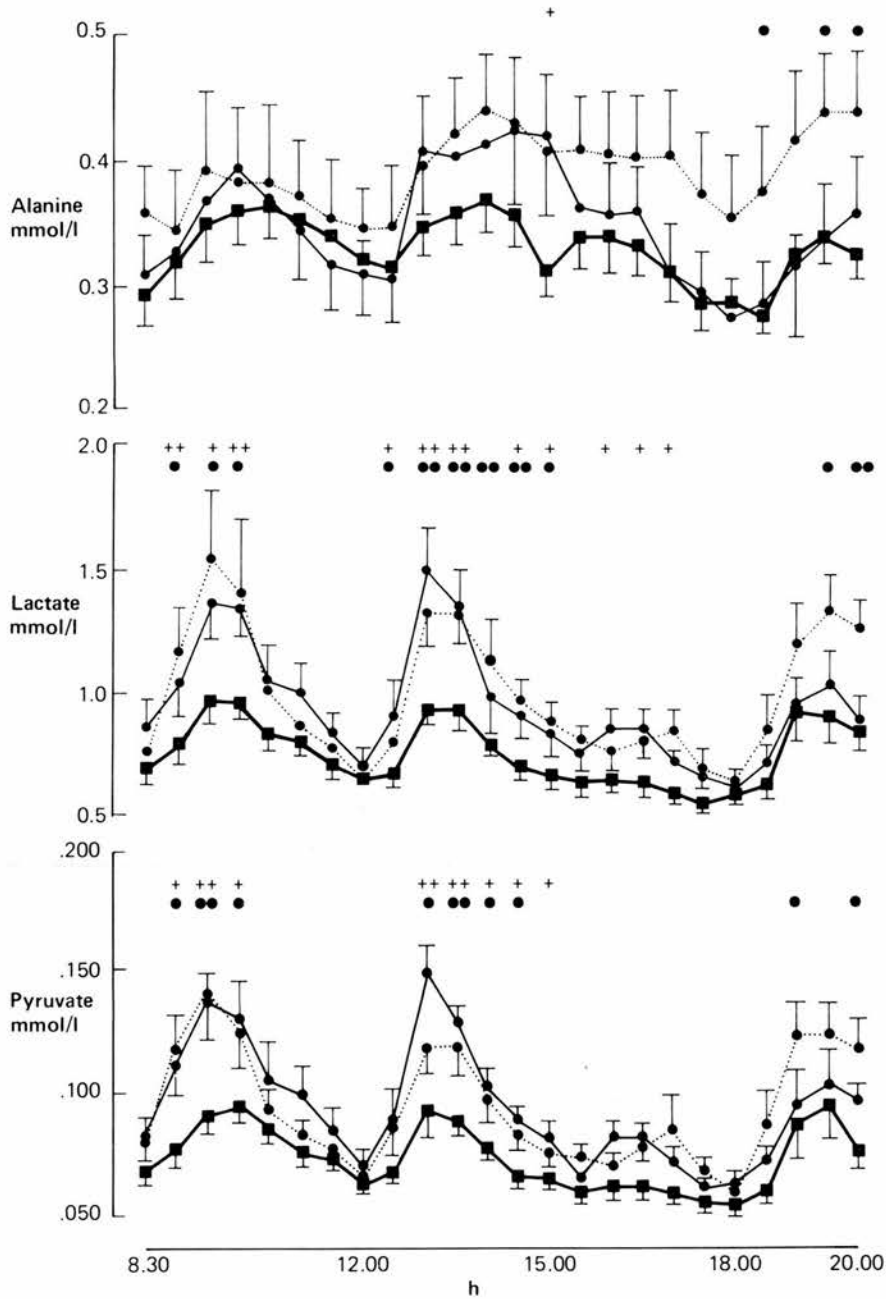
Figure 2. Blood alanine, lactate and pyruvate profiles in hyperthyroid subjects before and during therapy and in controls. See legend to Fig. 1 for symbols and conditions.

pressed to values below those found in controls (Fig. 3 and Table 2).

Insulin and growth hormone concentrations were unaffected by therapy. In untreated hyperthyroidism neither serum thyroxine nor triiodothyronine levels correlated with fasting, peak or mean 12 h glucose or gluconeogenic precursor levels. However, serum triiodothyronine levels correlated positively with peak prelunch blood glycerol ($r = 0.63$, $P < 0.05$) and plasma NEFA levels ($r = 0.62$, $P < 0.05$).

In treated hyperthyroid and control subjects thyroid hormone concentrations did not correlate with fasting,

peak or mean 12 h values for any of the hormones or metabolites measured.

Discussion

The present study showed fasting hyperglycaemia in hyperthyroid subjects. This could be a consequence of diminished utilization or increased hepatic glucose production. The latter has been noted by some [2, 19], but not all [20] workers. Increased glucose production may result from increased gluconeogenesis and/or increased glycogenolysis. Hepatic glycogen stores are
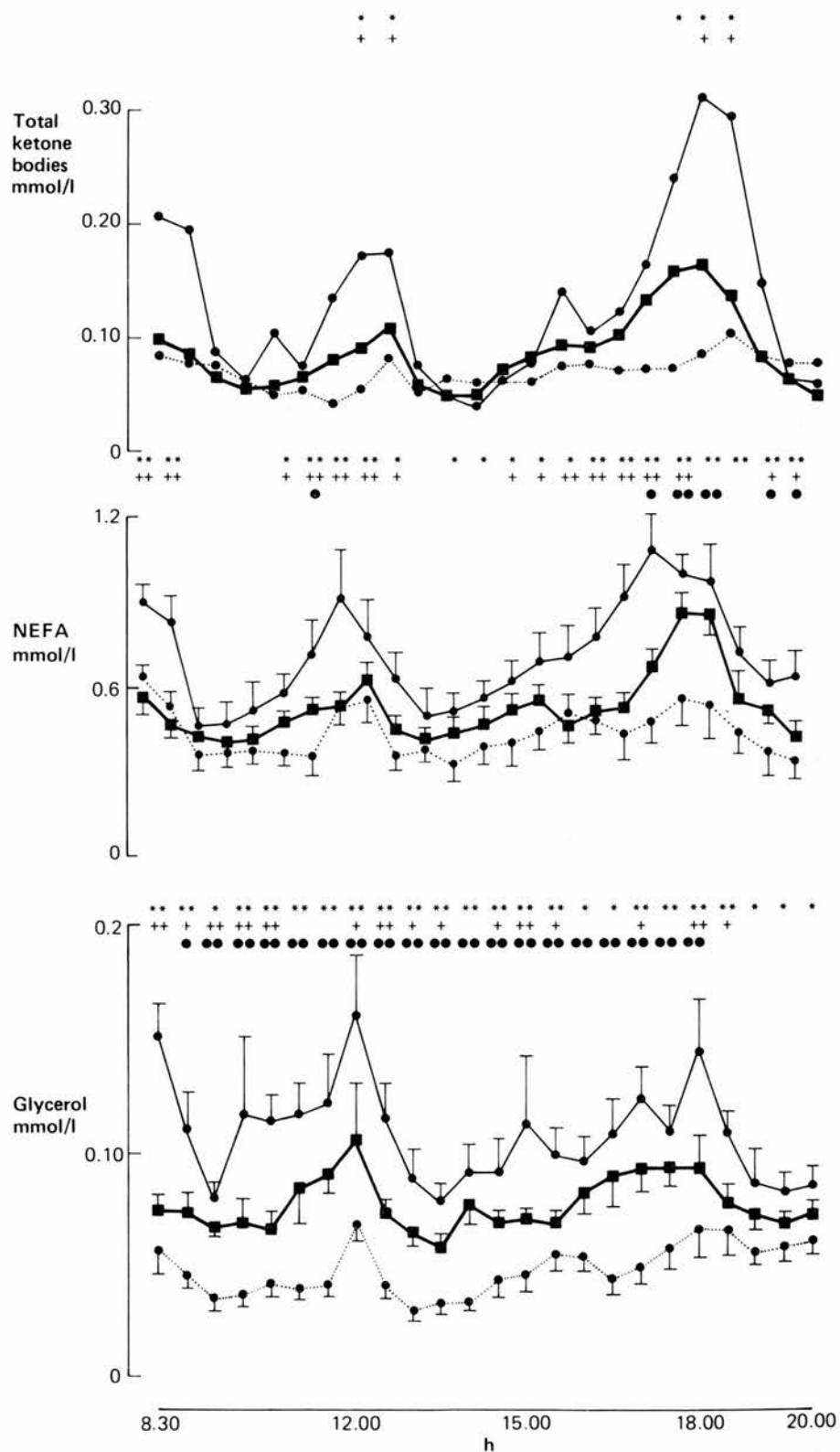
Figure 3. Diurnal profiles of blood ketone bodies, plasma non-esterified fatty acids and blood glycerol in hyperthyroid subjects before and during therapy and in controls. See legend to Fig. 1 for symbols and conditions.

decreased in hypothyroid man [21] although whether this is because of increased utilization or decreased synthesis is uncertain. Recently, studies in overnight fasted hyperthyroid man have shown increased splanchnic uptake of the gluconeogenic precursors, lactate, pyruvate, glycerol and alanine, largely due to increased fractional extraction of these precursors but also, to a smaller extent, to increased hepatic blood flow [20]. Similarly, the finding of increased glucose production from glucose derived 3-carbon interme-diates in both man [22] and animals [19] suggests an increase in gluconeogenesis. Confirmatory evidence has been obtained from studies with perfused rat livers at high concentrations of gluconeogenic precursors [23, 24] although not at physiological levels [25].

In the present study, postprandial as well as fasting hyperglycaemia was noted. Peak blood glucose con-centrations occurred at the same time as in controls indicating that rapid absorption of carbohydrate was not the major factor; a suggestion made by others on the basis of glucose tolerance tests [26].

Normal serum immunoreactive insulin levels were also found. The combination of fasting hyperglycae-mia, meal intolerance and normal circulating insulin levels suggests insulin resistance. Studies of insulin sensitivity in hyperthyroid man have been equivocal showing a normal [27], increased [5] or decreased [28] blood glucose response to exogenous insulin. Enhanced insulin degradation in hyperthyroidism, noted by some [29] but not all workers [30], may not explain the coexistence of normal insulin and elevated blood glucose concentrations as insulin degradation and biological effect are inextricably linked [31].

The important recent demonstration of elevated circulating proinsulin concentrations with low C-pep-tide levels suggests that true insulin secretion may be diminished in hyperthyroid man [32]. Proinsulin cross reacts with insulin in the assay used in our radioim-munoassay and many other studies. Thus 'true' insulin levels may have been decreased in our patients. However, even if proinsulin secretion were normal, the fact that insulin levels were not raised in the presence of hyperglycaemia suggests undersecretion of insulin. The mechanism of insulin hyposecretion is uncertain but has been attributed to increased adrenergic tone [33].

Recent work has suggested a role for glucagon in glucose intolerance of hyperthyroidism [1], some hy-perthyroid subjects showing elevated basal plasma glucagon levels with impaired glucagon suppression following oral glucose challenge. In addition, in-creased sensitivity to the cyclic AMP mediated effects of glucagon has been proposed [34]. This may be important in decreasing glycogen stores in the liver of hyperthyroid man and animals.

The elevated blood lactate and pyruvate responses to meals is not readily explained. Blood lactate levels are also increased in response to exercise in hyperthyroid man [35]. This hyperlactataemia is abolished by β-adrenergic blockade and increased adrenergic tone

has been suggested as a mechanism [36]. Our subjects were resting and a thyroid hormone mediated increase in peripheral glycolysis is a more likely mechanism. Such an effect has been demonstrated *in vivo* [37].

The elevated circulating concentrations of glycerol and NEFA after an overnight fast and before lunch and dinner reflect increased adipose tissue lipolysis. Increased lipolysis in hyperthyroidism has long been recognized [7, 16] and NEFA [2] and glycerol turnover [38] are elevated in fasting hyperthyroid man. The correlations observed between thyroid hormone con-centrations and the premeal peaks for NEFA and blood glycerol in this suggest a direct effect of thyroid hormones or potentiation of the lipolytic action of other hormones, particularly the catecholamines. Although plasma catecholamine levels are not in-creased in hyperthyroidism [39] increased lipolysis may be due to increased β adrenergic responsiveness [40]. Enhanced ketogenesis with fasting has been recog-nized in hyperthyroidism for many years [11]. In-creased NEFA supply combined with relative insulin deficiency are important and an additional direct ketogenic effect of thyroid hormone excess on the liver has recently been described [41].

Antithyroid treatment with carbimazole and thy-roxine replacement therapy did not restore blood glucose or gluconeogenic precursor concentrations to normal. Similarly Kreines *et al.* [42] and Cavagnini [43] reported persistent intolerance to oral glucose after antithyroid therapy while Saunders *et al.* [2] reported normalization of fasting glucose turnover by carbima-zole but blood lactate levels remained elevated. The reasons for the persisting abnormalities are not appar-ent. All patients were clinically and biochemically euthyroid and the decline in blood glycerol, ketone body and plasma NEFA levels (to subnormal levels in the case of glycerol) suggests that therapy adequately suppressed lipolysis and ketogenesis. No direct effects of carbimazole on intermediary metabolism, indepen-dent of its effect on thyroid function have been reported and the effects of methimazole, a related drug, on glucose turnover in the rat are restored to normal by thyroxine [19]. Insulin secretion, assessed by peripheral insulin concentrations did not alter although the proportion of 'true' insulin to proinsulin may have increased. It is still possible, however, that there may have been residual pancreatic hyposecretion or, particularly had 'true' insulin secretion increased, persistent insulin resistance. These may have contri-buted to the persistent carbohydrate intolerance but does not explain the elevated post-prandial lactate and pyruvate concentrations during antithyroid therapy. Finally, the duration of therapy (6·7 months) may have been inadequate. Studies in the rat, however, suggest that normalization of glucose metabolism occurs after 5 weeks [19]. Longer-term follow-up studies will be necessary to resolve this question.

Acknowledgments

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Glucose turnover and indices of recycling in thyrotoxicosis and primary thyroid failure

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Summary

1. Glucose turnover was examined by using simultaneous bolus intravenous tracer administration of both [3-^{3}H]glucose and [1-^{14}C]glucose in hyperthyroid and hypothyroid subjects before and during appropriate therapy and in matched controls.

2. In hyperthyroid subjects, total glucose turnover (as indicated by analysis of decay of [3-^{3}H]glucose specific radioactivity) was increased ($18 \cdot 1 \pm 1 \cdot 0$ vs $12 \cdot 4 \pm 0 \cdot 9$ μmol kg^{-1} min^{-1}, $P < 0 \cdot 01$), as was the metabolic clearance rate for glucose ($3 \cdot 5 \pm 0 \cdot 3$ vs $2 \cdot 4 \pm 0 \cdot 2$ ml kg^{-1} min^{-1}, $P < 0 \cdot 01$).

3. Glucose turnover rates determined with [1-^{14}C]glucose as tracer were lower than total glucose turnover rates in all subjects. This difference represents an index of recycling of the ^{14}C label between glucose and glucose-derived C$_3$ intermediates.

4. The recycling index was increased in hyperthyroidism both in amount ($4 \cdot 7 \pm 0 \cdot 6$ vs $1 \cdot 3 \pm 0 \cdot 3$ μmol kg^{-1} min^{-1}, $P < 0 \cdot 001$) and when expressed as a percentage of total glucose turnover (27 ± 3 vs $10 \pm 2\%$, $P < 0 \cdot 001$).

5. Turnover rates determined by [1-^{14}C]glucose represent irreversible glucose disposal and were similar in hyperthyroid and control subjects (hyperthyroid $13 \cdot 4 \pm 1 \cdot 1$ vs control $11 \cdot 2 \pm 0 \cdot 9$ μmol kg^{-1} min^{-1}). The major fraction of the increase in total glucose turnover could thus be accounted for by increased cycling of glucose.

6. Total glucose turnover, metabolic clearance and indices of recycling returned to normal with treatment of hyperthyroidism.

7. In hypothyroidism, total glucose turnover (hypothyroid $8 \cdot 2 \pm 0 \cdot 3$ vs control $11 \cdot 7 \pm 1 \cdot 2$ μmol kg^{-1} min^{-1}, $P < 0 \cdot 01$) and metabolic clearance (hypothyroid $1 \cdot 6 \pm 0 \cdot 1$ vs control $2 \cdot 3 \pm 0 \cdot 2$ ml kg^{-1} min^{-1}, $P < 0 \cdot 001$) were decreased and returned to normal with thyroxine therapy. Indices of glucose recycling were normal.

Key words: glucose turnover, hyperthyroidism, hypothyroidism, recycling.

Introduction

Thyroid hormones have long been known to influence carbohydrate metabolism. Fasting blood glucose concentrations may be elevated in hyperthyroidism [1, 2] and decreased in hypothyroidism [3, 4], although reports are conflicting. Intolerance to orally [5–8] or intravenously [2] administered glucose may also be present.

Administration of pharmacological amounts of thyroid hormones to animals has produced more clear-cut results. Thus increases in glycogenolysis [9], gluconeogenesis [10] and glucose turnover [11] have all been reported. Such experiments have also demonstrated increased activity of certain substrate cycles, such as those between glucose and glucose 6-phosphate [11] and between triacylglycerols and non-esterified

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fatty acids [12]. In man increased glucose turnover has been reported in hyperthyroidism and normal turnover in hypothyroidism with the use of [3-³H]glucose as tracer [13], and para-doxically decreased splanchnic glucose output in hyperthyroidism with the use of catheterization techniques [14], but substrate cycling between glucose and glucose-derived C_3 intermediates has not been examined.

In the present study we have investigated glucose turnover in fasting hyperthyroid and hypothyroid man before and during treatment with [3-³H]glucose as tracer to indicate total glucose turnover. In addition, the possibility of cycling of glucose-derived C_3 intermediates was investigated by simultaneous analysis of decay of [1-¹⁴C]glucose specific radioactivity.

Subjects and methods

Six hyperthyroid subjects [90 ± 3% of ideal body wt. before therapy, 99 ± 1% of ideal body wt. after therapy (means ± SEM, five subjects only), were compared with six age-matched normal controls (95 ± 2% of ideal body wt.) (Table 1). Hyperthyroidism was caused by Graves' disease in all subjects, on the basis of clinical features and antibodies to thyroid tissue components. No subject had a thyroid nodule on ⁹⁹Tc scanning. Hyperthyroid subjects were re-studied after 5–8 months (mean 5·9 months) of antithyroid therapy (carbimazole 30–40 mg daily from diagnosis, with L-thyroxine 100–150 µg daily added after 4–6 weeks). Circulating thyroxine, tri-iodothyronine and thyrotrophin concentrations were normal at the time of re-investigation.

Seven hypothyroid patients [121 ± 3% of ideal body wt. before therapy and 110 ± 2% of ideal body wt. after therapy (six subjects only)] were compared with six normal controls (111 ± 4% of ideal body wt.) (Table 1). Hypothyroid subjects were older than the controls, although the difference was not significant. Hypothyroidism was secondary to Hashimoto's thyroiditis in all cases. Patients were re-studied after 6 months' therapy with L-thyroxine, 100–200 µg daily, when circulating thyroxine, tri-iodothyronine and thyrotrophin concentrations were within the normal range.

No subject was on any medication at the time of initial study, and none had any other known disease or a family history of diabetes. All subjects consumed in excess of 300 g of carbohydrate daily for at least 48 h before the study. Informed oral consent was obtained from every subject for the study, which was approved by the Newcastle Area Health Authority (Teach-

ing) and the D.H.S.S. Isotope Advisory Panel. One subject from each group of patients was unavailable for repeat study.

Protocol

After a 10 h (overnight) fast, indwelling intravenous Teflon cannulae (Venflon; Viggo, Helsinborg, Sweden) were positioned in both antecubital veins at 08.00 h. Subjects remained recumbent throughout, the test and basal samples for hormones and metabolites were taken at 08.25 and 08.30 h. Bolus intravenous injections of 50 µCi (1·85 mBq) of [3-³H]glucose and 25 µCi (0·925 mBq) of [1-¹⁴C]glucose (The Radio-chemical Centre, Amersham, Bucks., U.K.) were given at 08.30 h. Blood samples (10 ml) were taken at 1, 2, 3, 4, 5, 6, 8, 10, 12·5, 15, 20, 30, 40, 50, 60, 75, 90 and 120 min after injection for the determination of glucose specific radioactivity, intermediary metabolites and hormones.

Chemical methods

Blood (1 ml) for glucose, lactate, pyruvate and glycerol determinations was taken into chilled perchloric acid (0·3 mol/l) and assayed by automated fluorimetric enzymic methods [15]. Serum insulin [16], thyrotrophin [17] and tri-iodothyronine [18] were assayed by double-antibody radioimmunoassay and serum thyrox-ine by radioimmunoassay (Malinckrodt-RIA MAT). Blood samples (2·25 ml) for glucagon were taken into chilled tubes containing 0·25 ml of aprotinin (2500 kallikrein-inhibitory units) and 25 µmol of EDTA and assayed by wick chromatography [19]. For measurement of radioactivity in glucose samples were taken into heparinized tubes and the plasma was separated and stored at −20°C until assay. Plasma (0·5 ml) was deproteinized by adding 1 ml of 5·36% (w/v) Ba(OH)$_2$, 1 ml of 5% (w/v) ZnSO$_4$,7H$_2$O [20] and 7·5 ml of distilled water. The supernatant was passed through a column containing 5 ml of ion-exchange resin (AG2-X 8/B; Bio-Rad Laboratories, Richmond, CA, U.S.A.). The column was washed with 10 ml of distilled water, and the eluate was freeze-dried, resuspended in toluene-based scintillant (Cocktail T; Hopkin and Williams, Chadwell Heath, Essex, U.K.) and its radioactivity counted in a scintillation counter (Intertechnique, St Albans, Herts., U.K.), with the use of an external standard to correct for efficiency and overlapping. Recovery of stan-dards was 93% (range 90–100%) for [3-³H]glucose and 94% (91–100%) for [1-¹⁴C]glucose.

Calculations

Curves for [³H]glucose and [¹⁴C]glucose specific radioactivity (SA) against time were constructed. Values for glucose turnover, mean residence time, glucose pool and glucose space were calculated as described by Shipley & Clarke [21] by the formulae:

Glucose turnover (RT)

$$= \frac{\text{Injected dose}}{\int_0^\infty \text{Glucose SA} \times \text{d}t}$$

Mean residence time (MRT)

$$= \frac{\int_0^\infty (t \times \text{Glucose SA} \times \text{d}t)}{\int_0^\infty \text{Glucose SA} \times \text{d}t}$$

Glucose pool = Glucose RT × MRT

Glucose space

$$= \frac{\text{Glucose pool} \times 100}{\text{Body wt.} \times \text{Glucose concentration}}$$

Metabolic clearance rates (MCR) were given by the formula:

$$\text{MCR} = \frac{\text{Glucose RT}}{\text{Glucose concentration}}$$

Values for glucose mean residence time, pool size, space and metabolic clearance rates quoted in the text are those calculated from the data given by analysis of [3-³H]glucose specific radioactivity. Radioactive glucose 'recycling', as defined by Streja *et al.* [22], was estimated by the difference between glucose turnover calculated by [3-³H]glucose and that by [1-¹⁴C]glucose [Recycling = RT(³H) − RT(¹⁴C)]. 'Recycling' was also expressed as a percentage of total [RT(³H)] glucose turnover.

$$\left(\% \text{ 'recycling'} = \frac{\text{RT}(^3\text{H}) - \text{RT}(^{14}\text{C})}{\text{RT}(^3\text{H})} \times 100 \right)$$

Double-exponential functions were fitted to the specific radioactivity (SA) − time (*t*) curves for [³H]- and [¹⁴C]-glucose by linear least-squares regression by using an iterative program on a mini-computer (Apple II Europlus; Cupertino, CA, U.S.A.). For the number of data points ($n = 17$) polyexponential regression provided a better estimate of *t* than did the trapezoidal rule where SA approximated towards zero. The correlation of curve fit was 0·95 (0·92–0·99).

Statistical analyses were performed by using Student's paired and unpaired *t* tests as appropriate. Serum insulin and plasma glucagon concentrations showed logarithmic distribution, and statistical analyses were performed on log-transformed data. Correlations were sought by the least-squares method or the Spearman ranking method as appropriate. Values in the text are given as means ± SEM or as means and ranges.

Results

In both hyperthyroid and hypothyroid subjects serum thyroxine, tri-iodothyronine and thyrotrophin concentrations returned to normal with treatment (Table 1). Fasting blood glucose concentrations and blood concentrations of the gluconeogenic precursors lactate, pyruvate and alanine were similar in hyper- and hypo-thyroid subjects and control subjects and were unaltered by therapy (Table 2). In hyperthyroidism fasting blood glycerol concentrations were elevated compared with control values (0·12 ± 0·02 vs 0·06 ± 0·02 mmol/l, $P < 0.05$) and fell to normal with therapy. Blood glycerol concentrations were normal in hypothyroidism. Serum insulin and plasma glucagon concentrations were similar in all groups. Total glucose turnover [RT(³H)] was increased in hyperthyroidism (18·1 ± 1·0 vs 12·4 ± 0·9 μmol kg⁻¹ min⁻¹, $P < 0.01$) and decreased in hypothyroidism (8·2 ± 0·3 vs 11·7 ± 1·2 μmol kg⁻¹ min⁻¹, $P < 0.01$) (Tables 3 and 4) and returned to normal with treatment. Glucose turnover rates derived from the [1-¹⁴C]glucose data [RT(¹⁴C)] were similar in hyperthyroid and control subjects (hyperthyroid 13·4 ± 1·1 vs control 11·2 ± 0·9 μmol kg⁻¹ min⁻¹), although a small but significant decrease was seen after antithyroid therapy (12·3 ± 1·2 μmol kg⁻¹ min⁻¹, $P < 0.05$). In hypothyroid subjects RT(¹⁴C) was decreased (6·9 ± 0·4 vs 10·2 ± 0·8 μmol kg⁻¹ min⁻¹, $P < 0.01$) (Table 4) and increased with thyroxine therapy (8·2 ± 0·8 μmol kg⁻¹ min⁻¹).

The difference between RT(³H) and RT(¹⁴C) was increased 4-fold in hyperthyroidism (4·7 ± 0·6 vs 1·3 ± 0·3 μmol kg⁻¹ min⁻¹, $P < 0.001$) with a 2·7-fold increase in the % recycling index

$$\left(\frac{\text{RT}(^3\text{H}) - \text{RT}(^{14}\text{C})}{\text{RT}(^3\text{H})} \times 100 \right)$$

(Table 3). Both returned to normal with therapy. By contrast, apparent recycling was not significantly altered in hypothyroidism (Table 4). Metabolic clearance rate for glucose was also increased (3·5 ± 0·3 vs 2·4 ± 0·2 ml kg⁻¹ min⁻¹,

TABLE 1. *Characteristics of hyper- and hypo-thyroid patients before and during therapy and of controls*

***P < 0·001 untreated patients vs appropriate controls. †††P < 0·001 untreated vs treated patients.

	Hyperthyroid	Hyperthyroid during therapy	Control	Hypothyroid	Hypothyroid during therapy	Control
Sex	3M, 3F	3M, 2F	4M, 2F	2M, 5F	2M, 4F	4M, 2F
Age	46 ± 3	44 ± 3	43 ± 6	59 ± 3	58 ± 4	45 ± 6
% of ideal body wt.	90± 3	99 ± 1	95 ± 2	122 ± 3	110 ± 2	111 ± 4
Serum thyroxine (nmol/1)	197 ± 17***	84 ± 8†††	90 ± 8	24 ± 7***	110 ± 5†††	104 ± 10
Serum tri-iodothyronine (nmol/1)	6·2 ± 0·7***	2·4 ± 0·2†††	1·6 ± 0·2	0·8 ± 0·1***	2·0 ± 0·1†††	2·0 ± 0·2
Serum thyrotrophin (m units/1)	1·3 ± 0·6	3·3 ± 1·5	2·9 ± 0·5	26·0 ± 2***	3·7 ± 0·9†††	2·9 ± 0·5

TABLE 2. *Fasting metabolite and hormone concentrations in hyperthyroid and hypothyroid subjects and controls shown as means ± SEM or means (and ranges)*

*P < 0·05 hyperthyroid subjects vs controls. †P < 0·05 hyperthyroid subjects before vs during therapy.

Hormone or metabolite	Hyperthyroid before therapy	Hyperthyroid during therapy	Controls	Hypothyroid before therapy	Hypothyroid during therapy	Controls
Blood glucose (mmol/1)	5·4 ± 0·3	5·3 ±0·5	5·2 ± 0·9	5·1 ± 0·1	5·0 ± 0·2	4·9 ± 0·2
Blood lactate (mmol/1)	0·67 ± 0·10	0·84 ± 0·11	0·91 ± 0·08	0·91 ± 0·05	0·79 ± 0·04	0·75 ± 0·05
Blood pyruvate (mmol/1)	0·06 ± 0·01	0·07 ± 0·01	0·10 ± 0·01	0·08 ± 0·01	0·07 ± 0·01	0·07 ± 0·01
Lactate/pyruvate ratio	11·3 ± 0·2	12·1 ± 2·3	10·7 ± 2·6	12·4 ± 0·6	11·4 ± 0·8	9·2 ± 1·2
Blood alanine (mmol/1)	0·27 ± 0·03	0·29 ± 0·03	0·34 ± 0·02	0·36 ± 0·01	0·34 ± 0·02	0·33 ± 0·02
Blood glycerol (mmol/1)	0·12 ± 0·02*	0·05 ± 0·01†	0·06 ± 0·02	0·07 ± 0·01	0·07 ± 0·01	0·07 ± 0·01
Serum insulin (m units/1)	5 (1–10)	7 (0–12)	6 (1–8)	7 (1–14)	5 (2–11)	7 (0–11)
Plasma glucagon (pg/1)	28 (12–40)	30 (18–50)	26 (13–36)	21 (10–30)	28 (2–57)	26 (20–31)

TABLE 3. *Glucose turnover rates, 'recycling', mean residence time, pool, space and metabolic clearance rates in hyperthyroid subjects before and during therapy and in controls*

*P < 0·05, **P < 0·01 and ***P < 0·001, untreated patients vs treated patients. †P < 0·05, ††P < 0·01 and †††P < 0·001, untreated patients vs controls.

	Absolute glucose turnover [RT(^{3}H)] (μmol kg^{-1} min^{-1})	^{14}C-derived glucose turnover [RT(^{14}C)] (μmol kg^{-1} min^{-1})	RT(^{3}H) − RT(^{14}C) (amount of glucose 'recycled') (μmol kg^{-1} min^{-1})	$\frac{RT(^3H) - RT(^{14}C)}{RT(^3H)} \times 100$ (% of glucose 'recycled')	Mean residence time (min)	Glucose pool (μmol/kg)	Glucose space (% of body wt.)	Metabolic clearance rate (ml kg^{-1} min^{-1})
Hyperthyroid (n = 6)	18·1 ± 1·0††	13·4 ± 1·1	4·7 ± 0·6†††	27 ± 3†††	74 ± 4††	1330 ± 93	26 ± 3	3·5 ± 0·3†
Hyperthyroid during therapy (n = 5)	13·7 ± 1·3***	12·3 ± 1·2*	1·3 ± 0·4*	10 ± 3*	81 ± 3	1113 ± 87**	23 ± 2	2·7 ± 0·4**
Control (n = 6)	12·4 ± 0·9	11·2 ± 0·9	1·3 ± 0·3	10 ± 2	97 ± 5	1183 ± 56	24 ± 1	2·1 ± 0·2

TABLE 4. *Glucose turnover rates, 'recycling', mean residence time, pool, space and metabolic clearance rates in hypothyroid subjects before and during therapy and in controls*

For symbols and conditions see the legend to Table 3.

	Absolute glucose turnover (μmol kg^{-1} min^{-1})	^{14}C-derived glucose turnover (μmol kg^{-1} min^{-1})	RT(^{3}H) − RT(^{14}C) (amount of glucose 'recycled' (μmol kg^{-1} min^{-1})	$\frac{RT(^3H) - RT(^{14}C)}{RT(^3H)} \times 100$ (% of glucose 'recycled')	Mean residence time (min)	Glucose pool (μmol/kg)	Glucose space (% of body wt.)	Metabolic clearance rate (min kg^{-1} min^{-1})
Hypothyroid (n = 7)	8·2 ± 0·3††	6·9 ± 0·4††	1·3 ± 0·8	16 ± 3	146 ± 14†	1195 ± 102	24 ± 2	1·6 ± 0·1†††
Hypothyroid during therapy (n = 6)	9·9 ± 0·6*	8·7 ± 0·8	1·2 ± 0·3	13 ± 3	119 ± 13	1172 ± 110	24 ± 1	2·0 ± 0·1*
Control (n = 6)	11·7 ± 1·2	10·2 ± 0·8	1·5 ± 0·4	12 ± 2	101 ± 7	1190 ± 134	24 ± 2	2·3 ± 0.2

$P < 0.05$) and decreased (1.6 ± 0.1 vs 2.3 ± 0.2 ml kg^{-1} min^{-1}, $P < 0.001$) in hyper- and hypothyroidism respectively. Glucose space was similar in all groups and was unaffected by treatment. In hyperthyroid subjects the glucose pool was not significantly different from control values, although a 15% decrease ($P < 0.01$) was observed after antithyroid therapy. Values for the glucose pool were normal in hypothyroid subjects. Mean residence time was decreased in hyperthyroid subjects. Mean residence time was decreased in hyperthyroid subjects (74 ± 4 vs 97 ± 5 min, $P < 0.01$) and increased in hypothyroid subjects (145 ± 14 vs 107 ± 7 min, $P < 0.05$). Normal values were restored with therapy.

Serum thyroxine and tri-iodothyronine concentrations did not correlate within patient or control groups with any parameter of glucose metabolism assessed. Similarly no correlations were observed for body weight in the hypothyroid group and their controls, where the latter tended to be younger and lighter than the hypothyroid group before treatment.

Discussion

Glucose tritiated in the 3-position measures total glucose turnover in post-absorptive man [13, 22]. The tritium on [3-^{3}H]glucose is lost at the triose phosphate level, so that turnover estimates made with the use of this labelled glucose represent glucose metabolized to this level and beyond. Turnover estimates made with the use of [1-^{14}C]glucose are lower owing to carbon-recycling back to glucose at any level in the glycolytic-gluconeogenic pathway [22, 23, 24], although the bulk of this is considered to occur from lactate and pyruvate [22]. The difference between the turnover estimates with these two forms of labelled glucose is therefore thought to provide an index of the amount of glucose cycled to C_3 intermediates and back.

Total glucose turnover was increased by 48% in hyperthyroidism and decreased by 30% in hypothyroid subjects. Similar findings have been obtained by other workers using isotopic methods in man [13] and animals [11]. Some 60% of the increase in glucose turnover noted in this study could be accounted for by the increase in recycling and only 40% by an increase in glucose metabolism by other routes. In contrast with our findings, a decrease in hepatic glucose production has recently been observed in hyperthyroid man with the use of catheterization techniques to obtain estimates of splanchnic glucose exchange [14]. The reason for these disparate findings is unclear, as the hyperthyroid subjects studied

appear to be similar in age, weight and severity of disease. In other conditions, both techniques may be expected to produce similar estimates of glucose production [25] and there is no evidence for an increase in renal glucose output in hyperthyroidism. A possible explanation may reside in the dietary preparation before study. A carbohydrate intake in excess of 300 g daily for 48 h before investigation may have ensured greater glycogen stores in the patients reported in the present study.

Increased glucose turnover may result from an increase in either glycogenolysis or gluconeogenesis. After an overnight fast, hepatic glycogen stores are diminished in hyperthyroidism [26], and increased gluconeogenesis is considered the major factor [14]. Rat liver perfusion experiments have shown thyroid-hormone-dependent increases in glucose production from lactate [27], alanine [28] and glycerol [29], and the activities of certain gluconeogenic enzymes [27, 30], particularly pyruvate carboxylase [31], are raised in the livers of hyperthyroid animals. In the present study, fasting blood glycerol concentrations were elevated in hyperthyroidism, as found by other workers [32, 33]. Glycerol is metabolized largely to glucose and its uptake is proportional to blood concentration [34]. Circulating concentrations of the other gluconeogenic precursors were normal in the present study, but flux was not measured. Others have, however, reported increased splanchnic fractional uptake of these substrates in hyperthyroid man, with a return to normal with treatment [14].

Absolute amounts of glucose recycled were increased 3.6-fold in hyperthyroidism, and percentage recycling also rose (2.7-fold). This process is energetically wasteful but represents only a trivial amount of energy lost: 43.9 kJ (10.5 kcal)/24 h, assuming ATP equivalent of 31.4 kJ (7.5 kcal)/mol and wastage of 4 ATP molecules per molecule of glucose recycled as the sole energy deficit. However, isotopic estimation of recycling may underestimate total chemical recycling because of interchange between labelled and unlabelled carbon atoms in substrate pools such as that for oxaloacetate [35]. Furthermore the isotopes used in the present study will not estimate glucose cycled in 'futile' cycles such as that between glucose and glucose 6-phosphate, activity of which may be increased by thyroid hormones [11]. The mechanisms controlling such 'futile' cycles are unclear but are independent of catecholamine action in thyroid excess in animals [36].

Increased hepatic glucose production has been attributed to increased glucagon secretion [37].

Circulating glucagon concentrations were, however, normal in the present and one other study [14]. Alternatively increased secretion or increased sensitivity to catecholamines has been postulated [38]. Catecholamine concentrations were not measured in the present study, but glucose turnover in hyperthyroidism is not restored to normal by β-adrenergic blockade [13].

Similarly, alterations in circulating insulin concentrations cannot be implicated, in that we and others [39] have found them to be normal. However, changes in portal insulin and glucagon concentrations cannot be excluded. It has been suggested that alterations in insulin-sensitivity may occur, although the evidence is conflicting, with enhanced [40, 41], normal [42] and diminished [2, 43] insulin-sensitivity all having been reported in hyperthyroidism. We have recently found normal sensitivity to insulin in hyperthyroid subjects with the use of a glucose clamp technique (unpublished observations).

In the present study peripheral disposal of glucose, assessed by glucose clearance, was diminished in hypothyroidism and elevated in hyperthyroidism, with both abnormalities reversed by treatment. However, a major abnormality of insulin action is unlikely in view of the normal blood glucose concentrations in both hyperthyroid and hypothyroid subjects.

Finally, body weight is a determinant of glucose turnover and glucose turnover may be diminished in the grossly obese [44]. In the present study, with a smaller range of body weights, no correlation between weight and glucose turnover was seen within the control subjects. Patients were matched by weight to separate control groups, and differences in turnover are therefore more likely to be related to thyroid status.

In conclusion, glucose turnover and recycling were markedly enhanced in hyperthyroid subjects. The greater proportion of the increase in turnover was due to the increase in recycling of C_3 intermediates. These abnormalities could not be accounted for by alterations in glucose space or changes in the two main glucoregulatory hormones insulin and glucagon. In hypothyroid subjects glucose turnover, representing both production and utilization, was decreased. In both groups of subjects values returned to normal with treatment. The precise mechanisms of these changes remain to be elucidated.

Acknowledgments

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INSULIN SENSITIVITY IN HYPERTHYROIDISM: MEASUREMENT BY THE GLUCOSE CLAMP TECHNIQUE

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SUMMARY

Sensitivity to porcine insulin has been compared in overnight fasted hyper-thyroid and control subjects using a euglycaemic clamp technique. Basal values for blood glucose, lactate, pyruvate, alanine, serum insulin and C-peptide were similar in the two groups, whilst blood glycerol (hyperthyroid 0.11 ± 0.02 (mean $\pm$ S.E.) vs. control 0.06 ± 0.01 mmol/l, $P < 0.01$) and blood 3-hydroxybutyrate (0.28 [0.03–0.79, range] vs 0.09 [0.01–0.29] mmol/l, $P < 0.05$) were increased in hyperthyroidism. During the 2 hour insulin infusion (0.05 U/kg/h), serum insulin plateaued at the same level (44 ± 4 vs 44 ± 1 mU/l) and insulin metabolic clearance rates were similar (1.19 ± 0.10 vs 1.25 ± 0.03 l/min). Serum C-peptide levels also decreased by similar amounts (40 ± 8 vs $47 \pm 6\%$). The amount of glucose infused to maintain euglycaemia was identical during the second hour of insulin infusion (290 ± 50 vs 330 ± 30 mg/kg) as were the increments in lactate and pyruvate concentrations. Blood glycerol values decreased in both groups although values in hyperthyroid patients remained significantly higher than in controls. 3-Hydroxybutyrate concentrations fell to similar values in the two groups. These findings suggest that insulin-stimulated glucose metabolism and inhibition of ketogenesis is normal in hyperthyroidism.

The blood glucose concentration of hyperthyroid man has been reported to be elevated following the ingestion of glucose (Holdsworth & Besser, 1968; Kreines *et al.* 1965) and mixed meals (McCulloch *et al.* 1982) in most, but not all (Macho 1936; Gorowski & Wolanska 1957) studies. The cause of this hyperglycaemia remains uncertain. Insulin sensitivity in hyperthyroidism has been assessed as normal (Ambrosolini *et al.* 1956), diminished (Doar *et al.* 1969; Holdsworth and Besser, 1968) or enhanced (Elgee & Williams, 1955; Maracek & Feldman 1973; Elrick *et al.* 1961). This probably reflects the

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variety of techniques used to measure insulin sensitivity as well as species differences between man (Holdsworth & Besser 1968; Kreines *et al.* 1965; McCulloch *et al.* in press) rat (Ambrosalini *et al.* 1956; Elgee & Williams, 1955) and rabbit (Maracek & Feldman, 1973). Where glucose tolerance is impaired, the finding of normal (Holdsworth & Besser, 1968, McCulloch *et al.*, 1982) or elevated (Doar *et al.* 1969) peripheral insulin levels has been taken to indicate insulin resistance, even where the pattern of insulin secretion has not been examined. Measurement of the rate of fall or nadir of blood glucose concentrations after injection of exogenous insulin in man has produced contradictory results, sometimes suggesting enhanced (Elrick *et al.* 1961) and sometimes decreased (West *et al.* 1975) sensitivity to insulin. Such studies are however complicated by the counter-regulatory response to hypoglycaemia which is very variable in both time and degree.

The euglycaemic clamp technique allows assessment of insulin sensitivity in the absence of stimulated counter-regulatory hormone secretion (Alberti *et al.* 1979; De Fronzo *et al.* 1979; Home *et al.* 1982). We have therefore used this technique, with the aid of a blood glucose-controlled glucose infusion system (Biostator, Miles) to compare insulin sensitivity in normal and hyperthyroid subjects. Insulin sensitivity is defined as the rate of glucose infusion required to maintain the blood glucose level in the face of a constant infusion of insulin. The response of intermediary metabolites to the insulin infusion has also been examined.

MATERIALS AND METHODS

Subjects

Six female patients (age [mean ± SE] 28 ± 2 years, % ideal body weight 100 ± 3) with hyperthyroidism due to Graves' Disease (diffusely enlarged hyperaemic thyroid glands with no nodules on Technicium scanning) were compared with a matched group of seven healthy female controls (age 24 ± 1 years, % ideal body weight 100 ± 2). The study was approved by the local Ethical Committee and informed consent obtained from all subjects. Patients and controls were all within 10% of ideal body weight, and their ages ranged from 22–36 years. They were asked to maintain their normal diet for three days before the study and to fast from midnight on the evening beforehand.

No subject was taking oral contraceptive pills or any other drug and none was known to be suffering from any disease other than thyrotoxicosis.

Protocol

On the morning of the study, three PTFE cannulae (Venflon, Viggo, Helsinborg) were inserted i.v. into each subject. One cannula, in an antecubital fossa, was used for intermittent blood sampling for hormones and metabolites and was flushed with 0·15 mol/l saline after use. A second cannula, inserted retrogradely and more distally on the same arm was used for continuous sampling of arterialised blood by the biostator. Arterialisation of venous blood was achieved using a heated hand warming system (Abumrad *et al.* 1981). The third cannula, on the contralateral arm, was used for all infusions. A period of one hour was allowed for equilibration following cannulation, calibration of the machine and to achieve stability of the glucose sensor response.

At the end of calibration (time 0 min) highly purified porcine insulin (Actrapid, Novo) was infused i.v. at a rate of 0·05 u/kg/h body weight. The biostator constants and

clamping technique were as described previously (Home *et al.* 1982). Blood glucose was clamped 0·28 mmol/l (5 mg/dl) below fasting levels to avoid stimulation of endogenous insulin during oscillation around the intended clamp value. The insulin infusion continued for 120 min and blood samples were taken at −30, 0 and every 15 min thereafter for 2 h. Blood for glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxy-butyrate was taken into chilled 0·5 mol/l perchloric acid and the extract stored at −20°C until analysis by fluorimetric methods (Lloyd *et al.* 1978). Serum insulin was measured by double antibody radioimmunoassay (Soeldner and Slone, 1965) (sensitivity 2 mU/l: intraassay coefficient of variation (CV) 7%) using human insulin as standard. C-peptide was measured by radioimmunoassay with ethanol precipitation (Heding, 1975) (sensitivity 0·02 nmol/l within assay CV 3%). Serum triiodothyronine (T_3) was measured by double antibody radioimmunoassay (Hesch and Evered, 1973) and serum thyroxine (T_4) by radioimmunoassay (Mallinkrodt RIA-MAT (R) T_4).

Statistics

Results were analysed by standard parametric methods and expressed as mean ±standard error unless otherwise indicated. Blood 3-hydroxybutyrate concentrations were log normally distributed and statistical analysis was performed on log transformed data. Student's independent *t*-test was employed to detect differences between groups. Insulin sensitivity was calculated as the amount of glucose infused to maintain euglycaemia in the second hour of insulin infusion (60–120 min) when the glucose infusion rate was relatively constant.

RESULTS

Serum thyroxine (hyperthyroid 276±33 vs control 107±4 nmol/l $P < 0.001$) and triiodothyronine (8·5±1·6 vs 2·0±0·1 nmol/l, $P < 0.001$) were increased in hyperthyroid subjects.

Basal values for blood glucose, (Fig. 1), lactate (Fig. 2), pyruvate (hyperthyroid 0·06±0·005 vs control 0·07±0·01 mmol/l) and alanine (hyperthyroid 0·23±0·03 vs control 0·28±0·03 mmol/l) were similar in the two groups, but blood glycerol (hyperthyroid 0·11±0·02 vs control 0·06±0·01 mmol/l, $P < 0.01$) and blood 3-hydroxy-butyrate (0·28 [0·03–0·79] vs 0·09 [0·01–0·29] mmol/l (mean and range), $P < 0.05$) were increased in hyperthyroidism (Fig. 2). Basal serum insulin (hyperthyroid 7±1 vs control 6±2 mu/l and C-peptide levels (0·32±0·06 vs 0·33±0·03 nmol/l) were however identical (Fig. 1). During insulin infusion, plateau serum insulin values were similar in the two groups (hyperthyroid 44± vs control 44±1 mU/l) (Fig. 1). Insulin metabolic clearance rates were also identical (1·21±0·10 vs 1·25±0·03 l/min). The degree of suppression of endogenous insulin secretion, as reflected by the fall in C-peptide levels (Fig. 1) was indistinguishable between patients and controls (hyperthyroid 40±8 vs control 47±6%). Blood glucose levels were satisfactorily controlled by the biostator (Fig. 1) with a mean deviation from intended values of 0·04 mmol/l. The coefficient of variation of blood glucose values for the period of the clamp was similar in both groups (hyperthyroid 6·3±1·5 vs control 5·1±1·6%). During the last 60 min blood glucose was 4·6±0·3 mmol/l in hyperthyroid subjects and 4·4±0·1 mmol/l in controls (Fig. 1). Feedback glucose infusion increased throughout the 2 h study period but most of this change occurred in the
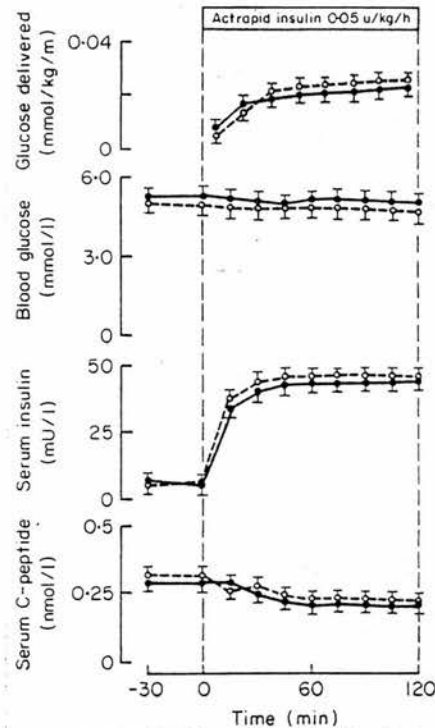
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Fig. 1. Glucose delivery to maintain blood glucose levels, blood glucose, serum insulin and C-peptide concentrations (mean ± SEM) during infusion of highly purified porcine insulin (0–120 min) in hyperthyroid (●) and control (O) subjects. Values for glucose delivery are expressed as mmol/kg/min and are shown as the mean of each 15 min infusion period.

first hour (Fig. 1). No significant difference was seen in the glucose required to maintain the clamp value at any time.

Blood lactate levels rose to the same extent in both groups (Fig. 2), and blood pyruvate followed a similar pattern. Blood alanine levels were unchanged in both groups during insulin infusion. Blood glycerol concentrations fell in both groups (Fig. 2), although levels in the hyperthyroid patients remained significantly higher than in the controls. Blood 3-hydroxybutyrate levels decreased to similar values in both groups (Fig. 2).

DISCUSSION

Glucose clamp techniques allow assessment of insulin activity on glucose metabolism without the interference of counter-regulatory responses to hypoglycaemia (De Fronzo *et al.* 1979; Olefsky & Kolterman 1981). For ethical reasons we have not performed repeated studies to obtain dose response curves, but the single dose chosen (0·05 u/kg/h) lies on the straight part of the curve in normal subjects (Massi-Benedetti *et al.* 1981). This infusion rate also gives circulating insulin levels comparable to those seen during glucose tolerance tests (Sestoft & Heding, 1981) and after normal meals (McCulloch *et al.* 1982). The highly purified porcine insulin used in this study is known to be identical in activity with human insulin when assessed using the same technique (Home *et al.* 1982).

a combination of insulin-induced inhibition of hepatic gluconeogenesis and increased hepatic and peripheral glycolysis. Blood alanine concentrations were unchanged by insulin infusion in both groups reflecting a balance of the hepatic and peripheral effects of insulin. 3-Hydroxybutyrate levels were, however, increased in hyperthyroid subjects but suppressed rapidly to control values during insulin infusion. Fasting hyperketonaemia has previously been reported in these patients (Beylot *et al.* 1980; Bartels *et al.* 1979) and ketone body levels are known to fall to normal values after mixed meals (McCulloch *et al.* 1982) as a result of the suppression of lipolysis and ketogenesis. Fasting blood glycerol levels were increased in the hyperthyroid patients but, in contrast to ketone bodies, did not decline to normal values during insulin infusion, although a significant decrease was apparent. This is consistent with recent studies using adipocytes from hyperthyroid patients which demonstrated impairment of the normal insulin-mediated suppression of glycerol release (Wennlund *et al.* 1981). In accord with this we have recently shown that blood glycerol levels fail to return to normal after mixed meals (McCulloch *et al.* 1982).

In summary insulin sensitivity in terms of overall glucose metabolism is normal in hyperthyroidism. Lipolysis is however enhanced at normal fasting insulin levels, and may not be totally suppressed by insulin levels effective in normal man.

ACKNOWLEDGEMENTS

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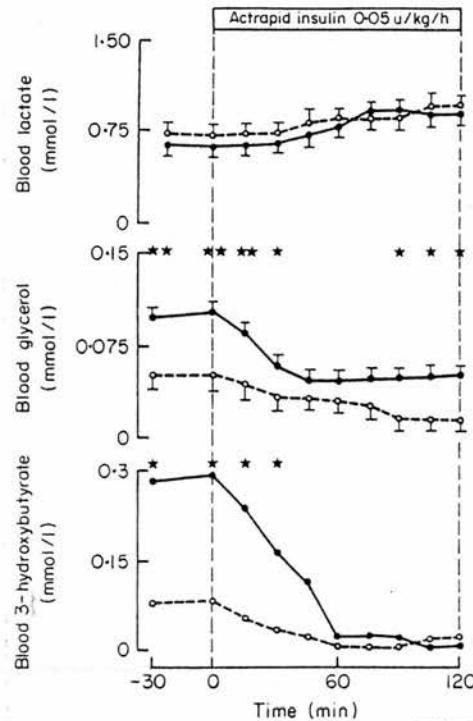
Fig. 2. Blood lactate, glycerol and 3-hydroxybutyrate concentrations during insulin infusion with maintainance of blood glucose levels by feedback glucose infusion. For lactate and glycerol mean $\pm$ SEM is shown and for 3-hydroxybutyrate mean value is given. ● = hyperthyroid and O = control. $P < 0.05$ hyperthyroid vs control (**$P < 0.01$).

In the current study, fasting blood glucose, serum insulin and C-peptide levels were identical in matched hyperthyroid and euthyroid women. No suggestion could be found of any difference in the rate of change or absolute level of glucose turnover during peripheral insulin infusion.

The published evidence for a disturbance of insulin secretion in human and experimental hyperthyroidism is contradictory, with enhanced (Doar *et al.* 1969; Yallow & Berson, 1960) diminished (Daweke *et al.* 1965; Andreani *et al.* 1970) or normal (Hales & Hyans, 1964) levels all being reported. Secretion of biologically inactive insulin in hyperthyroid patients may also be important. Sestoft and Heding (1981) have recently demonstrated that hyperthyroid patients secrete a higher proportion of proinsulin to insulin. Although proinsulin cross-reacts in the standard insulin radioimmunoassay, it has only 5% of the biological activity of insulin (Sönksen *et al.* 1973). Enhanced degradation of insulin has been reported in some studies (Elgee & Williams, 1955; Maracek & Feldman, 1973) but not all (Wajchenberg *et al.* 1978) although, it is clear from the present study that metabolic clearance rates are unchanged. Increased clearance of insulin cannot in any case explain glucose intolerance as both the action and degradation of insulin are effected via its receptors (Terris & Steiner, 1975).

Basal circulating lactate and pyruvate levels were also similar and rose to the same extent during insulin infusion in patients and controls. These increases presumably reflect

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EVIDENCE THAT THYROID HORMONES REGULATE GLUCONEOGENESIS FROM GLYCEROL IN MAN

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SUMMARY

We have previously reported that glucose production assessed using radioisotopic methods is 50% increased in hyperthyroidism but 30% decreased in hypothyroidism. These studies, however, do not distinguish between glycogenolysis and gluconeogenesis. In fasting man more than 80% of circulating glycerol is cleared by the liver and enters the gluconeogenic pathway. We have therefore measured glycerol clearance following bolus intravenous glycerol administration as an indirect assessment of gluconeogenic capacity. Hyperthyroid and hypothyroid subjects were compared with separate matched controls after an overnight fast. In hyperthyroid subjects blood glucose and blood glycerol were increased but lactate, pyruvate, and alanine concentrations were normal. Glycerol clearance was increased in hyperthyroidism and followed a double exponential decay with a shortened second component half-time. Endogenous glycerol production was increased three-fold. In hypothyroidism fasting circulating levels of glucose, lactate, pyruvate, alanine, and glycerol were normal but glycerol clearance was diminished. Both first and second component half-times were prolonged in hypothyroidism and endogenous glycerol production was decreased by 50%. Thus in hyperthyroidism glycerol clearance is greatly enhanced whilst in hypothyroidism glycerol clearance is diminished. The magnitude of the changes suggests that alterations in gluconeogenesis are probably the major factors concerned in the reported increase and decrease in glucose production in hyperthyroidism and hypothyroidism respectively.

Total glucose production is increased in hyperthyroidism (Perez *et al.*, 1980; Saunders *et al.*, 1980; McCulloch *et al.*, 1983) and decreased (McCulloch *et al.*, 1983) or normal

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(Saunders *et al.*, 1980) in hypothyroid man and blood glycerol concentrations may be elevated in the former state (Tibbling, 1969; McCulloch *et al.*, 1982). The relative contributions of glycogenolysis and gluconeogenesis to these changes in glucose production, however, are not known.

Animal and *in vitro* studies have suggested that thyroid hormones may regulate gluconeogenesis from the major gluconeogenic precursors lactate (Menahan & Weiland, 1969; Weinberg & Utter, 1979; Okajima & Ui, 1979), alanine (Ness *et al.*, 1969), and glycerol (Sestoft *et al.*, 1977). In the rat, thyroid hormones cause the induction of L-glycerophosphate dehydrogenase in liver mitochondria (Lee *et al.*, 1959), the activity of this enzyme playing a central role in the regulation of glycerol utilization by determining the rate of carbon transfer into gluconeogenesis (Schimassek *et al.*, 1963; Sestoft *et al.*, 1977). In normal man and animals glycerol is highly gluconeogenic (Nikkila & Ojala, 1964; Bortz *et al.*, 1972) and is metabolized to glucose in preference to the other gluconeogenic precursors (Steele *et al.*, 1971). In the fasting state 90–100% of circulating glycerol is converted to glucose (Bortz *et al.*, 1972; Shaw *et al.*, 1976), conversion increasing as blood concentrations rise (Winkler *et al.*, 1970). The liver is responsible for 70–90% of total glycerol uptake (Larsen, 1963; Gidez & Karnowsky, 1954; Swick & Nakao, 1954; Schambye *et al.*, 1954) with the remainder metabolized by the kidneys (Borchgrevink & Havel, 1963; Swanson & Thompson, 1969), small intestine and other tissues (Saunders & Dawson, 1967; Haessler & Isselbacher, 1963; Lin, 1977). Clearance of glycerol from blood following intravenous glycerol loading may therefore provide an estimate of the gluconeogenic capacity of the liver in normal man and in patients with thyroid dysfunction.

We have therefore examined glycerol in patients with thyrotoxicosis, primary thyroid failure and in normal controls using a bolus intravenous technique with subsequent analysis of decay of blood glycerol levels.

MATERIALS AND METHODS

Seven hyperthyroid (six female, one male) and six hypothyroid (six female) patients were compared with separate groups of matched controls (seven male and eight female) (Table 1). Hyperthyroidism was due to Graves' disease in all cases (on the basis of clinical features and the absence of thyroid nodules on ^{99}Tc scanning). Hypothyroidism was due to autoimmune thyroid failure in all patients (on the basis of clinical features and the presence of circulating antibodies to thyroid tissue). No subject had any evidence of hepatic or renal impairment and none was taking any drug prior to the study. Informed consent was obtained from all subjects for the study which was approved by the Newcastle Area Health Authority (Teaching) Ethical Committee.

Studies were performed on recumbent subjects after an overnight (10 h) fast. Subjects had abstained from alcohol for at least 48 h and had a daily carbohydrate intake in excess of 250 g for this period. Bilateral indwelling intravenous cannulae (Venflon, Viggo Helsinborg) were inserted using 1% lignocaine as local anaesthetic. Cannula patency was maintained by flushing with 0·154 mol/l saline after every sample.

Basal samples for hormones and metabolites were withdrawn 25 and 30 min after cannulation. An intravenous bolus injection of glycerol (0·1 mmol/kg body weight) diluted with water to isotonicity was then administered over 15 s and further blood samples were taken 1, 2, 3, 4, 5, 6, 8, 10, 12·5, 15, 20, 30, 40, 50, and 60 min after injection.

Venous blood (1·0 ml) was mixed with chilled 0·5 mol/l perchloric acid. Blood glucose, glycerol, lactate, pyruvate alanine, and 3-hydroxybutyrate were measured by an automated fluorimetric enzymatic method (Lloyd *et al.*, 1978). Serum insulin (Soeldner & Slone, 1965), triiodothyronine (Hesch & Evered, 1973), and thyroid stimulating hormone (Hall *et al.*, 1971) were measured by double antibody radioimmunoassay and serum thyroxine by radioimmunoassay (Mallinkrodt, RIA-MAT (R) T4).

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using Student's unpaired *t*-test. Correlations were sought by the least-squares method. 3-Hydroxybutyrate concentrations were log-normally distributed and statistical analyses were performed on log-transformed data.

Mathematical analysis

The decay of blood glycerol following bolus intravenous injection was closely approximated by a double exponential function of the form $A_w - k_1t + B_e - k_2t$, where A, B, K_1, and k_2 are constants. Curve-fitting was performed by computer to the points of the blood glycerol decay curve, after subtraction of basal glycerol concentrations. The area under the curve was calculated by integration of the equation for curve fit (Bateman *et al.*, 1981). Metabolic clearance rate (MCR) is equal to the dose of glycerol injection divided by the area under the glycerol decay curve:

$$MCR \ (l/min) = \frac{mmol/injected}{(mmol/l/min)}.$$

Endogenous glycerol production (EPR) is equal to the product of metabolic clearance and basal glycerol concentration:

$$EPR \ (mmol/min) = MCR \times Basal \ [glycerol].$$

It is assumed in these calculations that the endogenous production rate of glycerol remains constant throughout the experimental period. Volume of distribution of glycerol was calculated using the intercepts and exponents for the α and β phases of the glycerol decay curve (Gibaldi, 1969).

RESULTS

Serum thyroxine and triiodothyronine concentrations were increased in hyperthyroid and decreased in hypothyroid patients when compared to controls (Table 1). Serum thyroid stimulating hormone concentrations were increased in hypothyroidism.

Peak blood glycerol concentrations following glycerol injection ranged between 0·35 and 1·20 mmol/l and were not significantly different between the groups. Glycerol injection had no effect on any of the other metabolite concentrations measured in any group nor on peripheral insulin levels.

Hyperthyroid subjects

Fasting blood glucose ($5·4 \pm 0·2$ *v.* $4·6 \pm 0·1$ mmol/l, $P < 0·01$) (Table 2) and blood glycerol ($0·12 \pm 0·01$ *v.* $0·07 \pm 0·01$ mmol/l, $p < 0·01$) (Table 3) concentrations were increased in hyperthyroid subjects but basal circulating lactate, pyruvate, alanine, 3-hydroxybutyrate, and insulin levels were similar to controls (Table 2).

The double exponential decay curve for injected glycerol is clearly demonstrated in Fig.

Table 1. Clinical details of hyperthyroid, hypothyroid, and control subjects. Results are mean $\pm$ SEM

	Age (years)	Per cent of ideal body weight	Serum thyroxine (nmol/l)	Serum triiodo-thyronine (nmol/l)	Serum thyroid stimulating hormone (mU/l)
Hyperthyroid	34±4	104±3	181±13***	4·5±0·3***	1·0±0·02
Control	34±3	108±4	97±9	2·0±0·2	1·5±0·2
Hypothyroid	48±1	123±5	28±9***	0·9±0·2**	40·1±7·1***
Control	45±2	118±4	89±8	2·0±0·2	1·6±0·5

** $P < 0.01$ *v.* appropriate controls.
*** $P < 0.001$ *v.* appropriate controls.

Table 2. Fasting hormone and metabolite concentrations (mean $\pm$ SE) in hyperthyroid, hypothyroid, and control subjects. For 3-hydroxybutyrate mean (range) is given

Subjects	Blood glucose (mmol/l)	Blood lactate (mmol/l)	Blood pyruvate (mmol/l)	Blood alanine (mmol/l)	Blood 3-hydroxybutyrate (mmol/l)	Serum insulin (mU/l)
Hyperthyroid	5·4±0·2**	0·94±0·15	0·09±0·01	0·28±0·02	0·08(0·03–0·18)	7·8±1·0
Control	4·6±0·1	0·88±0·16	0·07±0·01	0·30±0·04	0·08(0·03–0·18)	8·8±1·5
Hypothyroid	5·1±0·4	1·10±0·07	0·10±0·01	0·31±0·02	0·04(0·01–0·14)	9·4±1·8
Control	4·9±0·2	0·99±0·15	0·08±0·04	0·34±0·04	0·05(0·005–0·14)	8·4±1·3

** $P < 0.01$ hyperthyroid subjects *v.* controls.

1 ($r = 0.98 \pm 0.003$). Values for the second exponential constant (k_2) were increased in hyperthyroidism (0.138 ± 0.022 *v.* 0.070 ± 0.010, $p < 0.05$) and as a result the second component half-time was shorter (5.9 ± 1.0 *v.* 11.9 ± 0.3 min, $P < 0.05$). Glycerol clearance rate was also increased in hyperthyroidism (48.7 ± 5.7 *v.* 27.2 ± 2.6 ml/kg/min, $P < 0.005$) as was endogenous glycerol production (5.7 ± 0.8 *v.* 2.1 ± 0.3 μmol/kg/min, $P < 0.01$) (Table 3).

Hypothyroid subjects

In hypothyroidism, fasting circulating concentrations of glucose, lactate, pyruvate, alanine, glycerol, 3-hydroxybutyrate, and insulin were similar to control values (Table 2). Both the first ($P < 0.05$) and second component ($P < 0.01$) half-times were significantly prolonged in hypothyroidism (Table 3). Glycerol clearance rate was decreased (hypothyroid 17.1 ± 0.7 *v.* control 25.0 ± 1.8 ml/kg/min, $P < 0.01$) and endogenous glycerol production rate was diminished (1.1 ± 0.5 *v.* 2.1 ± 0.3 μmol/kg/min, $P < 0.01$) in hypothyroid patients.

Table 3. Glycerol kinetic data in hyperthyroid, hypothyroid, and respective control subjects

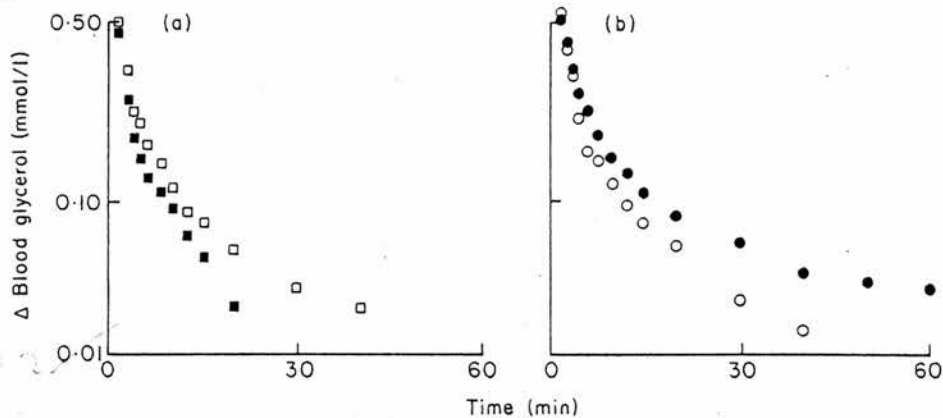| Subject group	Basal blood glycerol (mmol/l)	Metabolic clearance rate (ml/kg/min)	Volume of distribution (l/kg)	k_1 (min^{-1})	k_2 (min^{-1})	Half disappearance time for 1st component (min)	Half disappearance time for 2nd component (min)	Endogenous production rate (µmol/kg/min)
Hyperthyroid (n=7)								
Mean	0·12	48·7	0·54	2·05	0·138	0·7	5·9	5·7
SEM	0·01	5·7	0·06	0·81	0·022	0·2	1·0	0·8
Controls (n=7)								
Mean	0·07	27·2	0·63	0·83	0·070	1·1	11·9	2·1 (2)
SEM	0·01	2·6	0·08	0·02	0·010	0·2	2·3	0·3 (4)
P	<0·01	<0·005	NS	NS	<0·05	NS	<0·05	<0·01
Hypothyroid (n=6)								
Mean	0·06	17·1	0·60	0·46	0·030	1·7	27·9	1·1
SE $t_{1/2}$	0·01	0·7	0·04	0·08	0·006	0·3	4·7	0·2
Controls (n=8)								
Mean	0·08	25·0	0·52	1·01	0·076	0·92	10·8	2·1
SE $t_{1/2}$	0·01	1·8	0·09	0·17	0·010	0·22	1·6	0·2
P	NS	<0·01	NS	<0·05	<0·01	<0·05	<0·01	<0·01

NS, $P > 0·05$.

Fig. 1. Change in blood glycerol concentration from basal levels following bolus intravenous glycerol (0·1 mmol/kg) administration in (a) hyperthyroid (■) and control (□) and (b) hypothyroid (●) and control (○) subjects. Blood glycerol is shown on a logarithmic scale.

Correlations

Correlations between glycerol kinetic data, metabolic status, and thyroid hormone levels did not reach significance in either patient or control groups.

DISCUSSION

These studies show that in man hyperthyroidism is associated with enhanced glycerol clearance after bolus injection of glycerol. No estimate of basal glycerol clearance is possible from our data but a 170% increase in endogenous production with only a 70% increase in fasting levels implies enhanced basal glycerol clearance and hence, perhaps, increased gluconeogenesis. Converse changes were noted in hypothyroid subjects.

Fasting blood glucose concentrations were increased in hyperthyroidism in this, as in other studies (Ikejiri *et al.*, 1978; McCulloch *et al.*, 1982) whilst lactate pyruvate and alanine concentrations were normal. Alone amongst the gluconeogenic precursors, glycerol levels were increased, presumably secondary to enhanced lipolysis (Rich *et al.*, 1959; Brodie *et al.*, 1966; Tibbling, 1969; McCulloch *et al.*, 1982). In hypothyroidism blood glucose, lactate, pyruvate, and glycerol levels were normal, as has previously been reported (McCulloch *et al.*, 1981).

Hepatic glucose production measured isotopically has been shown to be increased in hyperthyroidism (Perez *et al.*, 1980; Saunders *et al.*, 1980; McCulloch *et al.*, 1983) and diminished (McCulloch *et al.*, 1983) or normal (Saunders *et al.*, 1980) in hypothyroidism. Such data does not distinguish between glycogenolysis and gluconeogenesis nor does it give information about the capacity of those two processes. In fasting man, glycerol taken up by the liver enters the gluconeogenic pathway and thus clearance of an intravenous glycerol load may provide an estimate of gluconeogenic capacity. Previous studies of gluconeogenesis in patients with thyroid disease are few. In hyperthyroidism, splanchnic balance studies suggest that hepatic uptake of all the major gluconeogenic precursors is increased (Wahren *et al.*, 1981). For lactate, pyruvate, and the alanine this is achieved by an increase in fractional extraction whilst for glycerol, increased arterial concentrations

combined with a 20% increase in hepatic blood flow are responsible. Apart from a single study of congenital hypothyroidism (Hayek, 1979) showing normal gluconeogenesis from from alanine, there have been no estimates of gluconeogenesis in hypothyroid man.

The metabolic clearance rate for glycerol is the best index of glycerol disappearance in the kind of study used here, as its measurement

$$\text{(amount of glycerol administered} / \int_0^\infty \Delta \text{ blood glycerol)}$$

is independent of any descriptive compartmental model and its estimate is valid even if glycerol elimination occurs outside the sampling compartment (Wilkinson & Shand, 1971). The amount of glycerol used in this study was chosen to give peak blood glycerol values between 0·4 and 1·5 mmol/l. This avoided the very high blood glycerol levels which have been reported to cause renal damage in man and animals (Hagnevick *et al.*, 1974; Anderson *et al.*, 1950). Much higher doses than those used in this study have been used therapeutically in man without adverse effects (Senior & Loridan, 1968).

Glycerol clearance rate was increased by 80% in hyperthyroidism and decreased by 30% in hypothyroidism. The mechanism(s) underlying these changes are uncertain. Glycerol taken up by the liver is first phosphorylated by glycerol kinase and enters the gluconeogenic pathway after oxidation by glycerol-3-phosphate dehydrogenase (G3PD). Glycerol kinase is subject to feedback inhibition by glycerol-3-phosphate (Robinson & Newsholme, 1969; Sestoft & Fleron, 1975). In the rat, the activity of mitochondrial G3PD is thyroid hormone dependent (Hoch, 1974). It has long been considered an intracellular marker of thyroid hormone action and alteration of mitochondrial G3PD activity in our subjects is thus a possible explanation of the observed changes in glycerol metabolic clearance rates. However, Nolte *et al.* (1972), in an as yet unconfirmed study, failed to demonstrate any increase in mitochondrial G3PD activity in thyrotoxic man.

Altered hepatic blood flow may also contribute to our findings. A 20% increase in hepatic blood has previously been directly demonstrated in hyperthyroid patients (Wahren *et al.*, 1981). Circulating (albeit venous) glycerol concentrations of glycerol were increased by 70% in our thyrotoxic subjects. Assuming a similar 20% increase in hepatic blood flow, this would provide a 100% increase in hepatic glycerol delivery in hyperthyroidism. Fractional Glycerol extraction need not therefore be increased to account for increased glycerol clearance rates of 80%. This is compatible with the data of Tibbling (1969) who demonstrated that fractional glycerol turnover was not significantly increased in thyrotoxic humans. Similarly, in hypothyroidism cardiac output, stroke volume (Graetinger *et al.*, 1958) and flow through many organs is diminished (Scott *et al.*, 1962; Stewart & Evans, 1942; Zondek, 1967) and may contribute to the decreased glycerol clearance rates reported here. In keeping with the changes in glycerol clearance rates, the fractional rate constants (k_1 and k_2) were approximately doubled in hyperthyroidism and halved in hypothyroidism with appropriate reciprocal changes in the calculated first and second half disappearance times. These changes may result from either haemodynamic factors, primary changes in hepatic glycerol uptake, or a combination of these two factors.

Calculated endogenous glycerol production rates for normal subjects (2·1 μmol/kg/min) were similar to those quoted in other studies using different methods (2·2 μmol/kg/min: Johnston *et al.*, 1983; 1·6 μmol/kg/min: Bortz *et al.*, 1972). Endogenous production rates were increased three-fold in hyperthyroidism and decreased by 50% in

hypothyroidism. These results are entirely consistent with previous studies showing lipolysis to be increased in hyperthyroidism (Rich *et al.*, 1959; Brodie *et al.*, 1982; McCulloch *et al.*, 1982) and decreased in hypothyroidism (McCulloch *et al.*, 1981; Goodman & Bray, 1966).

Volumes of distribution for glycerol, although variable, were not significantly different between the groups suggesting that the changes in glycerol elimination were not secondary to altered distribution space in any patient group.

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COBELLI, C., R. NOSADINI, G. TOFFOLO, A. McCULLOCH, A. AVOGARO, A. TIENGO, AND K. G. M. M. ALBERTI. *Model of the kinetics of ketone bodies in humans.* Am. J. Physiol. 243 (Regulatory Integrative Comp. Physiol. 12): R7–R17, 1982.— The kinetics of ketone bodies was studied in normal humans by giving a combined bolus intravenous injection of labeled acetoacetate ($[^{14}C]$AcAc) and D(−)-β-hydroxybutyrate (β-$[^{14}C]$-OHB) to seven subjects after an overnight fast, on two different occasions, and by collecting frequent blood samples for 100 min. Kinetic data were analyzed with both noncompartmental and compartmental modeling techniques. A four-compartment model, representing AcAc and β-OHB in blood and two equilibrating ketone body compartments, inside the liver and extrahepatic tissues, was chosen as the most reliable mathematical representation; it is physiologically plausible and was able to accurately fit the data. The model permitted evaluation of the in vivo rate of ketone body production in the liver, the individual plasma clearance rates of AcAc and β-OHB, their initial volumes of distribution, and the transfer rate parameters among the four ketone body compartments. Moreover, the model provided estimates of the components of the rates of appearance of AcAc and β-OHB in plasma due to newly synthesized ketone body from acetyl-CoA in the liver, and to interconversion and recycling in the liver and extrahepatic tissues. The model also was used to evaluate other methodologies currently employed in the analysis of ketone body turnover data: the conventional approach based on use of the combined specific activity of AcAc and β-OHB required assumptions not satisfied in vivo, leading to substantial errors in key parameter estimates.

ketone body turnover; acetoacetate; hydroxybutyrate; mathematical modeling; compartmental model; model identification; parameter estimation; tracer kinetics

IN THE POSTABSORPTIVE STATE, ketone bodies play a minor role in supplying energy needs in mammals (35), but in other metabolic conditions ketone bodies are a major metabolic substrate (32). Isotopic techniques provide relatively noninvasive methods for measuring rates of production and utilization of ketone bodies in vivo (11), and infusion of either [3-^{14}C]acetoacetate ($[^{14}C]$-AcAc) or D(−)-β-[3-^{14}C]hydroxybutyrate (β-$[^{14}C]$OHB) has been widely used in animals and in humans (4, 6, 11, 22, 23, 33). However this approach is possibly not without

serious difficulties. Acetoacetate (AcAc) and D(−)-β-hydroxybutyrate (β-OHB) are interconverted too rapidly to be considered independent substrates, but too slowly for their specific activities (SA) to become equal (8). All investigators except Barton (6, 7), Heath and Barton (20), and Bates (10) attempt to circumvent this difficulty using the combined SA of AcAc and β-OHB for quantifying total ketone body (TKB) turnover. But it is not clear whether this approach is valid. It has recently been suggested that this approach may lead to incorrect disposal rates and plasma clearance rates (8).

Despite these unsolved methodological issues TKB kinetics has been investigated both in steady and nonsteady state in a variety of metabolic conditions (3, 5). Moreover conflicting results have been reported concerning several kinetic parameters such as volume of distribution, ranging from 10 to 50% of body weight (5); number of compartments, ranging from 1 to 3 (3, 9, 20, 23), and ratio of plasma clearance rates of AcAc and β-OHB ranging from 1 to 3 (4, 8). Whereas in rats a variety of models have been proposed and quantified in the steady state (6–9, 20), a thorough quantitative analysis of ketone body kinetics has never been performed in man.

Barton (8) recently suggested that either separate injections of $[^{14}C]$AcAc and β-$[^{14}C]$OHB at different times or simultaneous administration of doubly labeled ketone bodies could provide a more accurate means of estimating the individual plasma clearance rates and more generally the kinetics of the two ketone bodies in steady state. In this study we have adopted the first of these two suggestions along with appropriate mathematical modeling techniques. Our results indicate that a four-compartment model can describe adequately the kinetics of AcAc and β-OHB in normal humans after an overnight fast, thus providing a new basis for quantifying ketone body turnover in vivo.

MATERIALS AND METHODS

Subjects

Four male and three female volunteers [46 ± 7 (SE) yr] were each judged normal on the basis of medical history and physical examination. Preliminary blood

tests, which were normal in each subject, included blood urea nitrogen, Na$^+$, Ca^{2+}, blood sugar, total protein, total bilirubin, serum glutamine-oxalacetic transaminase, hemoglobin, and white cell count. All subjects were within 10% of ideal body weight but *subject GB*, who was 21% overweight, according to Metropolitan Life Insurance Table (1967). All subjects followed a normocaloric diet with at least 250 g of carbohydrates in the 4 days before the study. No subject was taking any form of medication and they were all afebrile at the time of the study. No alcohol was consumed in the 24 h before the study. All subjects were advised of the nature, scientific goals, and possible hazards of the studies prior to consenting to participation.

Experimental Procedures

The day of the experiment the subjects were admitted at 7 A.M. to the Metabolic Units after a 12-h overnight fast. Lidocaine [Xylocaine, Astra; 1% weight/volume (wt/vol)] was used to anesthetize the skin and one Venflo cannula (1.1 mm OD) was inserted into an antecubital vein for the 8 A.M. bolus intravenous injection of the tracer. A second cannula (1.1 mm OD) was inserted into a dorsal hand vein of the other arm in a retrograde manner to the most distal site from which sampling was possible, and the hand was placed in a warming chamber at 60°C (18, 27). The cannula was filled with 0.5–0.7 ml of isotonic saline and the first 1 ml of blood sampled was subsequently discarded at each sample time. At 7:30 A.M. a 12-ml blood sample was taken for the radioactivity recovery calculation. Two basal samples (6 ml) were collected just before the tracer was injected. Other 6-ml samples were collected 1, 2, 3, 4, 5, 6, 8, 10, 12.5, 15, 20, 25, 30, 40, 50, 60, 70, 80, and 100 min after the injection of the tracer.

Subjects were asked to urinate before the study, and at the end of the study new urine was collected and immediately neutralized and chilled. The seven subjects were injected the first time with about 50 μCi sodium [^{14}C]AcAc diluted in 10 ml saline. Under the same metabolic and experimental conditions, about 50 μCi of sodium β-[^{14}C]OHB diluted in 10 ml saline was similarly injected. The order of injections was performed randomly. During the experiment, the subjects remained at rest in an armchair.

Preparation of Tracer Infusate

Ethyl[3-^{14}C]acetoacetate (250 μCi, sp act 8.9 mCi/mmol, Radiochemical Center, Amersham, UK) was added to 100 μmol of freshly redistilled carrier ethyl acetoacetate. Hydrolysis was accomplished as described elsewhere (28). After neutralization with 0.2 M HCl and addition of 5 ml of saline, the solution was washed 6 times with 15 ml of diethylether and treated for 45 min with a stream of N$_2$, keeping the solution in crushed ice. To check whether acetone was still present in the solution, 5 μCi of [2-^{14}C]acetone (sp act 59 mCi/mmol, Radiochemical Centre, Amersham, UK) were added to 5 ml of saline and passed through the steps cited above. No residual radioactivity was found in the solution after such treatment. The radiochemical purity of sodium [3-^{14}C]-

acetoacetate was determined in each experiment (2: and was between 92 and 96% (28) after extraction wit 10 ml ether 6 times.

D-β-[3-^{14}C]OHB (250 μCi) (sp act 56 mCi/mmol, Ra diochemical Center, Amersham, UK) were diluted wit 25 ml of saline containing 100 μmol of DL-sodium β-OHI Labeled AcAc and β-OHB solutions were passed twic through a Millipore filter (0.22 μm), kept frozen in steri syringes at −20°C, and used within 7 days. The concer tration of cold carrier AcAc and β-OHB was determine in the tracer solution of each subject (28). The mas magnitude of both carriers was about 15–20 μmol, bot in the linear perturbation range.

Analytic Procedures

Each 6-ml blood sample was immediately and thor oughly mixed with 5 ml 20% (wt/vol) perchloric aci kept in crushed ice until the end of the study, an neutralized with 20 and 5% (wt/vol) KOH. The radioat tivity concentrations of [^{14}C]AcAc and of β-[^{14}C]OHI and the cold AcAc and β-OHB concentrations were de termined as described elsewhere (28). Neither [^{14}C]ace tone nor acetone was measured. Recoveries of [^{14}C]AcA and β-[^{14}C]OHB were determined within each set c analyses by adding portions of diluted infusates to whol blood. Recoveries of [^{14}C]AcAc were 82–87% when co rected for radiochemical purity and 79–86% for β-[^{14}C OHB. Efficiency of the liquid-scintillation counting pro cedure was determined by use of [^{14}C]toluene as a external standard, and each sample count was thus co rected for quenching. The SA of AcAc and β-OHB i urine was measured in the same manner as in blood. A analyses were performed in duplicate within 8 h after th end of blood sample collection.

Data Analysis

Two linear modeling methods were used to account fc the kinetic data of labeled AcAc and β-OHB after injec tion of [^{14}C]AcAc and β-[^{14}C]OHB, namely th "noncompartmental" (or "model independent") and th compartmental modeling approach (14) (see MODELIN STUDIES. RATIONALE AND RESULTS).

Noncompartmental analysis was carried out as pro posed by Rescigno and Gurpide (34) and the computa tional aspects of the method are briefly summarized i the APPENDIX. This approach extracts information esser tially from the areas under the experimental curves with out proposing a specific model structure. A number c assumptions are however necessary to carry out th analysis (APPENDIX). In this study these were *1*) extrap olation to $t \to \infty$ was done through an exponential func tion determined from the last four data points; *2*) extrap olation to $t \to 0$ and initial slope were obtained throug an exponential function determined from the first thre points; *3*) linear interpolation between data points wa performed.

Compartmental modeling involves the assumption c a specific model structure and its numerical quantifica tion from the experimental data. A variety of physiolo ically based linear compartmental models of ketone bod kinetics was examined. The general theoretical modelir

framework and the identification/validation techniques employed in this study are described in Cobelli et al. (16). More specifically a priori identifiability of the various models has been examined according to techniques described by Cobelli and DiStefano (15). Parameter values of the models were estimated, using all available a priori constraints, using a nonlinear weighted least-squares parameter estimation technique (17). Errors in measurements of labeled AcAc and β-OHB concentrations were assumed to be uncorrelated and Gaussian, with zero mean and an experimentally determined standard deviation. The coefficient of variation of the data was inversely proportional to the square root of the measurements; a typical mean value of the range was 5%. Parameter accuracy estimates were obtained from the Fisher information matrix, the inverse of which is a lower bound on the parameter covariance matrix (19). The practical applicability of this Fisher information matrix approach was tested using Monte Carlo simulation techniques (14). Goodness-of-fit was expressed in terms of the weighted residual sum of squares of the differences between observed and predicted values (1). The Akaike information criterion (2) also was used to account for the relative contributions of goodness-of-fit and the number of parameters in evaluating the competing mathematical descriptions.

EXPERIMENTAL RESULTS

Fourteen experiments were used in our kinetic modeling studies, involving seven injections of [^{14}C]AcAc and seven of β-[^{14}C]OHB. In all subjects both the iv bolus injection of labeled AcAc and β-OHB were performed in random order on two different occasions. The mean concentrations of cold AcAc and β-OHB during each experiment are shown in Table 1. A steady-state condition was met during each experiment, and good experimental control of the subjects was obtained so that it could be reasonably assumed that the same (within 1 SD) steady state was achieved in each of the two experiments performed in each subject. A "mean steady state," c_1, c_2, was used in subsequent computations. A number of representative studies are shown in Fig. 1. For each subject the measured time courses of labeled AcAc and β-OHB after the two injections are shown. Labeled AcAc and β-OHB were not detectable in the urine of two subjects (*ED* and *GB*), whereas in the other five, it averaged 1.2 ± 0.3 (SE) % of the injected dose.

TABLE 1. *Steady-state values of unlabeled* $AcAc(c_1)$ and β-OHB(c_2)

Subj	μmol/ml			
	c_1	c_1	c_2	c_2
ED	0.036±0.006	0.027±0.010	0.046±0.010	0.057±0.003
GB	0.044±0.013	0.036±0.007	0.028±0.011	0.024±0.003
CF	0.048±0.011	0.073±0.018	0.108±0.042	0.106±0.028
EM	0.121±0.039	0.151±0.076	0.221±0.061	0.216±0.050
MS	0.140±0.059	0.116±0.049	0.113±0.054	0.147±0.044
AG	0.134±0.020	0.112±0.020	0.246±0.048	0.345±0.106
LT	0.115±0.032	0.104±0.026	0.238±0.038	0.250±0.059

Values are means ± SD of concentrations determined at each sampling time after labeled AcAc and β-OHB injection.

MODELING STUDIES. RATIONALE AND RESULTS

A Noncompartmental Analysis

The availability of data obtained from experiments involving intravenous tracer injection of AcAc and β-OHB and measurement of concentrations in blood of labeled AcAc and β-OHB allow estimation of various parameters of ketone body metabolism by the "two accessible pools" approach proposed in Rescigno and Gurpide (34). The approach is often referred to as noncompartmental, as it does not require a compartmental structure, i.e., only the two accessible compartments are evidenced (blood AcAc and blood β-OHB), embedded in a network of an undetermined number of connected compartments.

The model is shown in Fig. 2. A similar structure has been originally quantified in the rat, using a somewhat different set of experimental conditions, by Barton and colleagues (6, 7). We have quantified this model in the seven humans from our two input-four output data sets

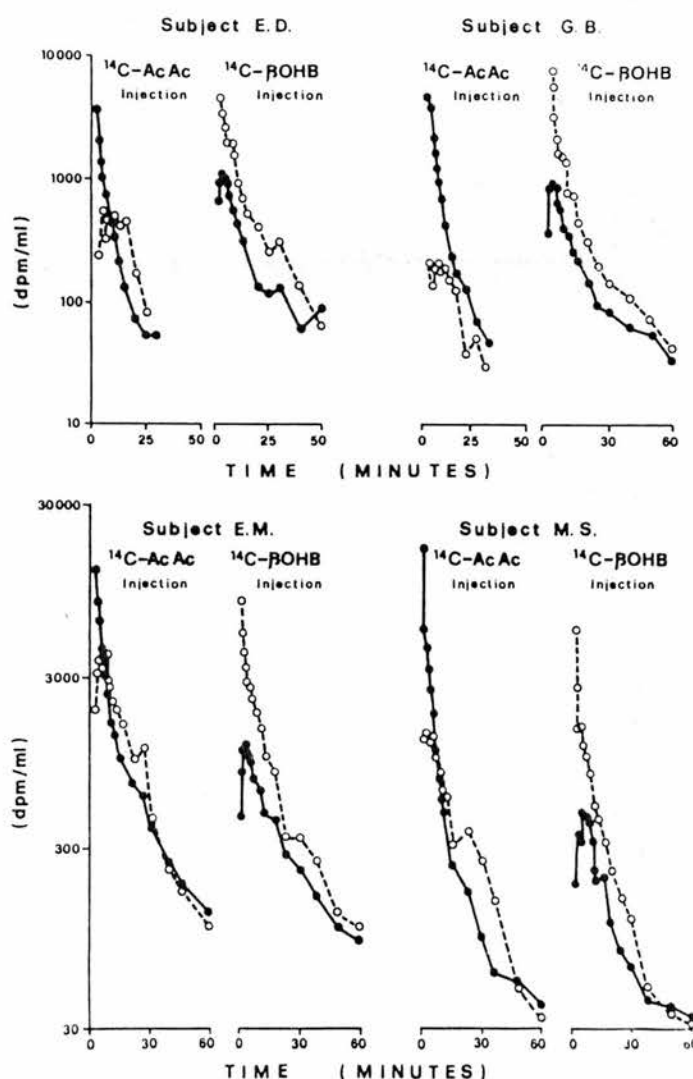FIG. 1. Blood concentration of labeled acetoacetate and D-β-hydroxybutyrate in 4 subjects following tracer injection of acetoacetate ([^{14}C]AcAc) and D-β-hydroxybutyrate (β-[^{14}C]OHB). ●——●, Blood concentration of [^{14}C]AcAc; ○——○, blood concentration of β-[^{14}C]OHB.

(APPENDIX), and the estimated parameters of AcAc and β-OHB metabolism are reported in Table 2. These include: the rate of appearance (Ra$_1$, Ra$_2$) of AcAc and β-OHB in plasma, decomposed into their components due to recycling (Ra$_{1r}$, Ra$_{2r}$), interconversion (Ra$_{1i}$, Ra$_{2i}$), and de novo synthesis (Ra$_{1n}$, Ra$_{2n}$); the compartment sizes (Q$_1$, Q$_2$) of AcAc and β-OHB; and the plasma clearance rates (PCR$_1$, PCR$_2$) of AcAc and β-OHB per se, i.e., not due to interconversion (8). While providing a quantitative picture of ketone body metabolism, this approach implies a number of structural and computational assumptions that are not necessarily valid (13). Moreover, the approach does not make full use of the information available from kinetic studies. This led us to propose more detailed, physiologically based models.
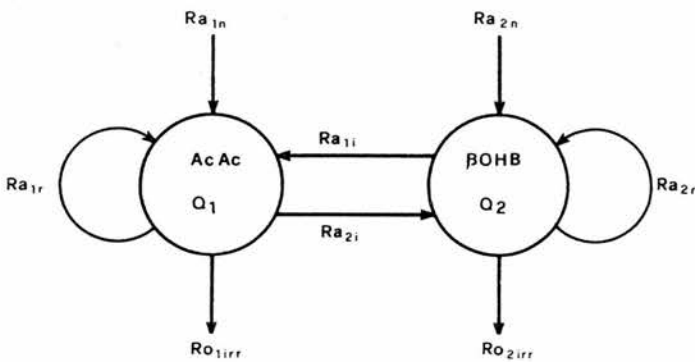

FIG. 2. Model structure adopted for noncompartmental analysis of AcAc and β-OHB kinetics. AcAc and β-OHB accessible pools are represented embedded in a multicompartment system. Ra$_{1n}$, Ra$_{2n}$ denote the de novo synthesized AcAc and β-OHB appearing in blood; Ra$_{1r}$, Ra$_{2r}$ rate of AcAc and β-OHB recycling; Ra$_{1i}$, Ra$_{2i}$ rate of interconversion of 2 ketones; R$_{01irr}$, R$_{02irr}$ their irreversible rate of removal, and Q$_1$, Q$_2$ the AcAc and β-OHB compartment sizes.

Compartmental Modeling

The use of a structured compartmental approach has definite advantages when the purpose of modeling is understanding of the system (14). A schematic of the biochemical pathways of ketogenesis is shown in Fig. 3, and the specific interactions between AcAc and β-OHB are indicated in Fig. 4. We have examined, starting with the basic structure of Fig. 4, a number of physiologically based linear compartmental models. The six-compartment model was too complex to be resolved from our kinetic data (unpublished observations).

A four-compartment model. Evidence exists that AcAc and β-OHB rapidly equilibrate within the liver, and also inside extrahepatic tissues (24, 29). Therefore the four-compartment model of Fig. 5 was considered. This model depicts the two individual ketones in blood and assumes the existence of two other compartments (liver and extrahepatic tissues), where the two ketone bodies interconvert very rapidly. This configuration is consistent (16) with the experimental finding of isotopic disequilibrium following infusion (or injection) in blood of labeled ketones (26) as the tracer enters in a compartment different from blood, which is the accessible pool for test input and measurement. The equations of the model are

$$\mathring{q}_1(t) = -(k_{31} + k_{41})q_1(t) + k_{13}q_3(t) + k_{14}q_4(t) + u_1(t), \qquad q_1(0) = 0 \tag{1}$$

$$\mathring{q}_2(t) = -(k_{32} + k_{42})q_2(t) + k_{23}q_3(t) + k_{24}q_4(t) + u_2(t), \qquad q_2(0) = 0 \tag{2}$$

$$\mathring{q}_3(t) = k_{31}q_1(t) + k_{32}q_2(t) - (k_{13} + k_{23})q_3(t), \qquad q_3(0) = 0 \tag{3}$$

TABLE 2. *Rate of appearance of AcAc(Ra$_1$) and of β-OHB(Ra$_2$) in plasma*

Subj	μmol·min^{-1}·m^{-2}				μmol·min^{-1}·m^{-2}				mmol·m^{-2}		ml·min^{-1}·m^{-2}	
	Ra$_1$	Ra$_{1r}$	Ra$_{1i}$	Ra$_{1n}$	Ra$_2$	Ra$_{2r}$	Ra$_{2i}$	Ra$_{2n}$	Q$_1$	Q$_2$	PCR$_1$	PCR$_2$
ED	68.93	11.27	22.98	34.68	115.31	43.32	13.93	58.06	0.140	0.151	1410.5	951.7
GB	57.99	8.54	8.71	40.74	35.78	11.35	5.41	19.02	0.163	0.081	1100.8	605.0
CF	98.68	16.70	41.54	40.44	122.18	13.51	5.84	102.83	0.276	0.617	1258.4	627.5
EM	354.50	47.22	178.22	129.06	467.74	141.71	161.65	164.38	1.048	1.276	1070.8	676.5
MS	409.61	27.86	101.67	280.07	346.11	119.24	115.21	111.66	1.360	0.499	2082.3	963.1
AG	410.16	67.98	243.47	98.70	613.97	200.63	138.86	274.48	1.170	0.700	1653.0	574.9
LT	331.11	68.52	173.25	89.34	474.51	163.58	95.51	215.41	0.931	0.539	1525.8	564.3
Mean	247.28	35.44	109.98	101.86	310.80	99.05	76.63	135.12	0.726	0.552	1443.1	709.0
±SD	±163.87	±25.85	±90.42	±86.30	±221.37	±76.18	±67.03	±89.19	±0.518	±0.395	±353.6	±173.6

Components due to recycling (Ra$_{1r}$, Ra$_{2r}$), to interconversion (Ra$_{1i}$, Ra$_{2i}$), and to newly synthetized ketone bodies (Ra$_{1n}$, Ra$_{2n}$); compartment pool sizes (Q$_1$, Q$_2$) and plasma clearance rates (PCR$_1$, PCR$_2$) of AcAc and β-OHB are shown (cf. model structure of Fig. 1).
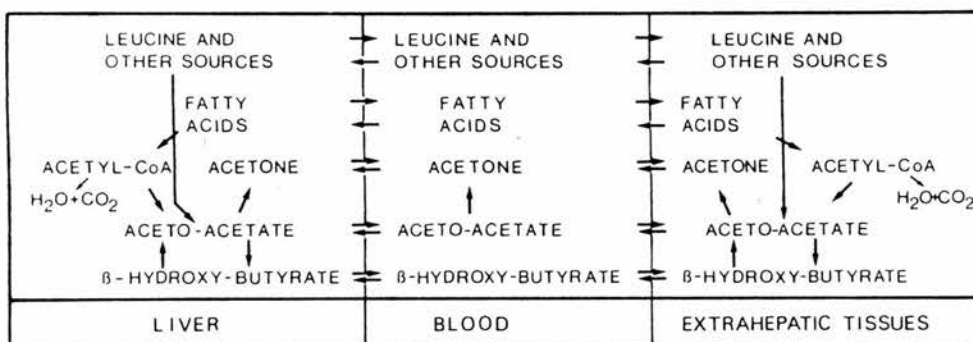

FIG. 3. Biochemical pathways of ketone body metabolism in whole animal.

$$\dot{q}_4(t) = k_{41}q_1(t) + k_{42}q_2(t) - (k_{14} + k_{24} + k_{04})q_4(t), \tag{4}$$
$$q_4(0) = 0$$

$$y_1(t) = \frac{1}{V_1} q_1(t) \tag{5}$$

$$y_2(t) = \frac{1}{V_2} q_2(t) \tag{6}$$

$$y_3(t) = \frac{1}{V_1} q_1(t) \tag{7}$$

$$y_4(t) = \frac{1}{V_2} q_2(t) \tag{8}$$

where $q_i(t)$ is the amount of tracer in the ith compartment; $u_1(t)$, $u_2(t)$ are the impulsive inputs of [^{14}C]AcAc and β-[^{14}C]OHB, respectively; $y_1(t)$, $y_2(t)$ are the measurements (outputs) of labeled AcAc and β-OHB concentration after [^{14}C]AcAc injection, and $y_3(t)$, $y_4(t)$ are those after β-[^{14}C]OHB injection; k_{ij} are the nonnegative transfer rate parameters, and V_1, V_2 are the initial distribution volumes of the two ketones.

The unknown parameters of this model are the distribution volumes, V_1, V_2, and the transfer rate parameters, k_{ij}. This model is uniquely identifiable (15); parameter estimates and their accuracies are shown in Table 3. The four-compartment model exhibits very good performance
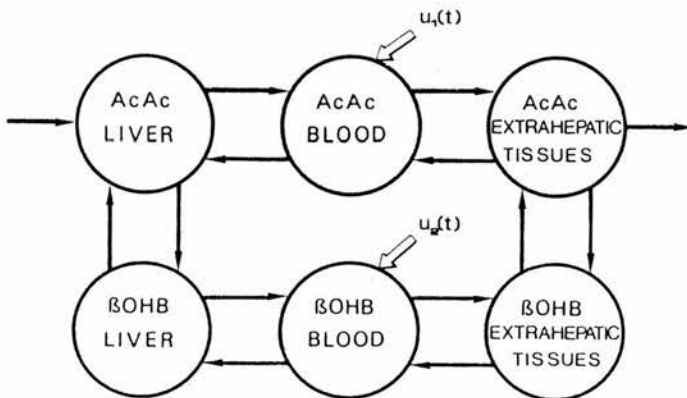
in terms of quality of identification results (i.e., parameter accuracy). It is of interest to note that, in four cases, a negligible recycling of either AcAc (k_{14}) or β-OHB (k_{24}) resulted, from extrahepatic tissues back to blood.

A three-compartment model. Negligible recycling of both AcAc and β-OHB from extrahepatic tissues back to plasma has been reported in the rat (10, 21, 26). We have tested this possibility with the model, i.e., parameters k_{14} and k_{24} were both set equal to zero, thus leading to the three-compartment model of Fig. 6 (16). This model is uniquely identifiable (15), is consistent with the isotopic disequilibrium evidence (26), and has already been proposed for the rat (10). The identification results for this model are shown in Table 4. For each subject very accurate parameter estimates were obtained, and it is of interest to note that this model provides, for those individuals with negligible recycling from extrahepatic tissues (*subj ED, MS, AG,* and *LT* of Table 3), parameter values in good agreement with the corresponding parameters of the four-compartment model.

Comparison of the four- with the three-compartment model. The identification results support well the point
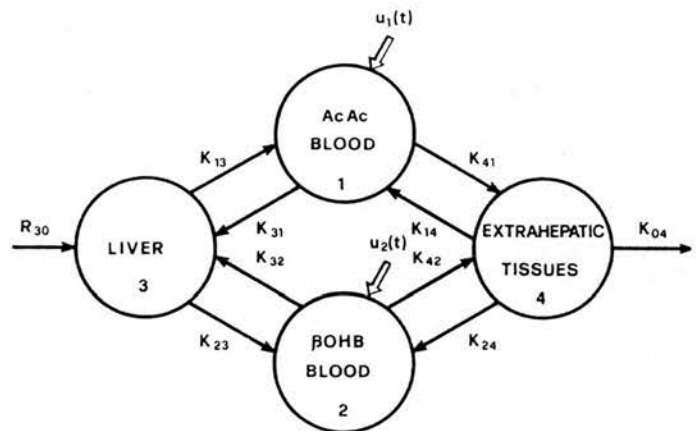

FIG. 5. A 4-compartment model. This model, besides taking into account acetoacetate and β-hydroxybutyrate in blood, depicts 2 ketone body compartments, liver and extrahepatic tissues. It is assumed that in liver (*compartment 3*) and extrahepatic tissues (*compartment 4*), the 2 ketones equilibrate rapidly. R_{30} is rate of production of acetoacetate and β-hydroxybutyrate inside liver; k_{31}, k_{13}, k_{41}, k_{14}, k_{32}, k_{23}, k_{42}, k_{24}, and k_{04} are transfer rate parameters and $u_1(t)$, $u_2(t)$ as in legend to Fig. 4.

FIG. 4. A 6-compartment model of AcAc and β-OHB kinetics. $u_1(t)$, $u_2(t)$ are tracer injection inputs of [^{14}C]AcAc and β-[^{14}C]OHB.

TABLE 3. *Numerical estimates and their accuracies of initial distribution volumes and transfer rate parameters of four-compartment model of Fig. 5*

Subj	1/m²		min⁻¹								
	V_1	V_2	k_{41}	k_{31}	k_{13}	k_{23}	k_{32}	k_{42}	k_{14}	k_{24}	k_{04}
ED	3.2 (8)*	2.1 (4)	0.464 (6)	0.200 (6)	0.056 (6)	0.114 (6)	0.529 (4)	0.527 (4)	0.282 (38)	0.†	2.546 (43)
GB	3.9 (4)	3.2 (3)	0.387 (4)	0.083 (10)	0.050 (10)	0.029 (10)	0.117 (8)	0.316 (4)	1.073 (48)	0.288 (52)	3.360 (52)
CF	3.8 (10)	3.7 (6)	0.508 (10)	0.016 (28)	0.013 (14)	0.028 (14)	0.097 (8)	0.234 (6)	0.120 (10)	0.017 (10)	0.263 (8)
EM	6.4 (3)	3.0 (4)	0.356 (6)	0.138 (10)	0.106 (6)	0.137 (6)	0.534 (6)	0.467 (8)	0.233 (12)	0.531 (12)	0.721 (18)
MS	3.4 (6)	1.6 (4)	0.450 (4)	0.256 (4)	0.090 (6)	0.080 (6)	0.697 (4)	0.749 (4)	0.	0.072 (36)	0.925 (36)
AG	3.7 (5)	2.4 (4)	0.548 (4)	0.332 (6)	0.034 (13)	0.106 (13)	0.498 (4)	0.455 (7)	0.157 (11)	0.	0.306 (13)
LT	2.7 (6)	1.8 (5)	0.513 (3)	0.395 (6)	0.049 (10)	0.120 (10)	0.652 (4)	0.495 (9)	0.075 (17)	0.	0.337 (25)
Mean	3.9	2.5	0.461	0.203	0.050	0.088	0.446	0.463	0.277	0.130	1.204
±SD	±1.2	±0.8	±0.070	±0.135	±0.037	±0.044	±0.242	±0.164	±0.363	±0.205	±1.242

* Accuracy of parameter estimates expressed as % coefficient of variation in parentheses, i.e., (standard deviation of parameter estimate/parameter estimate) × 100.   † In four cases the parameter was set equal to zero (k_{24} in *ED, AG,* and *LT*; k_{14} in *MS*) as suggested by the identification results of the complete model [estimated values were very small and inaccurate, respectively 0.001 (4,096), 0.007 (368), 0.009 (234), and 0.001 (2,082)].

that the four-compartment model, which is also more plausible in terms of physiological structure, is a robust representation of ketone bodies metabolism in vivo. When significant recycling of AcAc and β-OHB from extrahepatic tissues are present, the model is able to detect them. Also, from the results shown in Table 5, the four-compartment model emerges as the best representation, as it exhibits significantly lower values of AIC, the Akaike criterion for model discrimination (2). The ability of the four-compartment model to predict the kinetic data in two subjects is illustrated in Fig. 7.

Use of the four-compartment model for quantifying in vivo ketone body metabolism. Besides providing estimates of the initial distribution volumes and of the transfer rate parameters, the four-compartment model allows estimation of a number of important parameters of ketone body metabolism.

In addition to the sizes of the AcAc and β-OHB blood compartments, given by
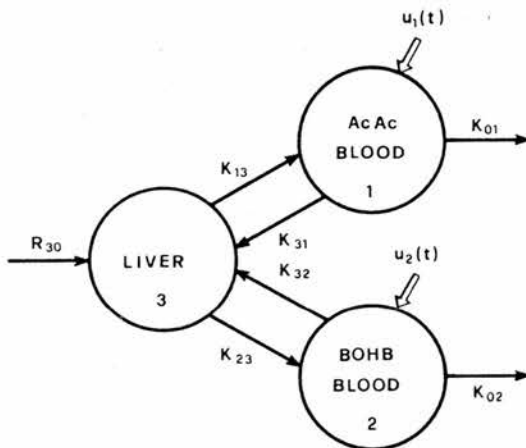
$$Q_1 = c_1 V_1 \tag{9}$$

FIG. 6. A 3-compartment model. In this kinetic representation, besides blood ketone body compartments, a rapidly equilibrating liver compartment for acetoacetate and β-hydroxybutyrate is assumed. R_{30}, $u_1(t)$, $u_2(t)$ as in legend to Fig. 5; k_{13}, k_{31}, k_{23}, k_{32}, k_{01} are transfer rate parameters. This model is a direct simplification of 4-compartment model (Fig. 5) with $k_{14} = 0$ and $k_{24} = 0$. Also, as parameter k_{04} is not identifiable it cannot be explicitly considered. Note that parameters k_{41} and k_{42} of Fig. 5 are indicated here as k_{01} and k_{02}.

$$Q_2 = c_2 V_2 \tag{10}$$

the steady-state *Eqs. 1–8* give the extrahepatic compartment size (Q_4), the rate of production of ketone bodies inside the liver (R_{30}), and the liver compartment size (Q_3)

$$Q_4 = \frac{k_{41}Q_1 + k_{42}Q_2}{k_{14} + k_{24} + k_{04}} \tag{11}$$

$$R_{30} = k_{04}Q_4 \tag{12}$$

$$Q_3 = \frac{k_{31}Q_1 + k_{32}Q_2 + R_{30}}{k_{13} + k_{23}} \tag{13}$$

Also, the plasma clearance rates PCR_1 and PCR_2, of AcAc and β-OHB per se, are

$$PCR_1 = \frac{k_{41}k_{04}}{k_{14} + k_{24} + k_{04}} V_1 \tag{14}$$

$$PCR_2 = \frac{k_{42} \cdot k_{04}}{k_{14} + k_{24} + k_{04}} V_2 \tag{15}$$

The model also provides an estimate of the true total distribution volume, V_D, of TKB. The total mass of AcAc plus β-OHB present in vivo is known and thus the true total distribution volume of TKB is given by

$$V_D = \frac{Q_1 + Q_2 + Q_3 + Q_4}{c_1 + c_2} \tag{16}$$

Finally, the model provides some insight into the relative role of liver and extrahepatic tissues in the interconversion and recycling of AcAc and β-OHB. The rates of appearance in plasma of AcAc, Ra_1, and β-OHB, Ra_2 (Fig. 1), can be split into their liver components (Ra_1^L, Ra_2^L) and extrahepatic tissue components (Ra_1^T, Ra_2^T). More precisely

$$
\begin{aligned}
Ra_1 &= Ra_1^L + Ra_1^T = Ra_{1r}^L + Ra_{1i}^L + Ra_{1n}^L + Ra_{1r}^T \\
&\quad + Ra_{1i}^T \\
&= Q_1 \frac{k_{13}k_{31}}{k_{13} + k_{23}} + Q_2 \frac{k_{13}k_{32}}{k_{13} + k_{23}} + \frac{R_{30}k_{13}}{k_{13} + k_{23}} \\
&\quad + \frac{k_{14}k_{41}}{k_{14} + k_{24} + k_{04}} Q_1 + \frac{k_{14}k_{42}}{k_{14} + k_{24} + k_{04}} Q_2
\end{aligned} \tag{17}
$$

TABLE 4. *Numerical estimates and their accuracies of initial distribution volumes and transfer rate parameters of three-compartment model of Fig. 6*

| Subj | \multicolumn{2}{c}{l/m^2} | \multicolumn{6}{c}{min^{-1}} | | | | | |
	V_1	V_2	k_{01}	k_{31}	k_{13}	k_{23}	k_{32}	k_{02}
ED	1.6 (6)*	1.8 (3)	0.637 (4)	0.272 (6)	0.056 (4)	0.136 (4)	0.669 (4)	0.575 (4)
GB	3.3 (4)	2.7 (4)	0.327 (4)	0.155 (8)	0.146 (4)	0.082 (4)	0.225 (4)	0.294 (4)
CF	5.3 (4)	3.0 (10)	0.278 (4)	0.036 (6)	0.109 (6)	0.174 (6)	0.226 (8)	0.271 (12)
EM	4.5 (3)	3.1 (3)	0.232 (4)	0.513 (4)	0.167 (4)	0.223 (4)	0.676 (4)	0.223 (4)
MS	3.0 (4)	1.7 (6)	0.441 (4)	0.309 (4)	0.078 (4)	0.080 (4)	0.645 (4)	0.647 (4)
AG	4.8 (4)	3.0 (4)	0.288 (4)	0.288 (4)	0.324 (4)	0.055 (4)	0.114 (4)	0.573 (4)	0.264 (4)
LT	3.3 (4)	1.9 (4)	0.368 (4)	0.387 (4)	0.061 (4)	0.121 (4)	0.739 (4)	0.392 (4)
Mean	3.7	2.5	0.367	0.285	0.096	0.133	0.536	0.381
±SD	±1.3	±0.6	±0.137	±0.155	±0.046	±0.051	±0.218	±0.167

* Accuracy of parameter estimates expressed as % coefficient of variation in parentheses, i.e., (standard deviation of parameter estimate/ parameter estimate) × 100.

$$Ra_2 = Ra_2^L + Ra_2^T = Ra_{2r}^L + Ra_{2i}^L + Ra_{2n}^L + Ra_{2r}^T + Ra_{2i}^T$$

$$= Q_2 \frac{k_{23}k_{32}}{k_{13}+k_{23}} + Q_1 \frac{k_{23}k_{31}}{k_{13}+k_{23}} + \frac{R_{30}k_{23}}{k_{13}+k_{23}} \qquad (18)$$

$$+ \frac{k_{24}k_{42}}{k_{14}+k_{24}+k_{04}} Q_2 + \frac{k_{24}k_{41}}{k_{14}+k_{24}+k_{04}} Q_1$$

In these equations L and T denote liver and extrahepatic

TABLE 5. *Akaike Information Criterion (AIC) for four- and three-compartment models*

Subj	½ AIC	
	Three-compartment model	Four-compartment model
ED	200.5	192.4
GB	231.8	222.8
CF	205.8	189.9
EM	273.7	259.8
MS	248.2	248.3
AG	267.8	257.8
LT	258.2	252.3

tissues, respectively; Ra_{1r}, Ra_{2r} are the fractions of AcAc and β-OHB appearing in plasma due to recycling; Ra_{1i}, Ra_{2i} are the fractions of AcAc and β-OHB appearing in plasma due to their interconversion; and Ra_{1n}, Ra_{2n} are the fractions of AcAc and β-OHB appearing in plasma from newly synthetized AcAc in the liver. Tables 6 and 7 show the numerical values of all these parameters for the seven normal humans.

It is of interest to compare the parameter values obtained from the noncompartmental approach and the four-compartment model (Tables 2, 6, and 7). The differences are almost entirely attributable to the different estimates that the two approaches provide for the initial distribution volumes (V_1, V_2) and the initial fractional rates of disappearance of labeled AcAc and β-OHB, $\mathring{y}_1(0)/y_1(0)$ and $\mathring{y}_4(0)/y_4(0)$; see APPENDIX. These are shown in Table 8. It appears that V_1 and V_2 are about the size of the blood volume in the four-compartment model.

DISCUSSION

The present study was undertaken to quantify ketone body metabolism in the normal human in steady state. This particular study has never been performed, although
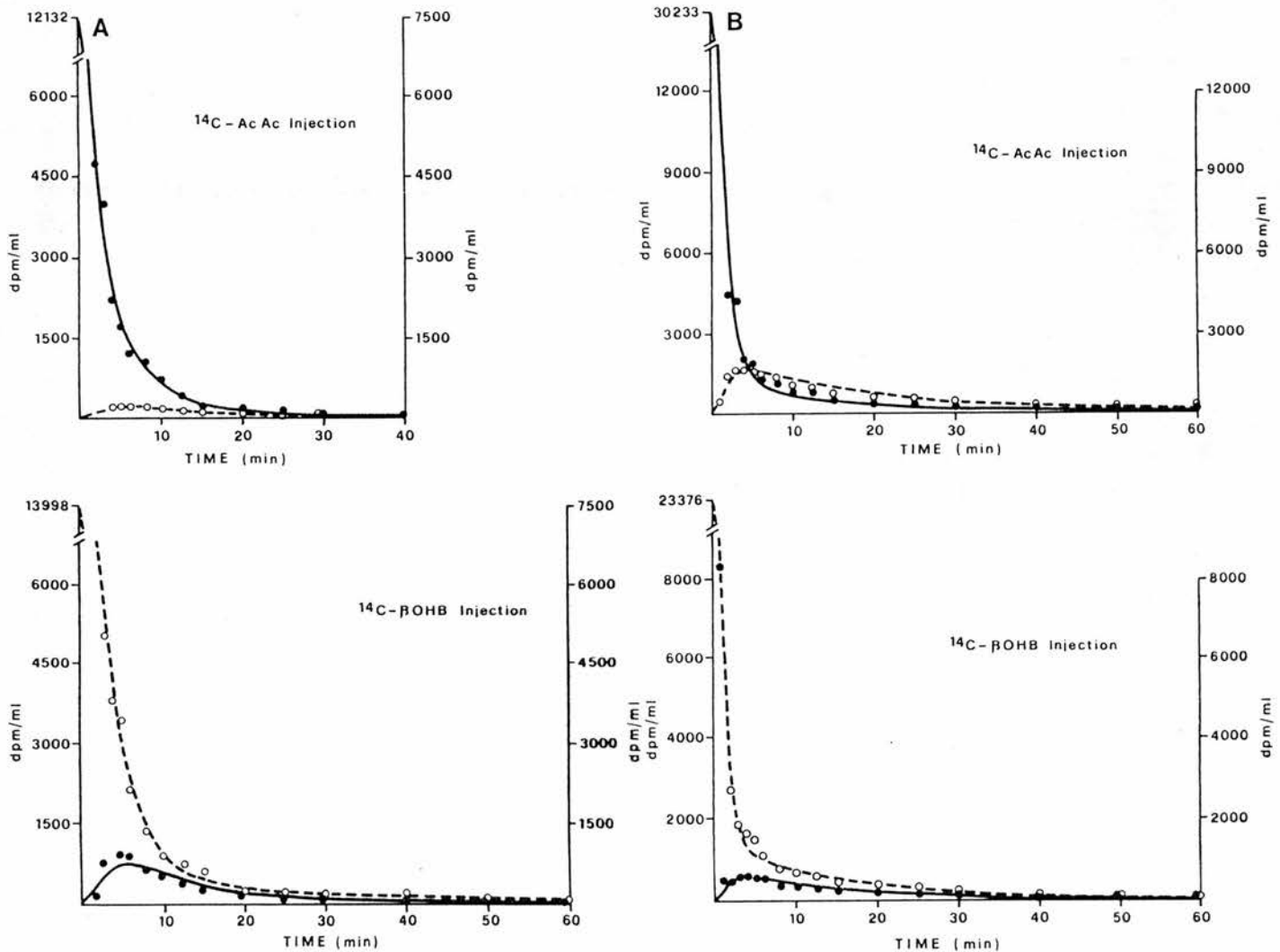

FIG. 7. Predictions of 4-compartment model for labeled acetoacetate (*continuous line*) and for labeled β-hydroxybutyrate (*dashed line*) concentrations in blood in 2 representative studies (A and B). Experimental data of [^{14}C]AcAc (●) and β-[^{14}C]OHB (○) are also shown.

ketone body kinetics have been investigated in a variety of other ways, both in steady and nonsteady states. We were prompted by wide discrepancies reported by several investigators for several parameters, such as initial and total distribution volumes, plasma clearance rates, and number and definition of ketone body compartments (3, 6, 9, 22, 23, 33). Another motivation was to investigate the validity of the generally accepted use of the combined SA of TKB, because, as stated recently (8), there is no proof that this mode of calculation is correct. It has been demonstrated in dogs in steady state that TKB turnover determined using TKB combined SA is, on the average, not too different from values obtained by hepatic catheterization (22). Because of the impossibility in the human of sampling in portal vein to obtain true portal vein/hepatic vein differences, we decided to circumvent this difficulty using a less invasive technique, injection of labeled AcAc and β-OHB in the same subjects, together with careful kinetic modeling. For each subject the time course of the four labeled AcAc and β-OHB concentrations after injection of both tracers have been simultaneously considered and used to develop what we propose as an adequate mathematical representation of ketone body metabolism. We have shown the four-compartment model to be an appropriate mathematical representation for studying the individual kinetics of AcAc and β-OHB in vivo: it is plausible from a physiological point of view, it is able to accurately predict the kinetic data, and its parameters can be accurately estimated. This model provides new quantitative insight into ketone body metabolism, allowing a number of variables and parameters to be estimated otherwise unaccessible to direct measurement.

Both noncompartmental and compartmental approaches have been evaluated. Noncompartmental modeling was carried out by the "two accessible pools" analysis proposed in Resigno and Gurpide (34). We have quantified this model (Fig. 1) from the four sets of labeled AcAc and β-OHB data. A similar model was proposed for the rat by Barton (6, 7), who used a different kinetic experiment to quantify it. Whereas this approach provides quantitative insight into the individual kinetics of AcAc and β-OHB, it implies a number of structural and computational assumptions, which are not necessarily valid (13). Particularly critical in this study were the required extrapolation to $t \rightarrow 0$ of the disappearance curves of labeled AcAc and β-OHB, and the computation of their derivatives, thus confirming an intrinsic computational difficulty of the noncompartmental method noted in the original paper (34). The intrinsic ambiguity in these parameter estimates (Table 8) affects the estimation of all the parameters, but particularly the rate of recycling and the compartment sizes, which depend

TABLE 6. *Rate of production of ketone bodies inside liver (R_{30}), compartment sizes (Q_1, Q_2, Q_3, Q_4), plasma clearance rate of AcAc and β-OHB per se (PCR_1, PCR_2), and true total distribution volume (V_D) of AcAc plus β-OHB*

Subj	R_{30}, μmol· min^{-1}· m^{-2}	Q_1	Q_2	Q_3	Q_4	PCR_1	PCR_2	V_D, l/m^2
		mmol/m^2				ml·min^{-1}·m^{-2}		
ED	93.64	0.101	0.109	1.006	0.037	1,356.3	1,001.8	15.27
GB	61.36	0.156	0.083	1.068	0.018	1,063.9	723.2	20.01
CF	137.35	0.231	0.391	4.349	0.521	1,276.3	562.0	32.69
EM	295.68	0.862	0.646	3.133	0.410	1,096.7	670.6	11.80
MS	320.86	0.429	0.204	3.379	0.347	1,400.3	1,089.3	16.90
AG	376.14	0.458	0.697	6.286	1.229	1,352.5	709.9	20.74
LT	304.74	0.301	0.441	4.221	0.905	1,151.7	732.1	16.62
Mean	227.11	0.363	0.367	3.349	0.495	1,242.5	784.1	19.15
±SD	±125.88	±0.257	±0.247	±1.876	±0.442	±136.9	±189.2	±6.67

TABLE 7. *Rate of appearance of AcAc (Ra_1) and β-OHB (Ra_2) in plasma due to liver (Ra_1^L, Ra_2^L) and extrahepatic tissues (Ra_1^T, Ra_2^T)*

Subj	Ra_1^L / Ra_1^T	Ra_{1r}^L / Ra_{1r}^T	Ra_{1i}^L / Ra_{1i}^T	Ra_{1n}^L	Ra_2^L / Ra_2^T	Ra_{2r}^L / Ra_{2r}^T	Ra_{2i}^L / Ra_{2i}^T	Ra_{2n}^L
	μmol·min^{-1}·m^{-2}				μmol·min^{-1}·m^{-2}			
ED	56.50	6.65	18.98	30.87	114.88	38.58	13.53	62.77
	10.39	4.67	5.72		0.	0.	0.	
GB	53.01	8.09	6.15	38.76	30.90	3.59	4.72	22.59
	19.62	13.61	6.01		5.27	1.62	3.66	
CF	58.56	1.25	12.37	44.95	120.39	25.42	2.56	92.40
	62.63	35.21	27.42		8.83	3.87	4.96	
NM	330.66	51.77	150.18	128.72	428.90	194.79	67.15	166.96
	95.50	48.18	47.33		217.89	107.97	109.92	
GS	303.05	58.10	75.16	169.79	269.63	66.87	51.69	151.07
	0.	0.	0.		25.09	11.07	14.02	
AG	211.30	36.76	83.72	90.82	663.79	262.99	115.48	285.32
	192.37	85.08	107.29		0.	0.	0.	
LT	205.06	34.25	82.98	87.83	506.45	204.94	84.60	216.91
	68.07	28.17	39.90		0.	0.	0.	
Mean	174.02	28.12	61.36	84.53	304.99	113.88	48.53	142.57
±SD	±119.25	±22.93	±52.11	±51.34	±234.97	±104.04	±43.58	±91.15
Mean	64.08	30.70	33.38		36.73	17.79	18.94	
±SD	±66.37	±29.44	±37.34		±80.38	±39.96	±40.43	

Rates are decomposed into their components due to recycling (r), interconversion (i) and de novo synthesis (n).

TABLE 8. *Initial distribution volumes of AcAc (V_1) and β-OHB (V_2) and initial fractional rate of disappearance of AcAc [$\dot{y}_1(0)/y_1(0)$] and β-OHB [$\dot{y}_4(0)/y_4(0)$] estimated by noncompartmental and compartmental approach*

Subj	Noncompartmental Analysis				Four-Compartment Model			
	l/m²		min⁻¹		l/m²		min⁻¹	
	V_1	V_2	$\dot{y}_1(0)/y_1(0)$	$\dot{y}_4(0)/y_4(0)$	V_1	V_2	$\dot{y}_1(0)/y_1(0)$	$\dot{y}_4(0)/y_4(0)$
ED	4.5	2.9	0.492	0.765	3.2	2.1	0.664	1.056
GB	4.1	3.1	0.356	0.440	3.9	3.2	0.470	0.433
CF	4.6	5.8	0.357	0.198	3.8	3.7	0.524	0.331
NM	7.7	5.8	0.338	0.367	6.4	3.0	0.494	1.001
GS	10.6	3.8	0.301	0.693	3.4	1.6	0.706	1.446
AG	9.5	2.4	0.350	0.877	3.7	2.4	0.880	0.953
LT	8.5	2.2	0.356	0.880	2.7	1.8	0.908	1.147
Mean	7.1	3.7	0.364	0.603	3.9	2.5	0.664	0.910
±SD	±2.7	±1.5	±0.060	±0.268	±1.2	±0.8	±0.178	±0.395

strongly on extrapolations to $t \to 0$.

To avoid these limitations, and to make full use of the available kinetic information, physiologically based compartmental models appear to be more appropriate, and a four-compartment model (Fig. 3) provided the most accurate representation for studying the individual kinetics of AcAc and β-OHB. Two compartments refer to AcAc and β-OHB in blood, and the remaining two to lumped AcAc-β-OHB compartments in the liver and extrahepatic tissues, i.e., AcAc and β-OHB interconversion is assumed to be sufficiently rapid to consider AcAc and β-OHB as a single compartment.

The four-compartment model incorporates recycling of AcAc and β-OHB through the liver and extrahepatic tissues. Contradictory experimental evidence has been reported on the quantitative role played by these recycling processes in various metabolic conditions. Some authors found negligible recycling of β-OHB for the kidney in long-fasted humans (31). Others reported negligible recycling of AcAc from extrahepatic tissues in the rat (12) and, in other studies, both recycling fluxes have been neglected (9, 21, 26). Our modeling-based results confirm that, in some cases, the recycling flux of AcAc, and more often of β-OHB, from extrahepatic tissues are negligible.

We have also tested a simpler model structure, a three-compartment model (Fig. 4), which assumes negligible recycling of both AcAc and β-OHB from extrahepatic tissues back to plasma. This kinetic representation, originally proposed in rats (9), but afterwards ignored (10), has been shown to be inferior to the four-compartment model.

The four-compartment model provides a new quantitative description of ketone body metabolism in vivo. In addition to the initial distribution volumes (V_1, V_2) of AcAc and β-OHB, and the intercompartmental transfer rate parameters (k_{ij}) (Table 3), the model provides the following (Tables 6 and 7): compartment sizes of AcAc and β-OHB in plasma (Q_1, Q_2), and ketone bodies in liver (Q_3) and extrahepatic tissues (Q_4); the rate of production of ketone bodies inside the liver (R_{30}); and also the

plasma clearance rates of the individual ketones (PCR_1, PCR_2). Moreover, the rates of appearance of AcAc (Ra_1) and β-OHB (Ra_2) in plasma can be split into their liver (Ra_1^L, Ra_2^L) and extrahepatic (Ra_1^T, Ra_2^T) components, which in turn may be decomposed into their fractions due to recycling, interconversion, and de novo synthesis (Ra_{1r}^L, Ra_{1i}^L, Ra_{1n}^L, Ra_{2r}^L, Ra_{2i}^L, Ra_{2n}^L), as well as recycling and interconversion only (Ra_{1r}^T, Ra_{1i}^T; Ra_{2r}^T, Ra_{2i}^T). Useful information could be provided by the investigation of the relationships of these different sources in different metabolic conditions, such as starvation, diabetes, and obesity. For instance, a much larger increase in β-OHB than in AcAc in circulation has been reported in several ketotic states (35). The proposed model could provide some insights to explain at least partially this metabolic behavior. It allows quantitation of the individual PCR of the two ketones, and the rates of recycling and interconversion of AcAc and β-OHB, both in the liver and extrahepatic tissues.

The four-compartment model has been used to evaluate methodologies currently employed in the analysis of ketone body turnover data, in terms of physiological parameters. The conventional approach for quantitating ketone body metabolism is based on use of the combined specific activity of AcAc and β-OHB (3, 10, 23, 26); total ketone body concentration is considered directly and is used to estimate total ketone body production and plasma clearance rate. Inspection of *Eqs. 11–15* suggests that the conventional approach gives correct estimates of ketone body kinetics if and only if $V_1 = V_2$, $k_{41} = k_{42}$, and $k_{31} = k_{32}$. These conditions are not generally satisfied (Table 3). For instance, plasma clearance rates of AcAc and β-OHB (assumed to be equal in the conventional approach) are significantly different; in particular, the PCR of AcAc (PCR_1) is up to 100% greater than the PCR of β-OHB (PCR_2, Table 6). Even more remarkable differences in the PCR of the two ketones, e.g., PCR of AcAc = 2.5 PCR of β-OHB have been reported in humans fasted for 3 days (8). Thus use of the conventional approach based on the combined SA could lead to substantial errors both in assessing rates of production and plasma clearance rates, which could be even more marked in other metabolic conditions such as starvation and diabetic ketosis (35). Recently, Keller et al. (22) reported that tracer-determined TKB production using combined SA was higher by 3 and 8% in 48-h fasting dogs, and 11% lower in diabetic dogs, than net hepatic production as assessed by A-V difference techniques. However, it has to be pointed out that differences larger than 50% are present in individual data (22). Thus, calculations based upon the SA of total ketone bodies may be able to detect gross changes in production and utilization rates, but they can be seriously misleading in individual cases.

As cited above, conflicting results have been reported in the literature as concerns a number of parameters related to distribution volumes of ketone bodies. Our model serves to elucidate this matter. The estimated initial distribution volumes of AcAc (V_1) and of β-OHB (V_2) indicate that the blood compartment is essentially involved (Table 3).

The model also provides an estimate of the true total

distribution volume (V_D) of TKB (Table 6). Care must be taken in interpretation of the conventional recirculating volume (30) as the true total distribution volume of TKB (4, 23), because the recirculating volume gives only a lower bound on the true total distribution volume. The two coincide if and only if the plasma is the only compartment from which the substance can irreversibly disappear (personal communication, J. J. DiStefano III), which is not the case for ketone bodies. Available estimates on the recirculating volumes of TKB [$\cong 20\%$ of body weight (23)] seem to consistently underestimate the true total distribution volume provided by the model ($\cong 52\%$ of body weight).

In conclusion, the present study demonstrates that either separate injections of [^{14}C]AcAc and β-[^{14}C]OHB at different times in the same subject, or simultaneous administration of doubly labeled ketones are probably necessary to provide an adequate data base for kinetic modeling. A four-compartment model appears to be necessary for describing kinetic data in the normal human after an overnight fast. This model provided estimates of ketone body production in the liver, the plasma clearance of individual ketones, the initial and total distribution volumes, and the transfer rate parameters among the four ketone body compartments. Quantitative information is also provided on the compartment sizes and on the relative roles played by the liver and extrahepatic tissues in interconversion and recycling of the two ketones. This approach provides a new basis for the investigation of ketogenesis in a variety of metabolic conditions.

APPENDIX

For easier reference, the computational sequence adopted in the noncompartmental analysis of AcAc and β-OHB kinetics is reported.

Symbols

v_{ij}	rate of interconversion between *pool j* and *pool i* per unit of concentration in *pool j* (ml/min)
v_{0i}	rate of irreversible removal from *pool i* per unit of concentration in *pool i* (ml/min)
w_i	rate of recycling around *pool i* without going through the other accessible pool, per unit of concentration in *pool i* (ml/min)
D_1	dose of [^{14}C]AcAc introduced in *pool 1* at time $t = 0$ as an impulsive injection $u_1(t) = D_1\delta(t)$ where $\delta(t)$ is the Dirac function
D_2	dose of β-[^{14}C]OHB introduced in *pool 2* at time $t = 0$ as an impulsive injection $u_2(t) = D_2\delta(t)$
$y_1(t), y_2(t)$	concentrations of labeled AcAc and β-OHB in *pools 1* and *2*, respectively, after the injection $u_1(t)$
$y_3(t), y_4(t)$	concentration of labeled AcAc and β-OHB in *pools 1* and *2*, respectively, after injection $u_2(t)$
c_1, c_2	concentrations of cold AcAc and β-OHB in *pools 1* and *2*

By conservation of the tracers during the two experiments, the following four integral equations hold

$$D_1 = \int_0^\infty v_{01}y_1(t)dt + \int_0^\infty v_{02}y_2(t)dt \qquad (A1)$$

$$\int_0^\infty (v_{02} + v_{12})y_2(t)dt = \int_0^\infty v_{21}y_1(t)dt \qquad (A2)$$

$$D_2 = \int_0^\infty v_{01}y_3(t)dt + \int_0^\infty v_{02}y_4(t)dt \qquad (A3)$$

$$\int_0^\infty (v_{01} + v_{21})y_3(t)dt = \int_0^\infty v_{12}y_4(t)dt \qquad (A4)$$

The solution of the linear equation set *A1–A4* is given by

$$v_{01} = (D_1A_4 - D_2A_2)/(A_1A_4 - A_2A_3) \qquad (A5)$$

$$v_{02} = (D_2A_1 - D_1A_3)/(A_1A_4 - A_2A_3) \qquad (A6)$$

$$v_{21} = D_2A_2/(A_1A_4 - A_2A_3) \qquad (A7)$$

$$v_{12} = D_1A_3/(A_1A_4 - A_2A_3) \qquad (A8)$$

where A_i denotes the area under the concentration curve y_i

$$A_i = \int_0^\infty y_i(t)dt \qquad (A9)$$

To estimate the values of w_1, w_2, consider the following differential equations

$$V_1\dot{y}_1(t) = -v_{1\,tot}y_1 + f_1(t) \qquad (A10)$$

$$V_2\dot{y}_4(t) = -v_{2\,tot}y_4 + f_2(t) \qquad (A11)$$

where $v_{i\,tot}$ is the total rate of exit from *compartment i*, $f_i(t)$ is the total input into i of tracer from the other pools, and V_i is the initial distribution volume of *pool i*.

At time $t = 0$, $f_1(t)$ and $f_2(t)$ are equal to zero. Thus

$$V_1\dot{y}_1(0) = -v_{1\,tot}y_1(0) \qquad (A12)$$

$$V_2\dot{y}_4(0) = -v_{2\,tot}y_4(0) \qquad (A13)$$

Therefore, w_1 and w_2 both depend on the values of the decaying curves at time $t = 0$ and on their derivatives

$$w_1 = v_{1\,tot} - v_{01} - v_{21} = -\frac{\dot{y}_1(0)}{y_1(0)}V_1 - v_{01} - v_{21} \qquad (A14)$$

$$w_2 = v_{2\,tot} - v_{02} - v_{12} = -\frac{\dot{y}_4(0)}{y_4(0)}V_2 - v_{02} - v_{12} \qquad (A15)$$

Plasma clearance rates, rates of appearance, recycling and irreversible loss of AcAc and β-OHB can be estimated from v_{01}, v_{02}, v_{21}, v_{12}, w_1, w_2, c_1, and c_2 as follows

$$PCR_1 = v_{01} \qquad (A16)$$

$$PCR_2 = v_{02} \qquad (A17)$$

$$R_{a1i} = v_{12}c_2 \qquad (A18)$$

$$R_{a1r} = w_1c_1 \qquad (A19)$$

$$R_{01irr} = v_{01}c_1 \qquad (A20)$$

$$R_{a1n} = (v_{01} + v_{21})c_1 - v_{12}c_2 \qquad (A21)$$

$$R_{a2i} = v_{21}c_1 \qquad (A22)$$

$$R_{a2r} = w_2c_2 \qquad (A23)$$

$$R_{02irr} = v_{02}c_2 \qquad (A24)$$

$$R_{a2n} = (v_{02} + v_{12})c_2 - v_{21}c_1 \qquad (A25)$$

Finally, the pool sizes Q_1 and Q_2 can be estimated from

$$Q_1 = \frac{D_1}{y_1(0)}c_1 \qquad (A26)$$

$$Q_2 = \frac{D_2}{y_4(0)}c_2 \qquad (A27)$$

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