Effects of Thyroid Status on Glucose Cycling by Isolated Rat Hepatocytes

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The effects of alterations in thyroid status on glucose metabolism have been investigated in rat hepatocytes. Addition of 10 or 40 mmol/L glucose induced increases in respiration rate that were significantly larger in cells from hyperthyroid rats than from hypothyroid animals. The responses of hepatocytes from euthyroid rats were intermediate. In cells from hyperthyroid rats, most of the increase occurred upon addition of 10 mmol/L glucose, with only a further small stimulation resulting when glucose concentration was increased to 40 mmol/L. For a given glucose concentration, glycolytic rates, determined by measuring release of tritium from [6-^{3}H]glucose, were comparable in all thyroid states. Studies with 10 mmol/L [2-^{3}H]glucose showed that cycling between glucose-6-phosphate and glucose was almost twofold higher in euthyroid and hyperthyroid states as compared with the hypothyroid state, although the magnitude of the increase in cycling rate was only approximately 0.2 μmol glucose · min^{-1} · g^{-1}. When 40 mmol/L [2-^{3}H]glucose was added, over 44% of the glucose that was phosphorylated to glucose-6-phosphate was cycled back to glucose, but this cycling was independent of thyroid status. Cycling between fructose-1,6-bisphosphate and fructose-6-phosphate was negligible in all thyroid states. Rates of glycogen synthesis were comparable in hypothyroid and hyperthyroid states and slightly less than in the euthyroid state. Glycolytically formed pyruvate was cycled back to glucose in hepatocytes from hypothyroid, euthyroid, and hyperthyroid rats. During a 60-minute incubation period, cycling to glucose in the presence of 10 mmol/L or 40 mmol/L glucose was up to twofold higher in cells from euthyroid and hyperthyroid rats than in hepatocytes from hypothyroid animals. The measured increases in cycling rates induced by thyroid hormone were small and in theory could have been satisfied by a much smaller increase in respiration rate than was observed.

BASAL METABOLIC RATE, respiration rate, and heat production are stimulated in hyperthyroidism and decreased in hypothyroidism.[1-5] Although no consensus has yet been reached on the mechanism of the calorigenic action of thyroid hormone, a number of possibilities have been advanced.[1,3,5-7] One such possibility is substrate cycling. Substrate or "futile" cycles, by consuming cellular energy without apparently doing useful work, could contribute to the basal metabolic rate in the euthyroid state.[1,5,8-10] Well-documented examples of such cycles include the interconversion of glucose and glucose-6-phosphate and of fructose-6-phosphate and fructose-1,6-bisphosphate.[8-10] Experimental evidence also exists for cycling as a consequence of the concomitant operation of glycolysis and gluconeogenesis.[10-14] Any stimulation of substrate cycling by thyroid hormone would be expected to contribute to the increased basal metabolic rate associated with the hyperthyroid state. Conversely, the hypothyroid state might be anticipated to be associated with decreased cycling.

The possibility that administration of thyroid hormone might lead to an increase in substrate cycling has been investigated in fasted rats in vivo.[15,16] It was found that recycling of glucose via pyruvate, as well as cycling between glucose and glucose-6-phosphate[15,16] and between fructose-6-phosphate and fructose-1,6-bisphosphate,[16] was increased in hyperthyroid rats but decreased in hypothyroid rats relative to cycling rates found in normal animals. However, these studies in whole animals did not allow an unambiguous identification of the tissues responsible for substrate cycling, although it was proposed that thyroid hormone–induced alterations in glucose turnover reflected changes in activity of the Cori cycle between liver and extrahepatic tissues.[16] In this study, we have investigated the possible contribution of the liver to glucose turnover in different thyroid states by examining the cycling between glucose and glucose-6-phosphate and between fructose-6-phosphate and fructose-1,6-bisphosphate in hepatocytes derived from fasted hypothyroid, euthyroid, and hyperthyroid rats. In addition, we have examined the effect of thyroid status on the cycling of glycolytically formed pyruvate and lactate back to glucose.

MATERIALS AND METHODS

Materials

Collagenase, carbonic anhydrase, and enzymes necessary for the assay of cellular metabolites were obtained from Boehringer Mannheim (Sydney, Australia), as was bovine serum albumin (fraction V), which was defatted by the method of Chen.[17] Amyloglucosidase (EC 3.2.1.3) and 3,3′,5-triiodo-L-thyronine (T_3) were purchased from Sigma Chemical (Sydney, Australia). [1-^{14}C], [6-^{14}C], and [U-^{14}C]glucose were obtained from NEN (Sydney, Australia), as were HPLC-purified [2-^{3}H], [3-^{3}H], and [6-^{3}H]glucose. "Readysafe" scintillation fluid was from Beckman (Sydney, Australia). Other chemicals were of the highest quality commercially available.

Methods

Hepatocytes were prepared from 24-hour–fasted euthyroid, hypothyroid, and hyperthyroid rats of similar age (12 weeks) as previously described.[18] Hyperthyroidism was induced in rats (280 to 320 g) by four daily intramuscular injections of 170 ng T_3/g body mass, and rats were used for experimentation on the fifth day after commencement of administration of T_3. The T_3 solution was prepared by dissolving 1 mg T_3 in 50 μL 0.1-mol/L NaOH and diluting this solution to 2 mL with 0.9% (wt/vol) NaCl. Hypothy-

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roid rats were obtained by thyroid-parathyroidectomy of young rats (130 to 160 g). They were used 6 weeks later, when their body mass was 200 to 250 g, approximately 73% of that of normal rats of similar age.[19] In the postoperative weeks, the animals' drinking water was supplemented with 0.2% (wt/vol) calcium lactate. Incubation of isolated hepatocytes was performed for periods up to 120 minutes at 37°C as described previously,[20] and O_2 consumption in the presence of CO_2 was measured manometrically.[21] Respiration rates, which were recorded over the period from 10 to 60 minutes following cell addition to the incubation medium, were virtually constant. Reactions were terminated by addition to the incubation mixture of an equal volume of ice-cold 1-mol/L perchloric acid. The denatured protein was removed by centrifugation, the supernatants neutralized with 2 mol/L KOH, and the metabolites assayed according to standard methods[22] by means of a COBAS FARA automated analyzer (Roche Diagnostics, Basel, Switzerland). The data were transferred to a MicroVAX computer (Digital Equipment, Maynard, MA) for processing. Measurement of $^{14}CO_2$ derived from [U-^{14}C]glucose, [1-^{14}C]glucose, or [6-^{14}C]glucose was performed as described previously.[23] Glycogen was assayed as reported elsewhere.[19] Rates of glycogen synthesis are expressed as micromoles glucose equivalents per minute per gram wet mass.

To obtain an accurate estimation of glycolytic flux, the sum of rates of formation of ^3H_2O from added [6-^{3}H]glucose (1 μCi) and of accumulation of tritium in lactate, pyruvate, and amino acids[11,24,25] was measured. For convenience, glycolytic release of tritium from [6-^{3}H]glucose is termed glycolysis (^{3}H). In addition, the net rate of [U-^{14}C]glucose utilization (1 μCi) was determined by measuring the rate of formation of $^{14}CO_2$ together with the rates of accumulation of ^{14}C in lactate, pyruvate, and amino acids. Incorporation of ^{14}C into glycogen was excluded from this calculation. The difference between the rate of glycolysis (^{3}H) and the net rate of [U-^{14}C]glucose utilization provides a measure of the rate of cycling of glycolytic lactate and pyruvate back to glucose.[10-13] The rate of glucose phosphorylation was determined from the rate of release of tritium from [2-^{3}H]glucose (1 μCi).[10,25] Partial retention of tritium in glucose-6-phosphate can lead to an underestimation of the phosphorylation rate.[9] To minimize this error, the amount of tritium incorporated into glycogen was also determined.[25] To obtain a measure of flux through the triose-phosphate isomerase–catalyzed reaction, the rate of release of ^3H_2O from [3-^{3}H]glucose (1 μCi) was monitored.[10] All cycling rates are expressed in terms of glucose equivalents. Pentose phosphate pathway activity was assessed from relative rates of $^{14}CO_2$ generation from [1-^{14}C]glucose and [6-^{14}C]glucose.[26]

Measurement of the Metabolites of Isotopically Labeled Glucose

Portions (0.5 mL) of incubation mixtures were removed and mixed with 1.5 mL ice-cold ethanol to terminate reactions. The resulting precipitate was removed by centrifugation, and 1 mL of the supernatant was passed sequentially through three (0.9 × 2.5 cm) columns of Dowex AG 50W-X8 (H^+, 100 to 200 mesh), Dowex AG 1-X8 (acetate, 100 to 200 mesh), and Dowex AG 1-X8 (borate, 100 to 200 mesh).[27] Tritiated water derived from metabolism of the tritiated isotopes of glucose was not retained by the columns, and was recovered by thoroughly washing the columns with water. Glucose was adsorbed on the borate resin and could be eluted with 1 mol/L acetic acid. Lactate and pyruvate were adsorbed to the acetate resin and sequentially eluted with 1 mol/L acetic acid and 4 mol/L formic acid, respectively. Amino acids were adsorbed on the H^+ resin and eluted with 2 mol/L NH_4OH. Recoveries of ^{14}C from [U-^{14}C]glucose and of tritium from [6-^{3}H]glucose in products were over 98%. There was negligible incorporation of ^{14}C from [U-^{14}C]glucose into triglycerides and fatty acids, which were separated according to the method of Borgström.[28]

Liver parenchymal cells from hypothyroid rats are termed hypothyroid cells or hepatocytes, and when derived from hyperthyroid rats, hyperthyroid cells or hepatocytes. Cells from euthyroid rats are designated euthyroid cells or hepatocytes. All rates, including those relating to formation of isotopically labeled compounds, are expressed as micromoles per minute per gram wet weight.

RESULTS

Liver glucokinase (EC 2.7.1.1) in vitro exhibits an increased intracellular $[S]_{0.5}$ (substrate concentration yielding half-maximal reaction rate) for glucose of approximately 15 mmol/L.[29,30] Because of this, studies of glucose metabolism in isolated hepatocytes have often been performed with concentrations of added glucose as high as 60 mmol/L to obtain greater rates of glucose metabolism and more physiological glycolytic rates.[9,27] In these studies, we have conducted experiments with near-physiological concentrations of glucose, namely 10 mmol/L, where the rate of glycolysis is lower than that in vivo, as well as experiments where more physiological rates of glycolysis are attained, albeit with a nonphysiological concentration of glucose, 40 mmol/L.

O_2 Uptake in the Presence of Added Glucose

Hyperthyroid and hypothyroid hepatocytes incubated for 60 minutes in the absence of added substrate had rates of respiration 27% greater and 26% lower, respectively, than euthyroid hepatocytes (Table 1). Addition of glucose stimulated respiration above the respective endogenous rates, but to different degrees depending on thyroid status. Thus, 10 mmol/L glucose elevated the rate of O_2 uptake 11%, 18%, and 39% above the basal level in hypothyroid, euthyroid, and hyperthyroid hepatocytes, respectively. In consequence, the rate of respiration in the presence of 10 mmol/L glucose was more than twice as high in hyperthyroid hepatocytes as in hypothyroid cells and 49% greater than in euthyroid hepatocytes. Addition of glucose at a

Table 1. Effect of Thyroid Status on O_2 Uptake and $^{14}CO_2$ Production (μmol · min^{-1} · g^{-1}) by Hepatocytes in the Presence of Glucose

Thyroid Status	Glucose (mmol/L)	Rate of O_2 Uptake	Rate of $^{14}CO_2$ Formation
Hypothyroid	—	1.69 ± 0.09 (9)	
	10	1.87 ± 0.05 (5)	0.55 ± 0.07 (5)
	40	2.12 ± 0.09 (10)	0.82 ± 0.05 (6)
Euthyroid	—	2.29 ± 0.07 (11)*	
	10	2.71 ± 0.08 (9)*	0.60 ± 0.03 (5)
	40	3.10 ± 0.05 (45)*	1.61 ± 0.06 (22)*
Hyperthyroid	—	2.90 ± 0.09 (14)*	
	10	4.06 ± 0.09 (6)*	0.73 ± 0.03 (4)†
	40	4.31 ± 0.16 (11)*	2.20 ± 0.17 (7)*

NOTE. Data are the mean ± SE, with the number of separate experiments in parentheses. Significance was assessed by unpaired Student's *t* test.

*$P < .001$ for hyperthyroid *v* hypothyroid or euthyroid and for euthyroid *v* hypothyroid, under similar incubation conditions.

†$P < .02$ for hyperthyroid *v* hypothyroid.

concentration of 40 mmol/L (Table 1) increased O_2 uptake by the same amount above the level obtained with 10 mmol/L glucose in the hypothyroid or hyperthyroid state; thus, the percentage increase induced by the high glucose concentration was less in hyperthyroid than in hypothyroid hepatocytes. The smaller increase in respiration induced by glucose addition to hypothyroid cells was not due to a restriction in the electron transport chain, since the [3-hydroxybutyrate]/[acetoacetate] ratio, which reflects the mitochondrial [NADH]/[NAD^+] ratio,[31] reached steady state after approximately 20 minutes and was similar in hyperthyroid and hypothyroid hepatocytes, the values being 0.26 ± 0.01 (n = 27) and 0.25 ± 0.01 (n = 34), respectively.

There was a lag phase before $^{14}CO_2$ production reached maximal rates in euthyroid or hypothyroid hepatocytes, but $^{14}CO_2$ formation was linear from the commencement of incubation in hyperthyroid hepatocytes. Following the lag phase, the maximal rate of production of $^{14}CO_2$ from 10 mmol/L [U-^{14}C]glucose by hyperthyroid hepatocytes was 32% greater than by hypothyroid cells and 21% greater than by euthyroid cells, whereas in the presence of 40 mmol/L glucose, the rate of formation of $^{14}CO_2$ was almost three times that of hypothyroid cells and 36% higher than that of euthyroid hepatocytes (Table 1). Complete oxidation of 1 mol [U-^{14}C]glucose consumes 6 mol O_2 and generates 6 mol $^{14}CO_2$, yielding a $^{14}CO_2/O_2$ ratio of 1. The stimulation of O_2 uptake over endogenous levels, induced in hypothyroid cells by 10 mmol/L [U-^{14}C]glucose, was 0.18 μmol · min^{-1} · g^{-1}, which was considerably less than that expected for a 1:1 correspondence with $^{14}CO_2$ production. On the basis that $^{14}CO_2$ generation reflects [^{14}C]glucose oxidation, these results suggest that oxidation of endogenous substrates was partially suppressed by glucose addition. This conclusion could also be drawn on the basis of similar calculations made in the case of hypothyroid cells incubated with 40 mmol/L glucose and of euthyroid hepatocytes incubated in the presence of both 10 and 40 mmol/L glucose. On the other hand, O_2 uptake of hyperthyroid cells exposed to 10 mmol/L [^{14}C]glucose was 1.16 μmol · min^{-1} · g^{-1} above the endogenous rate, whereas the rate of $^{14}CO_2$ production was only 63% of this. Hence, oxidation of endogenous substrates must have been promoted by the glucose addition. However, 40 mmol/L glucose increased the respiratory rate of hyperthyroid cells 1.41 μmol · min^{-1} · g^{-1} above the endogenous rate, whereas the rate of $^{14}CO_2$ formation was 156% higher than this, indicating that in hyperthyroid cells oxidation of 40 mmol/L glucose must have suppressed a considerable portion of the oxidation of endogenous substrate.

Glucose Cycling Between Glucose and Glucose-6-phosphate

To examine the rate of cycling between glucose and glucose-6-phosphate, we compared rates of detritiation of [6-^{3}H]glucose and [2-^{3}H]glucose[9,10] in hypothyroid, euthyroid, and hyperthyroid hepatocytes. Rates of detritiation of both isotopes of glucose became constant after a lag phase of between 10 and 20 minutes. In the presence of 10 mmol/L glucose, the rate of glucose phosphorylation, given by the sum of the rate of ^3H_2O formation from [2-^{3}H]glucose and the rate of incorporation of [2-^{3}H]glucose into glycogen,[10,25] was faster in euthyroid and hyperthyroid than in hypothyroid hepatocytes (Table 2). Glycolysis (^{3}H) did not vary significantly with thyroid status (Table 2). Hence, cycling between glucose and glucose-6-phosphate, calcu-

Table 2. Metabolic Rates (μmol · min^{-1} · g^{-1}) in the Presence of 10 or 40 mmol/L Glucose

Metabolic Process	Interval (min)	10 mmol/L Glucose			40 mmol/L Glucose		
		Hypothyroid	Euthyroid	Hyperthyroid	Hypothyroid	Euthyroid	Hyperthyroid
Glycolysis (^{3}H)	10-60	0.28 ± 0.03 (5)	0.30 ± 0.01 (5)	0.31 ± 0.01 (4)	0.88 ± 0.08 (6)	1.01 ± 0.08 (22)	0.93 ± 0.05 (8)
Glucose phosphorylation	10-60	0.47 ± 0.04 (5)*	0.64 ± 0.01 (4)	0.67 ± 0.03 (4)	1.68 ± 0.10 (6)†	2.18 ± 0.07 (15)	1.66 ± 0.07 (8)†
^3H_2O from [3-^{3}H]glucose	10-60	0.28 ± 0.03 (5)	—	0.31 ± 0.02 (4)	0.96 ± 0.09 (3)	1.14 ± 0.10 (4)	0.95 ± 0.09 (4)
Glycogen synthesis	10-60	<0.04 (5)	0.11 ± 0.01 (8)	0	0.39 ± 0.07 (6)	0.51 ± 0.06 (22)	0.33 ± 0.04 (8)
Net utilization of	10-60	0.19 ± 0.03 (5)					
[U-^{14}C]glucose	10-20		0.26 ± 0.02 (6)	0.26 ± 0.04 (4)		0.91 ± 0.11 (6)	0.95 ± 0.07 (7)
	20-40		0.17 ± 0.02 (5)	0.18 ± 0.01 (5)	0.72 ± 0.05 (4)	0.82 ± 0.05 (6)	0.65 ± 0.06 (7)
	40-60		0.16 ± 0.02 (6)	0.14 ± 0.02 (5)	0.66 ± 0.04 (4)	0.52 ± 0.08 (6)	0.46 ± 0.03 (7)
	60-90					0.43 ± 0.06 (6)	
	90-120				0.43 ± 0.05 (3)		
Calculated glucose:G6P cycling	10-60	0.19	0.34	0.36	0.80	1.17	0.73
Calculated cycling of	10-60	0.09					
pyruvate and lactate to glucose	10-20		0.04	0.05		0.10	0
	20-40		0.13	0.13	0.16	0.19	0.28
	40-60		0.14	0.17	0.22	0.49	0.47
	60-90					0.58	
	90-120				0.45		

NOTE. Data are the mean ± SE, with the number of separate experiments shown in parentheses. Significance assessed by unpaired Student's *t* test.

*$P < .001$ for hypothyroid *v* euthyroid or hyperthyroid at 10 mmol/L glucose.

†$P < .001$ for hypothyroid and hyperthyroid *v* euthyroid at 40 mmol/L glucose.

lated as the difference between rates of glycolysis (^3H) and glucose phosphorylation,[9,10] was almost doubled in euthyroid and hyperthyroid as compared with hypothyroid hepatocytes, although the magnitude of the change was small (Table 2). The recycling rate for cells from fasted euthyroid rats is in good agreement with an earlier estimate of 0.39 $\mu mol \cdot min^{-1} \cdot g^{-1}$.[12]

The rates of both glucose phosphorylation and glycolysis (^3H) were approximately the same in hypothyroid and hyperthyroid hepatocytes but less than rates observed in euthyroid hepatocytes when cells were incubated with 40 mmol/L glucose (Table 2). Thus, in the presence of a concentration of glucose yielding physiological rates of glycolysis, the rate of cycling between glucose-6-phosphate and glucose was similar in hypothyroid and hyperthyroid states, but lower than in the euthyroid condition.

Cycling Between Fructose-6-phosphate and Fructose-1,6-bisphosphate

To measure cycling between fructose-6-phosphate and fructose-1,6-bisphosphate, hypothyroid and hyperthyroid hepatocytes were incubated in the presence of 10 or 40 mmol/L glucose labeled with tritium at either C3 or C6.[9,10] The difference between the rate of formation of ^3H_2O from [3-^{3}H]glucose and the glycolytic rate determined from detritiation of [6-^{3}H]glucose gives an estimate of the degree of cycling.[9,10] At each glucose concentration, the rate of ^3H_2O formation from [3-^{3}H]glucose was similar to or only marginally higher than the glycolytic rate and was substantially less than the rate of glucose phosphorylation (Table 2). Hence, cycling between fructose-6-phosphate and fructose-1,6-bisphosphate was minimal and independent of thyroid status. When 10 mmol/L glucose was perfused through the livers of fasted rats, a recycling rate of only 0.1 $\mu mol\ glucose \cdot min^{-1} \cdot g^{-1}$ was estimated.[10] This form of cycling was also negligible in hepatocytes isolated from 24-hour–fasted mice.[13]

Cycling of Glycolytically Produced Pyruvate and Lactate Back to Glucose

Determination of cycling of glycolytic products back to glucose requires measurement of the products of [U-^{14}C]glucose catabolism. In the presence of 10 mmol/L glucose, hypothyroid and euthyroid cells, following an initial lag period of 10 minutes, formed ^{14}C-labeled catabolic products (Fig 1) at a constant rate. The net rate of [U-^{14}C]glucose utilization was higher with 40 mmol/L glucose present (Fig 1) but was not constant, decreasing slightly between 40 and 60 minutes (hypothyroid) or decreasing to a constant rate beyond 40 minutes (euthyroid). In experiments in which incubation time was extended to 120 minutes, the net rate of [U-^{14}C]glucose utilization by hypothyroid cells decreased further during the second 60-minute period to a relatively constant value that was 40% lower than the rate measured in the period 20 to 40 minutes. The net rate of [U-^{14}C]glucose utilization in hyperthyroid cells, whether incubated with 10 or 40 mmol/L glucose (Fig 1), was also not constant over the incubation period, but decreased with time. Net rates of [U-^{14}C]glucose utilization under the various experimental circumstances are given in Table 2. The decrease in the rate of formation of ^{14}C-labeled products coincided with an approach to steady state of the levels of accumulated [^{14}C]lactate (Fig 2) and [^{14}C]pyruvate (not shown).

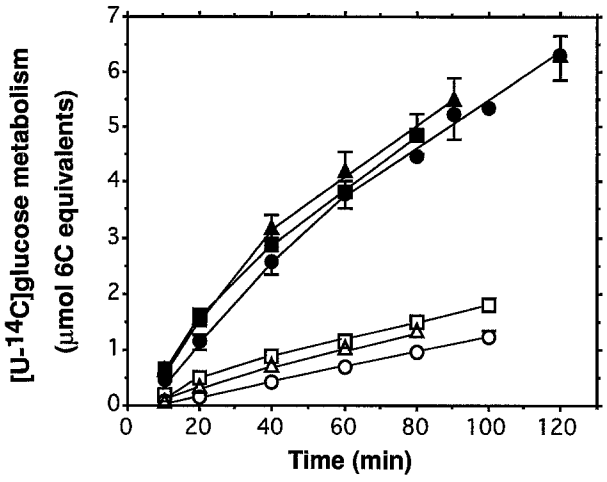

Fig 1. Metabolism of 10 and 40 mmol/L [U-^{14}C]glucose by hepatocytes from rats of different thyroid status. Hypothyroid (○,●), euthyroid (△,▲), and hyperthyroid (□,■) hepatocytes (100 mg wet weight) were incubated in a total volume of 2 mL at 37°C in the presence of 10 mmol/L (○,△,□) or 40 mmol/L (●,▲,■) [U-^{14}C]glucose and accumulation of ^{14}C-labeled products, excluding [^{14}C]glycogen was measured. Data are the mean ± SE from 4 experiments in each case.

On the basis of values obtained with [6-^{3}H]glucose and [U-^{14}C]glucose, the rate of cycling of glycolytically formed pyruvate and lactate back to glucose represented 32% of glycolysis (^3H) in hypothyroid cells metabolizing 10 mmol/L glucose (Table 2), but even so, the cycling rate was less than 0.1 $\mu mol \cdot min^{-1} \cdot g^{-1}$ and amounted to 64% or 53% of the

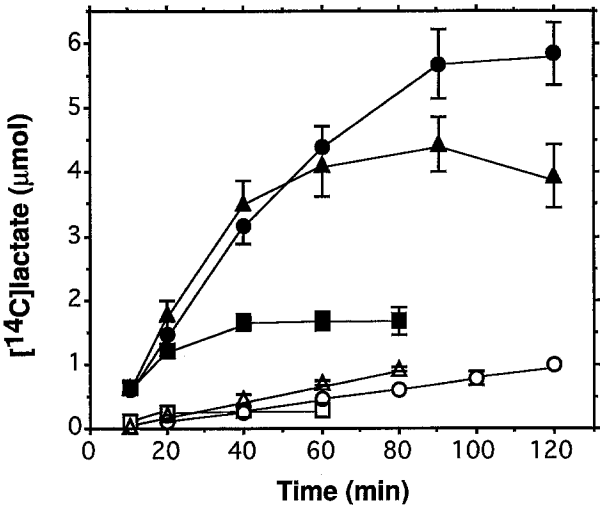

Fig 2. Formation of [^{14}C]lactate from [U-^{14}C]glucose in hepatocytes from rats of different thyroid status. Hypothyroid (○,●), euthyroid (△,▲), and hyperthyroid (□,■) hepatocytes (100 mg wet weight) were incubated at 37°C in the presence of 10 mmol/L (○,△,□) or 40 mmol/L (●,▲,■) [U-^{14}C]glucose and accumulation of [^{14}C]lactate was measured. Data are derived from experiments described in Fig 1.

cycling rates in euthyroid and hyperthyroid hepatocytes, respectively, during the period 40 to 60 minutes. In the presence of 40 mmol/L glucose, the rate of cycling increased with time to reach almost 50% of glycolysis (^3H) in both hyperthyroid and hypothyroid hepatocytes (Table 2), but attainment of this degree of cycling took twice as long in the latter cells and was achieved at a threefold higher steady-state concentration of [^{14}C]lactate (Fig 2). The quantity of enzymatically assayed lactate was consistently within 0.5 μmol of the measured [^{14}C]lactate (not shown). Euthyroid cells metabolizing 40 mmol/L glucose under steady-state conditions recycled pyruvate and lactate to glucose at a rate that was 57% of glycolysis (^3H).

Energy Cost of Increased Cycling in Hyperthyroid Cells

The maximal adenosine triphosphate (ATP) requirement of increased cycling in hyperthyroid relative to hypothyroid cells can be calculated on the basis of 1 mol ATP hydrolyzed per mol glucose phosphorylated and 6 mol ATP utilized per mol glucose synthesized from pyruvate or lactate.[32] On the other hand, glycolysis of 1 mol glucose can be expected to yield 2 mol ATP. In the presence of 10 mmol/L glucose, cycling between glucose-6-phosphate and glucose was 0.17 $\mu mol \cdot min^{-1} \cdot g^{-1}$ higher in hyperthyroid than in hypothyroid hepatocytes, and the maximal increase in the rate of conversion of pyruvate to glucose, in the period 40 to 60 minutes, was 0.08 $\mu mol \cdot min^{-1} \cdot g^{-1}$. The ATP requirement of increased cycling was thus 0.17 μmol ATP $\cdot min^{-1} \cdot g^{-1}$ (cycling between glucose-6-phosphate and glucose) plus 0.48 μmol ATP $\cdot min^{-1} \cdot g^{-1}$ (recycling of glucose via pyruvate), ie, a total increase of 0.65 μmol ATP $\cdot min^{-1} \cdot g^{-1}$. The ATP/O ratio in hepatocytes is believed to be less than the theoretical maximum and to vary with the state 3 respiration rate, so that an effective ATP/O ratio as low as 1.3 has been suggested for hepatocytes oxidizing glucose as the sole fuel.[33] Even using this minimal estimate, it can be calculated that an increase in respiration rate of no more than 0.25 $\mu mol\ O_2 \cdot min^{-1} \cdot g^{-1}$ would be required to support the energy needs of increased cycling. A corresponding increase of less than 0.1 μmol $O_2 \cdot min^{-1} \cdot g^{-1}$ can be calculated for hyperthyroid as compared with euthyroid hepatocytes. Similarly, in the presence of 40 mmol/L glucose, the greatest difference in cycling between hypothyroid and hyperthyroid states, in the period 40 to 60 minutes, would have accounted for an additional O_2 uptake of up to 0.58 $\mu mol\ O_2 \cdot min^{-1} \cdot g^{-1}$. Hence, the increased energy cost of stimulated cycling in hyperthyroid hepatocytes was relatively small, and in any case could have been satisfied by glycolytically formed ATP. Further, euthyroid cells incubated with 40 mmol/L glucose demonstrated rates of cycling that were slightly greater than those observed in hyperthyroid hepatocytes.

Other Modes of Glucose Utilization

Since metabolism of glucose via the pentose phosphate pathway also results in loss of tritium from C3,[9,10] the effect of thyroid status on pentose phosphate pathway activity was investigated in both hyperthyroid and hypothyroid hepatocytes. Cells were incubated for 60 minutes in the presence of either 40 mmol/L [1-^{14}C]glucose or [6-^{14}C]glucose and $^{14}CO_2$ production was measured. The proportion of glucose metabolized via the pentose phosphate pathway was calculated.[26] We found that less than 3% of glucose was utilized in the pentose phosphate pathway in both hyperthyroid and hypothyroid hepatocytes and less than 4% in euthyroid cells (results not shown). These low values compare well with that in a human study in which the pentose phosphate cycle contributed approximately 2% to glucose utilization regardless of thyroid status.[34]

In the presence of 10 mmol/L glucose, glycogen synthesis was almost negligible, whereas with added 40 mmol/L glucose, both hypothyroid and hyperthyroid hepatocytes maintained similar rates of synthesis of glycogen, albeit lower than that measured in euthyroid cells (Table 2). Hence, the increased oxidative metabolism in hyperthyroid cells cannot be ascribed to the energy requirements of an accelerated rate of glycogen synthesis.

DISCUSSION

Since the liver is known to be an important target of thyroid hormone action,[35] we examined the possibility that the hormone could bring about a sufficiently increased rate of hepatic glucose cycling to account for the stimulation of respiration observed in hyperthyroid hepatocytes. In the absence of added substrate, the respiratory rate of hyperthyroid hepatocytes from fasted rats is over 70% greater than that of hypothyroid cells. Under these conditions, the cells are depleted of glycogen and carbohydrate metabolism is negligible. It is likely therefore that endogenous lipid supplies the fuel for respiration in the absence of added glucose.[36] It follows that the marked stimulation of respiration induced by thyroid hormone under these conditions cannot be due to glucose cycling. Nevertheless, addition of glucose caused a substantial increase in O_2 uptake, which was much greater in hyperthyroid cells. The increase in O_2 uptake was almost maximal at 10 mmol/L glucose, a concentration less than the $[S]_{0.5}$ of glucokinase, as reflected by the low rates of glycolysis observed at this glucose concentration. The amount of $^{14}CO_2$ formed from the added glucose by hyperthyroid hepatocytes was only approximately 30% greater than the amount produced by hypothyroid cells and was 21% greater than that formed by euthyroid hepatocytes, whereas the increment in O_2 consumption induced by addition of 10 mmol/L glucose was more than six times higher than that in hypothyroid cells and almost three times higher than in euthyroid hepatocytes. These findings suggest that addition of 10 mmol/L glucose suppressed oxidation of endogenous substrates by hypothyroid and euthyroid cells, but stimulated this process in hyperthyroid hepatocytes. Since hyperthyroidism promotes lipolysis[1,37] and fatty acid oxidation[38,39] and since free fatty acids are the favored substrate for oxidative metabolism in the hyperthyroid state,[1] it is likely that 10 mmol/L glucose stimulates oxidation of endogenous fatty acids in hyperthyroid cells. The different effects of 10 mmol/L glucose on oxidation of endogenous substrates in different thyroid states are consistent with the view that the capacity

for electron transport along the respiratory chain is reduced in the hypothyroid and increased in the hyperthyroid state.[2]

That the disparity in the respiratory response of hypothyroid, euthyroid, and hyperthyroid hepatocytes to the addition of 10 mmol/L glucose was a consequence of mitochondrial factors is supported by the observation that the level of cycling and the rates of glycogen synthesis observed in all thyroid states were similar. Moreover, calculations based on the ATP demand of cycling show that differences in the degree of stimulation of respiration by glucose addition in the different thyroid states could not be accounted for by differences in the rates of cycling between glucose and glucose-6-phosphate, between fructose-1,6-bisphosphate and fructose-6-phosphate, or of glycolytic products back to glucose. This follows from the small increase in energy requirement of the minor changes in cycling rates resulting from addition of both 10 and 40 mmol/L glucose to hyperthyroid and euthyroid as compared with hypothyroid hepatocytes. Consequently, the contribution of hepatic glucose cycling to the calorigenic effect of thyroid hormone appears to be minimal.

Studies of glucose cycling in humans have been reported. Cycling has been demonstrated between glucose and glucose-6-phosphate,[40-43] between fructose-6-phosphate and fructose-1,6-bisphosphate,[34,40,41,43] and between glucose and glucose-derived C3 intermediates.[44] Thyroid hormone stimulated cycling between glucose and glucose-6-phosphate[40] and between glucose and glucose-derived C3 metabolites.[44] Evidence for a thyroid hormone–induced stimulation of cycling between fructose-6-phosphate and fructose-1,6-bisphosphate in humans is conflicting, with reports of both stimulation[40] and no effect in hyperthyroidism.[34] Although the glucose/glucose-6-phosphate cycle may represent approximately 17% of basal glucose production[43] and the fructose-6-phosphate/fructose-1,6-bisphosphate cycle 13% of the rate of glucose turnover in normal subjects,[34] the thermogenic cost to the individual is likely to be small in both euthyroid[42] and hyperthyroid[40] subjects. Consequently, results of human studies are in agreement with our data for rat hepatocytes, showing that hepatic glucose cycling of the types discussed plays a quantitatively minor role in thermogenesis in all thyroid states.

The small further increase in respiration induced by increasing glucose concentration from 10 to 40 mmol/L implies that the process responsible for stimulation of O_2 uptake is close to saturation at a much lower glucose concentration than that which saturates glucokinase, glycogen synthesis, and glycolysis. The increased respiration can be expected to be an indicator of increased energy production. However, it is not clear what cellular energy requirements have been increased in hyperthyroid cells. Our results excluded any significant diversion of glucose into fatty acids or triglycerides, as well as the possibility of increased glycogen deposition, in hyperthyroid cells. Although a thyroid hormone–induced increase in activity of the plasma membrane (Na^+/K^+)-transporting ATPase has been suggested to be a major calorigenic process,[7] we and others find that this ATPase has only a minor role.[19,45,46] An alternative possibility is that thyroid hormone–stimulated respiration is not tightly coupled.[19] Our earlier study identified a component of respiration that is apparently not linked to ATP synthesis and is increased in the hyperthyroid state.[19] Under conditions of maximal substrate-stimulated O_2 uptake, this component represented 20% of total respiration of hyperthyroid hepatocytes[19] and might make a significant contribution to thermogenesis. This may also be true of a portion of the increase in respiration induced by glucose addition to hyperthyroid cells. Although it has been suggested that such a component of respiration might reflect proton leak across the mitochondrial inner membrane,[47] this has not been shown conclusively and further studies are required. Other possible explanations for the increased respiration rate in hyperthyroid cells include increased substrate cycling between pyruvate and oxaloacetate[48] or between fatty acid and triglyceride.[49] In euthyroid rats, cycling between pyruvate and oxaloacetate accounted for approximately one third of the gluconeogenic flux, and this was increased fourfold to fivefold in the hyperthyroid state.[48] However, our results indicated similar rates of tritium release from [6-^{3}H]glucose or of ^{14}C from [U-^{14}C]glucose in euthyroid and hyperthyroid cells, making unlikely an increase in pyruvate-oxaloacetate cycling in the hyperthyroid state. We have previously not observed this cycling.[19] In an earlier study, a minor degree of cycling of triglyceride and fatty acid was indicated in the liver of thyroxine-treated rats.[49] Consistent with this is the finding that thyroid hormones increase lipogenesis in the rat,[37,50] and both adipose tissue and liver have been implicated in this process.[1,50] On the other hand, the metabolic cost of the increased lipogenesis in hyperthyroid rats accounted for only 3% to 4% of the thyroid hormone–induced increase in calorigenesis.

Accumulation of lactate to a steady-state concentration has previously been reported for euthyroid hepatocytes[14,27] and reflects a balance between rates of formation and removal of both pyruvate and lactate.[14] The further metabolism of lactate and pyruvate leads to CO_2 and amino acid production and to glucose formation.[13,14] The rate of cycling of pyruvate and lactate back to glucose increases as lactate and pyruvate accumulate and becomes maximal when lactate and pyruvate levels reach steady state. Given the similar glycolytic rates in hypothyroid, euthyroid, and hyperthyroid cells, the greater accumulation of lactate in hypothyroid cells must reflect a lower rate of removal of lactate and pyruvate, whereas maintenance of a much lower lactate concentration in hyperthyroid cells reflects a high rate of removal of these metabolites. Euthyroid cells show an intermediate position. Since similar mitochondrial [NADH]/[NAD^+] ratios in hyperthyroid and hypothyroid cells indicated that mitochondrial electron transport was not impaired in hypothyroid cells metabolizing glucose despite a much lower respiration rate than in hyperthyroid cells, dependence of the steady-state concentration of lactate on thyroid status may reflect an increased activity of the pyruvate carrier in response to thyroid hormone, as has been observed in rat heart.[51] In that study, the $[S]_{0.5}$ of approximately 200 μmol/L for mitochondrial pyruvate uptake was unaffected by thyroid status, whereas the

maximal rate was increased in the hyperthyroid state as compared with the normal.[51] The higher rates of formation of $^{14}CO_2$ from [U-^{14}C]glucose in hyperthyroid than in hypothyroid hepatocytes support this view, as does the requirement for a threefold higher concentration of lactate in hypothyroid as compared with hyperthyroid hepatocytes to achieve similar rates of cycling of pyruvate to glucose. Alternatively, the different steady-state concentrations of lactate may reflect a partial lack of coupling of ATP synthesis to respiration in hyperthyroid cells,[19,47] resulting in a stimulation of pyruvate oxidation, respiration, and $^{14}CO_2$ formation from [U-^{14}C]glucose.

In conclusion, although glucose causes a much greater increase in respiration rate in hyperthyroid than in hypothyroid hepatocytes, the observed thyroid hormone–induced increase in hepatic cycling of glucose was quantitatively small. A more complete explanation for the reported increase in glucose cycling in hyperthyroid rats in vivo[15,16] may well involve a participation between muscle and liver (Cori cycle). In keeping with this are the observations that thyroid hormone administration leads to both an increased glycolytic rate in muscle[52,53] and an increased gluconeogenetic rate in liver.[1,54]

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