Effects of hypothyroidism on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat

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1. The effects of hypothyroidism on the sensitivity of glycolysis and glycogen synthesis to insulin were investigated in the isolated, incubated soleus muscle of the rat. 2. Hypothyroidism, which was induced by administration of propylthiouracil to the rats, decreased fasting plasma levels of free fatty acids and increased plasma levels of glucose but did not significantly change plasma levels of insulin. 3. The sensitivity of the rates of glycogen synthesis to insulin was increased at physiological, but decreased at supraphysiological, concentrations of insulin. 4. The rates of glycolysis in the hypothyroid muscles were decreased at all insulin concentrations studied and the EC_{50} for insulin was increased more than 8-fold; the latter indicates decreased sensitivity of this process to insulin. However, at physiological concentrations of insulin, the rates of glucose phosphorylation in the soleus muscles of hypothyroid rats were not different from controls. This suggests that hypothyroidism affects glucose metabolism in muscle not by affecting glucose transport but by decreasing the rate of glucose 6-phosphate conversion to lactate and increasing the rate of conversion of glucose 6-phosphate to glycogen. 5. The rates of glucose oxidation were decreased in the hypothyroid muscles at all insulin concentrations.

INTRODUCTION

Previous studies have suggested that thyroid hormones influence the effect of insulin on the rate of glucose metabolism: in hyperthyroidism, the sensitivity of the rate of glucose production to insulin is decreased [1–6] whereas that of glucose uptake by skeletal muscle is increased [4,7]; in hypothyroidism, the rate of glucose production from the liver is decreased [8–11]. However, the effects of hypothyroidism on the sensitivity and responsiveness of glucose utilization to insulin have not been systematically studied. In hypothyroid patients, after administration of either intravenous glucose or insulin, and in the perfused rat hindquarter, the rate of utilization of glucose is either normal or decreased [11–16]. The effects of insulin on glucose utilization in isolated adipocytes from hypothyroid patients or rats are variable: the rate of glucose utilization is reported to be normal, decreased or increased [17–19]. The effects of insulin on glucose utilization in skeletal muscle have been investigated in only one study: markedly decreased rates of glycolysis and glycogen synthesis in soleus muscles isolated from hypothyroid rats were reported [19]. However, these experiments were performed in the presence of supraphysiological concentrations of insulin. The glycogen content is increased in soleus muscles isolated from hypothyroid rats [20,21] and it is normal in skeletal muscle of hypothyroid patients [22,23] which does not suggest that hypothyroidism should markedly decrease the sensitivity of the rate of this process to insulin. In view of the variability of the results in the above studies, it was decided to investigate the sensitivity of glucose utilization and glycogen synthesis to insulin in soleus muscles isolated from hypothyroid rats using a full range of insulin concentrations.

MATERIALS AND METHODS

Animals

Male Wistar rats (50–60 g and 160 g) were purchased from Harlane–Olac, Bicester, U.K., and they were kept in the Department's animal house with access to food and water *ad libitum*. Experimental hypothyroidism was induced by administration of 6-*n*-propyl-2-thiouracil (Sigma) for 4–5 weeks. The drug was dissolved in warm ethanol and was added in the drinking water at a final concentration of 0.5 mg/ml. Ethanol was also added in the drinking water of the control rats at the same final concentration (1.2%). Animals were fasted for 12–14 h prior to each experiment and they were killed by cervical dislocation.

Chemicals and enzymes

All chemicals, biochemicals and enzymes were obtained from sources given previously [24,25]. In addition, Dowex-2 (chloride form, dry mesh 200–400), aprotinin and amyloglucosidase were obtained from Sigma. D-[U- 14 C]Glucose and 2-deoxy-[2,6- 3 H]glucose were obtained from DuPont and Amersham International respectively. Sagatal (pentobarbitone) was obtained from May and Baker.

Incubation of muscles

Soleus muscle strips were prepared as previously described [25,26]. All muscles incubations were carried out in Krebs–Ringer bicarbonate buffer containing 5.5 mM-glucose, 1.5% (w/v) defatted bovine serum albumin, 0.5 μ Ci of D-[U- 14 C]glucose or 2-deoxy-[2,6- 3 H]glucose/ml and various concentrations of insulin (see the Results section and [27]).

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Metabolite and hormone determinations

The concentration of lactate in the incubation medium was assayed enzymically [28]. The rates of incorporation of ^{14}C -labelled glucose into glycogen (glycogen synthesis) and CO_2 were measured as previously described [29,30]. For calculation of the rates of $^{14}\text{CO}_2$ formation, the specific radioactivity of pyruvate was measured: the rates of CO_2 formation were never greater than 15% of the rate of lactate formation under any of the incubation conditions investigated. For determination of glycogen content the intact soleus muscles were digested for 1 h in 1 M-KOH at 70 °C. The digest was acidified with glacial acetic acid to pH 4.8, amyloglucosidase (in acetate buffer, pH 4.8) was added and the samples were incubated at 40 °C for 2 h to allow complete degradation of glycogen to glucose. The glucose concentration was measured enzymically [31,32].

The rates of hexose phosphorylation were determined using 2-deoxy[2,6- ^3H]glucose. A trace amount of 2-deoxy[2,6- ^3H]glucose was added to the incubation medium, which contained 5.5 mM-glucose. The muscles were incubated for 60 min. After incubation, muscles were removed, blotted and freeze-clamped, and the frozen muscles were powdered and homogenized in 6% (w/v) HClO_4 . Precipitated protein was removed by centrifugation. The supernatant was neutralized and 1 ml of that was placed on a 1.5 ml Dowex-2 (formate form) ion-exchange column to separate [^3H]deoxyglucose 6-phosphate from [^3H]deoxyglucose. The columns were washed first with 10 ml of distilled water containing 5 mM-D-glucose and then with 8 ml of 0.5 M-formic acid. Following the formic acid wash, the deoxyglucose 6-phosphate was eluted with 6 ml of 0.3 M-ammonium formate/1 M-formic acid. The radioactivity in 1 ml of samples of each eluate was measured in a liquid-scintillation counter [33,34]. The fluxes from glucose to hexose monophosphates (G , representing the sum of the rates of glucosyl units going into glycogen and through the glycolytic pathway) and from hexose monophosphates to glycogen (B) were calculated from the rates of total lactate formation (J), [^{14}C]lactate formation (λ), and ^{14}C incorporation into glycogen (γ) using the formulae:

$$G = [(J + B)/J]\lambda$$

and

$$B = J(\gamma/\lambda)$$

[35]. For measurements of the rate of conversion of glucose to lactate, [^{14}C]lactate was separated from other labelled compounds in the incubation medium using Dowex-2 ion-exchange chromatography [34].

For the determination of plasma levels of glucose [31], insulin [36], using rat insulin standards) and free fatty acids [37] the rats were anaesthetized with 40 μg of pentobarbitone/g body wt. and blood was taken from the iliac artery in a heparinized syringe. Blood was immediately centrifuged and plasma was stored at -20 °C with 1000 i.u. of aprotinin/ml until assayed. Plasma levels of tri-iodothyronine were measured using an Amerlex radioimmunoassay kit (Amersham International).

The results are presented as means $\pm$ S.E.M. Statistical significance was analysed using Student's non-paired t -test [38].

RESULTS AND DISCUSSION

It has been reported previously that changes in the rates of glucose transport in adipocytes isolated from hypothyroid rats may depend on the age of the rats when the hypothyroid treatment was initiated [17,19]. For this reason, both young and older rats were made hypothyroid (the body weights after treatment were 106 ± 2 g and 176 ± 2 g, respectively) and the results were compared to those obtained with age and weight-matched controls. However, no statistically significant differences with age were observed; therefore, results obtained from the soleus muscle incubations from the two types of animals, at the same insulin concentrations, are combined in Tables 2-4.

Hypothyroidism decreased the plasma levels of free fatty acids and increased those of glucose (Table 1). No statistically significant differences were observed in the plasma levels of insulin (Table 1). However, the rats were sampled after an overnight fast and it is possible that the insulin concentration in the fed state is increased.

Rates of glycogen synthesis

Hypothyroidism had no effect on the rate of glycogen synthesis at 1 μunit of insulin/ml but it decreased the rates at 1000 and 10000 μunits of insulin/ml in the isolated soleus muscle preparation (Table 2). Thus, at supraphysiological concentrations of insulin, the effect of insulin on the rate of glycogen synthesis in skeletal muscle is decreased in hypothyroidism, in confirmation of previous findings [19]. However, at physiological concentrations of insulin, the rates of glycogen synthesis were either normal (at 100 μunits of insulin/ml) or increased (at 10 and 40 μunits of insulin/ml) (Table 2). The latter finding is consistent with an increase in the glycogen content of the soleus muscles removed from hypothyroid animals in the postabsorptive state (4.99 ± 0.32 and 8.04 ± 0.89 mg of glycogen/g wet wt. in

Table 1. Effects of hypothyroidism on the plasma concentrations of tri-iodothyronine (T_3), glucose, insulin and free fatty acids in the rat

Values are presented as means $\pm$ S.E.M. with the number of separate animals given in parentheses. The statistical significance of the difference from controls is indicated by $^a P < 0.05$ and $^c P < 0.001$.

Animals	Plasma concentrations			
	T_3 (ng/ml)	Glucose (mg/dl)	Insulin ($\mu\text{units}/\text{ml}$)	Free fatty acids (mM)
Controls	0.80 ± 0.04 (5)	96 ± 3 (5)	15.50 ± 1.8 (5)	0.185 ± 0.02 (5)
Hypothyroid	0.05 ± 0.01 (11) ^c	104 ± 1 (5) ^a	9.84 ± 1.8 (5)	0.117 ± 0.01 (5) ^a

Table 2. Effects of hypothyroidism on the rates of lactate formation, glycogen synthesis and glucose oxidation in the stripped soleus muscle at various insulin concentrations

Values are presented as means $\pm$ S.E.M. with the number of separate incubations shown in parentheses. The statistical significance of the difference from controls is indicated by ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$.

Insulin (μ units/ml)	Rates of lactate formation (μ mol $\cdot$ h ⁻¹ $\cdot$ g ⁻¹)	
	Controls	Hypothyroid
1	9.41 $\pm$ 0.52 (17)	6.87 $\pm$ 0.50 (16) ^c
10	9.02 $\pm$ 0.35 (21)	7.01 $\pm$ 0.39 (16) ^c
40	10.68 $\pm$ 0.50 (6)	6.32 $\pm$ 0.36 (5) ^c
100	11.13 $\pm$ 0.62 (20)	7.60 $\pm$ 0.43 (14) ^c
1000	13.35 $\pm$ 0.65 (19)	7.99 $\pm$ 0.39 (15) ^c
10000	13.60 $\pm$ 0.85 (15)	8.27 $\pm$ 0.31 (16) ^c
	Rates of glycogen formation (μ mol of glucosyl units $\cdot$ h ⁻¹ $\cdot$ g ⁻¹)	
	Controls	Hypothyroid
1	2.49 $\pm$ 0.22 (19)	2.62 $\pm$ 0.19 (19)
10	2.53 $\pm$ 0.18 (22)	3.85 $\pm$ 0.36 (15) ^b
40	3.24 $\pm$ 0.08 (5)	3.94 $\pm$ 0.14 (5) ^b
100	5.29 $\pm$ 0.29 (23)	5.41 $\pm$ 0.38 (16)
1000	7.33 $\pm$ 0.37 (19)	5.95 $\pm$ 0.37 (16) ^a
10000	7.66 $\pm$ 0.40 (11)	6.65 $\pm$ 0.26 (12) ^a
	Rates of glucose oxidation (μ mol $\cdot$ h ⁻¹ $\cdot$ g ⁻¹)	
	Controls	Hypothyroid
1	0.781 $\pm$ 0.09 (8)	0.489 $\pm$ 0.08 (7) ^a
10	0.747 $\pm$ 0.07 (7)	0.365 $\pm$ 0.02 (6) ^c
100	0.867 $\pm$ 0.07 (8)	0.558 $\pm$ 0.08 (7) ^a
1000	0.898 $\pm$ 0.08 (7)	0.644 $\pm$ 0.10 (7) ^b
10000	0.918 $\pm$ 0.08 (7)	0.493 $\pm$ 0.03 (7) ^c

control and hypothyroid muscles respectively; the difference is statistically significant, $P < 0.01$); it is also supported by a recent report of increased activity of glycogen synthase in extracts of muscle from hypothyroid rats [39].

The rates of glycogen synthesis were also calculated from the rates of total lactate formation and [¹⁴C]glucose conversion to [¹⁴C]lactate (see the Methods section): qualitatively similar changes to those described above were obtained (Table 3).

Rates of glycolysis, glucose phosphorylation and glucose oxidation

The rate of lactate formation (glycolysis) at all insulin concentrations was decreased in soleus muscles from hypothyroid rats (Table 2). The concentration of insulin required to stimulate glycolysis half-maximally (EC_{50}), which is an index of the sensitivity of this process to insulin, was calculated from these data, assuming that the rate of lactate formation at 10000 μ units of insulin/ml is maximal. The value of the EC_{50} increased more than 8-fold in muscles from the hypothyroid rats (108 ± 19 and 879 ± 177 μ units/ml in control and hypothyroid muscles respectively; the difference is statistically significant, $P < 0.001$). This suggests that hypothyroidism decreases the sensitivity of glycolysis to insulin. It is considered that 10000 μ units/ml is maximal or near-maximal since it has been reported that very high

Table 3. Effects of hypothyroidism on the fluxes of glucose to hexose monophosphate (G) and hexose monophosphate to glycogen (B) in the stripped soleus muscle of the rat at various insulin concentrations

Values are presented as means $\pm$ S.E.M. with the number of separate incubations shown in parentheses. The statistical significance of the difference from controls is indicated by ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$.

Insulin (μ units/ml)	Flux of glucose to hexose monophosphate (μ mol of glucosyl units $\cdot$ h ⁻¹)	
	Controls	Hypothyroid
1	6.20 $\pm$ 0.39 (9)	7.00 $\pm$ 0.40 (9)
10	6.45 $\pm$ 0.45 (7)	8.02 $\pm$ 0.76 (8)
100	10.54 $\pm$ 0.69 (11)	9.36 $\pm$ 0.40 (16)
1000	12.50 $\pm$ 0.66 (11)	9.30 $\pm$ 0.49 (15) ^c
10000	12.67 $\pm$ 0.84 (6)	10.42 $\pm$ 0.31 (16) ^a
	Flux of hexose monophosphate to glycogen (μ mol of glucosyl units $\cdot$ h ⁻¹ $\cdot$ g ⁻¹)	
	Controls	Hypothyroid
1	3.90 $\pm$ 0.58 (8)	3.41 $\pm$ 0.33 (17)
10	3.24 $\pm$ 0.37 (11)	4.58 $\pm$ 0.52 (16) ^a
100	6.72 $\pm$ 0.50 (8)	6.15 $\pm$ 0.84 (14)
1000	10.40 $\pm$ 0.82 (10)	6.92 $\pm$ 0.65 (14) ^b
10000	10.26 $\pm$ 0.41 (4)	8.07 $\pm$ 0.74 (11) ^a

insulin concentrations (77 μ units/ml) did not increase the rate of glycolysis in the soleus muscles isolated from hypothyroid rats [19]. Indeed, if the rate of glycolysis in the hypothyroid muscles is higher at a higher concentration of insulin, the EC_{50} value calculated from the present results will be an underestimate.

The rates of glucose oxidation at all insulin concentrations were markedly decreased in muscles from hypothyroid rats (Table 2). These changes in soleus muscle, which has ~ 80 – 90 % Type I fibres, are likely to reflect changes in other muscles since hypothyroidism increases the proportion of Type I fibres in skeletal muscles of the rat [40].

The rate of glucose phosphorylation was measured directly by following the rate of accumulation of 2-deoxy[³H]glucose 6-phosphate in the muscle from 2-deoxy[³H]glucose added to the incubation medium (Table 4); it was also calculated from the rates of incorporation of [¹⁴C]glucose into glycogen and lactate (Table 3) (see the Methods section). There were no differences in the rates of glucose phosphorylation in the soleus muscles of hypothyroid rats compared to those in controls at 1, 10 and 100 μ units of insulin/ml, but the rates in the muscles from hypothyroid animals were decreased at supraphysiological levels of insulin (1000 and 10000 μ units/ml) (Tables 3 and 4). Hence, at physiological concentrations of insulin, hypothyroidism results in increased sensitivity of glycogen synthesis, decreased sensitivity of glycolysis but no change in the sensitivity of glucose phosphorylation to insulin. These findings suggest that, in skeletal muscle, this condition causes a marked redistribution of glucose residues away from the pathway of lactate formation towards that of glycogen synthesis. Furthermore, they suggest that

Table 4. Effects of hypothyroidism on the rates of ^3H -labelled 2-deoxyglucose phosphorylation in the stripped soleus muscle of the rat at various insulin concentrations

Values are presented as means $\pm$ S.E.M. with the number of separate incubations shown in parentheses. The statistical significance of the difference from controls is indicated by $^aP < 0.05$, and $^bP < 0.01$.

Insulin ($\mu\text{units/ml}$)	Rates of 2-deoxy-D- ^3H glucose phosphorylation (d.p.m. of 2-deoxy-D- ^3H glucose 6-phosphate $\cdot \text{h}^{-1} \cdot \text{mg}^{-1}$)	
	Controls	Hypothyroid
1	172 $\pm$ 10 (8)	152 $\pm$ 12 (7)
10	171 $\pm$ 13 (6)	191 $\pm$ 27 (8)
100	260 $\pm$ 17 (8)	258 $\pm$ 34 (8)
1000	331 $\pm$ 19 (8)	221 $\pm$ 23 (8) ^b
10000	357 $\pm$ 31 (8)	247 $\pm$ 39 (8) ^a

hypothyroidism has little effect on the sensitivity of glucose transport to insulin at physiological insulin concentrations. The decrease in glycolytic rate may be due to a decrease in activity of phosphofructokinase; recently it has been reported that the maximal activity of this enzyme was decreased in the heart of hypothyroid rats [41].

An increase in the sensitivity of the process of glycogen synthesis to insulin without an effect on glucose transport is possible because insulin affects these processes in different ways: the rate of glucose transport is increased by recruitment of more transporters to the cell membrane [42,43] whereas glycogen synthesis is influenced via covalent modification of glycogen synthase [44]. Furthermore, the stimulation of the rate of glycogen synthesis by insulin in the muscle can be metabolically independent of the effect of insulin on glucose transport, since glycogen synthase catalyses a flux-generating step [45]. Thus, adenosine receptor antagonists have previously been shown to increase the sensitivity of glucose transport and glycolysis to insulin without affecting the sensitivity of glycogen synthesis [46].

The effect of hypothyroidism on the binding of insulin to muscle was not studied in the present work since it was considered that the effects of hypothyroidism on the sensitivity of glucose metabolism in skeletal muscle to insulin are mediated through a postreceptor mechanism(s). The evidence in favour of this view is as follows: in comparison to controls, insulin has opposite effects on the rates of glycogen synthesis and glycolysis in the soleus muscles of hypothyroid rats (Table 2) although stimulation of both processes presumably requires the binding of insulin to its receptor; at physiological concentrations, insulin increased the rates of glucose phosphorylation in muscles from hypothyroid rats to the same extent as in muscles from control animals (Tables 3 and 4), which suggests that hypothyroidism did not change the binding of insulin to its receptor. Finally, in recent studies using adipocytes from hypothyroid subjects and rats, although the binding of insulin to its receptor

was decreased [47] or unchanged [19], the inhibition of the effect of insulin on glucose utilization was always much more pronounced, suggesting postreceptor defects. The biochemical mechanisms by which, in skeletal muscle, hypothyroidism decreases the sensitivity of glycolysis to insulin at all concentrations of the hormone but increases the sensitivity of glycogen synthesis to insulin at physiological levels of insulin are currently under investigation.

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