Sensitivity of the soleus muscle to insulin in resting and exercising rats with experimental hypo- and hyper-thyroidism

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1. The effects of hypothyroidism (caused by surgical thyroidectomy followed by treatment for 1 month with propylthiouracil) and of hyperthyroidism [induced by subcutaneous administration of L-tri-iodothyronine (T_3)] on glucose tolerance and skeletal-muscle sensitivity to insulin were examined in rats. Glucose tolerance was estimated during 2 h after subcutaneous glucose injection (1 g/kg body wt.). The sensitivity of the soleus muscle to insulin was studied *in vitro* in sedentary and acutely exercised animals. 2. Glucose tolerance was impaired in both hypothyroid and hyperthyroid rats in comparison with euthyroid controls. 3. In the soleus muscle, responsiveness of the rate of lactate formation to insulin was abolished in hypothyroid rats, whereas the sensitivity of the rate of glycogen synthesis to insulin was unchanged. In hyperthyroid animals, opposite changes were found, i.e. responsiveness of the rate of glycogen synthesis was inhibited and the sensitivity of the rate of lactate production did not differ from that in control sedentary rats. 4. A single bout of exercise for 30 min potentiated the stimulatory effect of insulin on lactate formation in hyperthyroid rats and on glycogen synthesis in hypothyroid animals. 5. The data suggest that thyroid hormones exert an interactive effect with insulin in skeletal muscle. This is likely to be at the post-receptor level, inhibiting the effect of insulin on glycogen synthesis and stimulating oxidative glucose utilization.

INTRODUCTION

In hypothyroidism, glucose tolerance and whole-body sensitivity to insulin are reduced [1–3]. In hyperthyroidism, glucose tolerance has also been found to be impaired [1,4], despite the increased glucose turnover [5,6] and elevated plasma insulin concentration [7]. Although the muscle constitutes the major site of glucose disposal, there have been few studies concerning the possible interaction of thyroid hormones with insulin in skeletal muscle. Czech et al. [8] demonstrated a diminished capacity of muscle to utilize glucose in the presence of insulin *in vitro* in rats made hypothyroid by administration of propylthiouracil. Recently, Dimitriadis et al. [9] reported that, in rats treated with tri-iodothyronine (T_3) for 5 or 10 days, the sensitivity of glycolysis and glucose oxidation in muscle to insulin are increased, but the sensitivity of glycogen synthesis in the same animals is reduced.

It was found in this laboratory that in normal rats, physical exercise enhances the sensitivity of soleus muscle to insulin [10]. There are no data, however, on whether this effect also occurs under conditions of thyroid-hormone excess or deficit.

Thus, the present study was designed (1) to compare the sensitivity of skeletal muscle to insulin in euthyroid, hypothyroid and hyperthyroid rats using the methodology of evaluating the action of insulin on the soleus muscle *in vitro*, and (2) to estimate the effect of physical exercise on the muscle insulin sensitivity in those three conditions.

MATERIALS AND METHODS

Male Wistar rats ($n = 180$) weighing 200 ± 20 g were used. They were housed in the Department's animal house and kept in groups in a temperature-controlled room at 22 °C with a light period from 06.00 to 18.00 h The animals were fed a standard laboratory rat chow and had access to water *ad libitum*. Sixty rats were surgically thyroidectomized, and then treated for 30 days with propylthiouracil (Frosst, Quebec, Canada), which was dissolved in drinking water (0.04 %) (hypothyroid group); 60 animals were injected subcutaneously with T_3 (L-tri-iodothyronine sodium B.P.; Glaxo Laboratories, Greenford, Middlesex, U.K.) at a dose of 75 µg/100 g body wt. daily for 3 days; while the remaining group of 60 rats served as controls.

Glucose tolerance was determined in 10 animals from each group after subcutaneous injection of 1 g of glucose per kg body wt. This simple method of glucose administration to rats gives reproducible results for glucose tolerance [11]. Blood samples for determining blood glucose (using the hexokinase method) were taken from the cut tail before glucose administration and then at 15, 30, 45, 60, 75, 90 and 120 min following the glucose load.

The remaining rats of each group were divided into two subgroups. One sub-group ($n = 25$) remained sedentary, whilst the other ($n = 25$) was exercised on a treadmill for 30 min (at 20 m·min^{-1}, 0° inclination) before being killed. Soleus muscles from both hindlimbs were taken from each rat immediately after decapitation,

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Abbreviation used: T_3, tri-iodothyronine.
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divided into two strips of 25–35 mg each, attached to steel clips, and transferred directly to 5 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 1 % defatted bovine serum albumin and 5 mM-glucose. The muscle strips were preincubated for 15 min at 37 °C, and then transferred to another flask containing [U-^{14}C]glucose (0.25 μCi·ml^{-1}) plus insulin at various concentrations (1–10^4 μunits·ml^{-1}) and incubated for a further 60 min. The flasks were gassed continuously with O_2/CO_2 (19:1) both during the preincubation and for the first 45 min of the 60 min incubation period. At the end of incubation, the muscles were removed from the flasks and freeze-clamped. [U-^{14}C]Glucose incorporation into glycogen was assessed according to Espinal et al. [12]. The perchloric acid-deproteinized medium was neutralized with KOH, and the precipitated KClO$_4$ was removed by centrifugation. In the supernatant, lactate concentration was determined spectrophotometrically, using the enzymic method [12].

Muscle insulin sensitivity was expressed as the concentration of insulin required to induce the half-maximal stimulation of lactate formation or glycogen synthesis. The results were obtained by the computer log–logit transformation of the relationship between insulin concentration in the incubation medium and the magnitude of the response, as described by Stupnicki [13].

In the majority of rats, blood samples were taken for estimation of the serum T$_3$, thyroxine and insulin concentrations by radioimmunoassay using available commercial kits (Institute of Atomic Energy, Swierk, Poland).

Statistics

Student's t test for unpaired data was used to evaluate the statistical significance of differences. The null hypothesis was rejected when $P < 0.05$. The results are presented as means ± S.E.M. throughout the paper.

RESULTS

Serum hormone concentrations

Serum T$_3$ and thyroxine concentrations were significantly reduced in hypothyroid rats (Table 1). In hyperthyroid animals the level of circulating T$_3$ was significantly elevated while that of thyroxine was diminished in comparison with controls. The basal serum insulin concentration was significantly increased in the hyperthyroid group.

Glucose tolerance

The initial blood glucose concentration was significantly lower ($P < 0.05$) in hypothyroid and higher ($P < 0.02$) in hyperthyroid rats in comparison with euthyroid controls (Fig. 1). The peak values attained
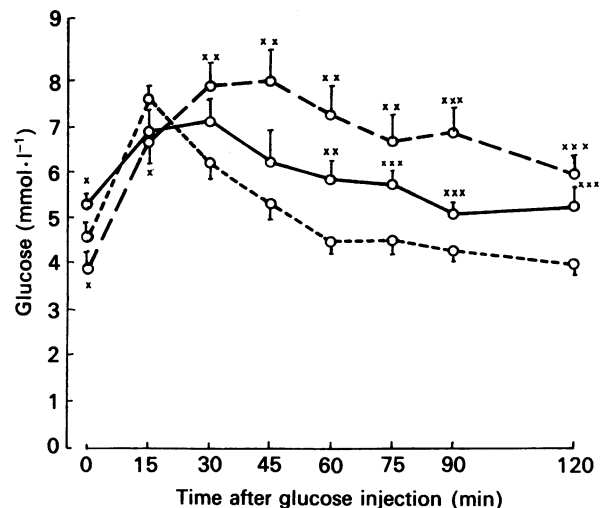
Fig. 1. Blood glucose concentration within 120 min of sub-cutaneous glucose injection in hypothyroid, hyperthyroid and control rats

The glucose injection was 1 g per kg body wt. ---, Hypothyroid; ——, hyperthyroid; ----, control (euthyroid). Values are means ± S.E.M. ($n = 10$). The significance of differences between experimental and control animals is marked by: $^xP < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$.

after glucose injection were similar ($P > 0.05$) in all groups; however, the rate of glucose decline was attenuated in both hypothyroid and hyperthyroid rats. The sums of glucose concentrations, calculated during 15–120 min following glucose injection, were significantly ($P < 0.001$) greater in hypothyroid (49.2 ± 0.5 mmol·l^{-1}) and in hyperthyroid rats (42.3 ± 0.5 mmol·l^{-1}) than in euthyroid animals (36.4 ± 0.5 mmol·l^{-1}).

Soleus muscle sensitivity to insulin in hypothyroid rats

In resting hypothyroid rats neither the unstimulated nor the insulin-stimulated rates of glycogen synthesis differed from those in controls (Fig. 2, Table 2). The post-exercise rate of glycogen synthesis in the soleus muscle was enhanced ($P < 0.001$), and the sensitivity of this process to insulin increased significantly in relation to the resting values both in hypothyroid and in control rats. However, this effect was more pronounced in the former group. In contrast with the results for euthyroid rats, no stimulation of lactate formation by insulin was found in the soleus muscles derived from hypothyroid rats. Exercise had no effect on the lactate formation response to insulin in either hypothyroid or control rats.

Table 1. Plasma concentrations of T$_3$, thyroxine (T$_4$) and insulin (IRI) at rest in control, hypothyroid and hyperthyroid animals

Values are means ± S.E.M. with the number of animals (n) in parentheses. Asterisks indicates values significantly different from the control group: $^*P < 0.05$, $^{***}P < 0.001$.

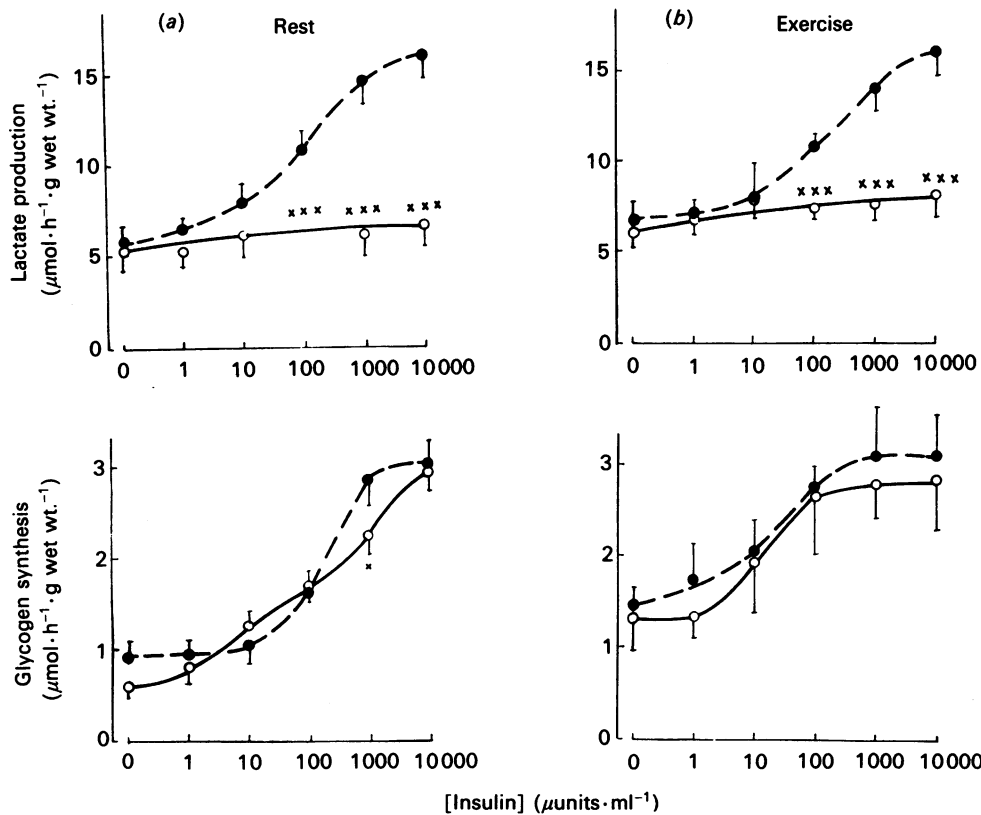| Hormone	Control	Hypothyroid	Hyperthyroid
T$_3$ (ng·ml^{-1})	1.48 ± 0.15 (40)	0.55 ± 0.039*** (29)	12.70 ± 2.57*** (27)
T$_4$ (ng·ml^{-1})	62.40 ± 2.41 (40)	39.79 ± 2.50*** (29)	31.42 ± 1.71*** (31)
IRI (μunits·ml^{-1})	33.07 ± 1.70 (34)	28.86 ± 2.22 (20)	42.48 ± 3.98* (20)

Fig. 2. Glycogen synthesis and lactate production responses to various concentrations of insulin in the soleus muscle from euthyroid (●) and hypothyroid (○) rats

Each point represents the mean value ($\pm$ s.e.m.) of 10–12 muscle preparations. Significant differences in the values of unstimulated lactate formation and glycogen synthesis between the control and hypothyroid rats are marked by: $^{\times}P < 0.05$, $^{\times\times}P < 0.01$, $^{\times\times\times}P < 0.001$.

Table 2. Sensitivity of lactate formation and glycogen synthesis to insulin in control, hypothyroid and hyperthyroid rats

Values are presented as logarithms of insulin concentration producing the half-maximal effect ($\pm$ s.e.m.), with the corresponding means re-transformed from logarithms (μunits·ml^{-1}) in parentheses. Asterisks denote significance of differences between euthyroid control and experimental groups: $*P < 0.05$, $***P < 0.001$. $\dagger(P < 0.001)$ denotes significance of differences between resting and exercising rats within the same group. L.R., lack of responsiveness.

Group	Resting conditions		After exercise	
	Lactate formation	Glycogen synthesis	Lactate formation	Glycogen synthesis
Control	1.97 ± 0.11 (94.0)	2.28 ± 0.07 (189.2)	2.13 ± 0.11 (133.7)	$1.54 \pm 0.09\dagger$ (34.7)
Hypothyroid	L.R.	2.21 ± 0.16 (161.8)	L.R.	$1.20 \pm 0.09\dagger*$ (15.9)
Hyperthyroid	2.00 ± 0.06 (99.1)	L.R.	$0.59 \pm 0.33\dagger***$ (3.9)	L.R.

Soleus-muscle sensitivity to insulin in hyperthyroid rats

The rate of glycogen synthesis in resting hyperthyroid rats was significantly reduced in comparison with control animals (Table 2; Fig. 3). No response of this process to insulin was found in either resting or exercising T_3-treated rats, although a tendency towards higher rates of glycogen synthesis without insulin was noted in muscles taken from exercised rats ($P > 0.05$).

The unstimulated rate of lactate formation in the soleus muscle derived from hyperthyroid sedentary rats was significantly higher than in controls ($P < 0.01$). However, the sensitivity of lactate production to insulin in hyperthyroid rats did not differ significantly from the control values (Table 1). Endurance exercise did not change the unstimulated rate of lactate formation in hyperthyroid rats, but markedly enhanced the sensitivity of this process to insulin.
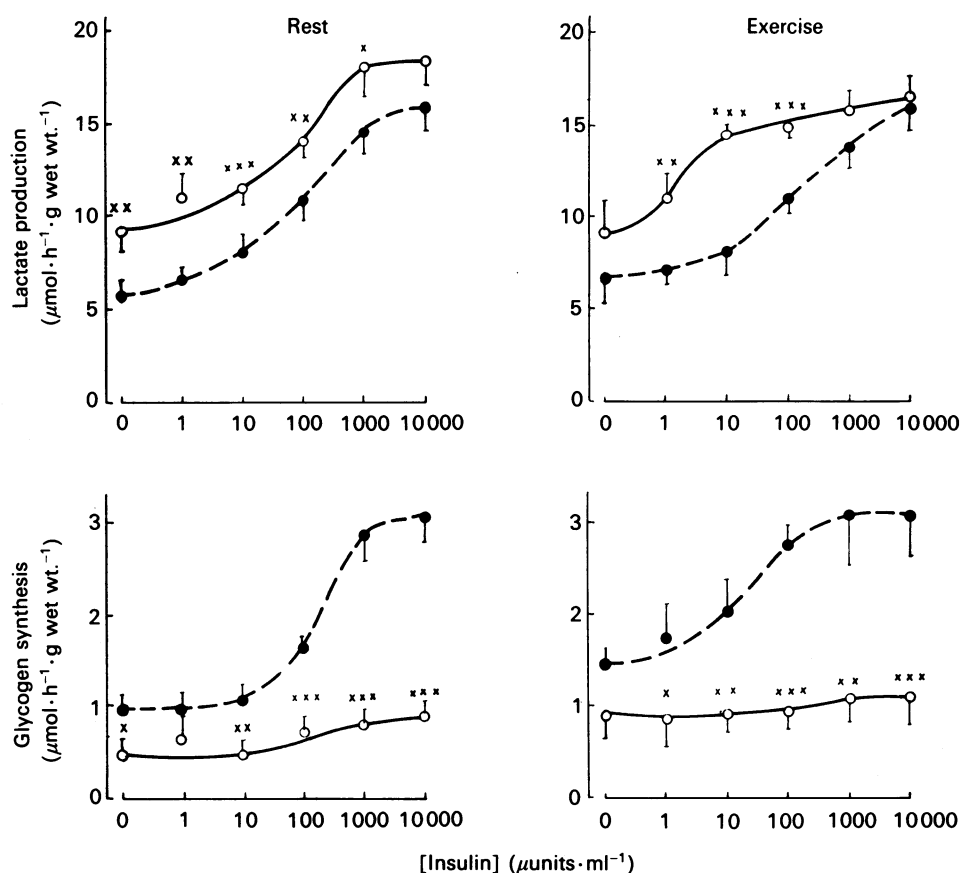
Fig. 3. Lactate-production and glycogen-synthesis responses to various concentrations of insulin in the soleus muscle from euthyroid (●) and hyperthyroid (○) rats

Each point represents the mean value ($\pm$ S.E.M.) of 10–12 muscle preparations. Significant differences in the values of unstimulated lactate formation and glycogen synthesis between the control and hyperthyroid rats are marked by: $^{x}P < 0.05$, $^{xx}P < 0.01$, $^{xxx}P < 0.001$.

DISCUSSION

In agreement with previous reports [1–3], the present study demonstrates that thyroid hormone deficiency impairs glucose tolerance. This was associated with a dramatic decrease in the responsiveness of lactate formation to insulin in the soleus muscle, which may indicate resistance of glucose transport to insulin. However, as suggested by Czech et al. [8], the possibility of an impaired glycolytic-enzyme system in hypothyroid animals cannot be excluded. There was no difference between the thyroid-hormone-deficient and euthyroid rats in the sensitivity of glycogen synthesis to insulin. Moreover, exercise performed by hypothyroid rats increased the sensitivity of glycogen synthesis to insulin to a greater extent than in controls.

In rats injected with T_3, basal blood glucose and insulin concentrations were elevated and glucose tolerance was impaired. The responsiveness of glycogen synthesis to insulin was abolished by T_3 administration. It is not apparent why in our work this effect was much more pronounced than in the studies by Dimitriadis et al. [9], in spite of similar doses of the hormone being administered. It cannot be excluded that subcutaneous administration of T_3 causes more sustained elevation of circulating T_3 than the intraperitoneal hormone injection used by Dimitriadis et al. [9]. The basal rate of muscle

lactate production was enhanced in T_3-injected rats, but the sensitivity of this process to insulin was increased. This may be related to the exercise-induced catecholamine release and an interaction of T_3 with the β-adrenergic receptors. Challiss et al. [14] have demonstrated that a β-adrenergic agonist increases the soleus muscle glucose utilization in response to insulin. Another possibility is that exercise decreases adenosine concentration in the muscle or reduces the sensitivity of adenosine receptors. Participation of adenosine in the control of insulin action on glucose transport and the rate of glycolysis has been well documented [15,16]. Furthermore, the increased sensitivity of lactate formation to insulin in rats after 10 days of T_3 treatment was found to be associated with a marked decrease in the muscle adenosine concentration [9].

The data obtained in the present study indicate that any deviation from the euthyroid state alters the responsiveness of muscle glucose metabolism to insulin. Under conditions of thyroid-hormone deficiency, insulin stimulation of lactate formation is abolished, while in the animals with experimental hyperthyroidism there is no responsiveness of glycogen synthesis to insulin. However, under each condition, one of the processes remains sensitive to insulin, and physical exercise enhances the insulin effect on this process to a greater extent than in euthyroid animals. In the case of T_3 excess this is

utilization of glucose in the glycolytic pathway, and in the case of the thyroid-hormone deficit it is glucose incorporation into glycogen. Therefore, it appears that an optimal level of thyroid hormones is necessary for maintaining a balance between the insulin-stimulated catabolic and anabolic utilization of glucose by skeletal muscles. The apparent dissociation between the changes in the responsiveness to insulin of lactate formation and glycogen synthesis in hypo- and hyper-thyroidism respectively suggests a post-receptor site of thyroid-hormone interaction with insulin.

REFERENCES

1. Lambreg, B. (1965) Acta Med. Scand. **178**, 351–362
2. Katsilambros, N., Ziegler, H., Schatz, H., Hinz, M., Maier, V. & Pfeiffer, E. F. (1972) Horm. Metab. Res. **4**, 377–379
3. Renold, A. E., Sverdlik, R. C., Andrade, L. L. & Rodriguez, R. R. (1972) Horm. Metab. Res. **4**, 373–376
4. Doar, J., Stamp, T., Wynn, V. & Audhya, T. (1969) Diabetes **18**, 633–639
5. Okajima, F. & Ui, M. (1979) Biochem. J. **182**, 565–575
6. Müller, M. & Seitz, H. (1980) Pflügers Arch. **386**, 47–52
7. Dimitriadis, G., Baker, B., Marsh, H., Mandarino, L., Rizza, R., Bergman, R., Haymond, M. & Gerich, J. (1985) Am. J. Physiol. **248**, E593–E601
8. Czech, M. P., Malbon, C. C., Kerman, K., Gitomer, W. & Pilch, P. E. (1980) J. Clin. Invest. **66**, 574–582
9. Dimitriadis, G. D., Leighton, B., Vlachonikolis, J. G., Parry-Billings, M., Challiss, R. A. J. & Newsholme, E. A. (1988) Biochem. J. **253**, 87–92
10. Langfort, J., Budohoski, L. & Newsholme, E. A. (1988) Pflügers Arch. **412**, 101–105
11. Budohoski, L., Challiss, R. A. J., Dubaniewicz, A., Kaciuba-Uściłko, H., Leighton, B., Lozeman, F. J., Nazar, K., Newsholme, E. A. & Porta, S. (1987) Biochem. J. **244**, 655–660
12. Espinal, J., Dohm, G. L. & Newsholme, E. A. (1983) Biochem. J. **212**, 453–458
13. Stupnicki, R. (1982) Endocrinologie **80**, 48–51
14. Challiss, R. A. J., Budohoski, L., Newsholme, E. A., Sennitt, M. V. & Cawthorne, M. A. (1985) Biochem. Biophys. Res. Commun. **128**, 928–935
15. Budohoski, L., Challiss, R. A. J., McManus, B. & Newsholme, E. A. (1984) FEBS Lett. **167**, 1–4
16. Challiss, R. A. J., Leighton, B., Lozeman, F. J. & Newsholme, E. A. (1987) in Topics and Perspectives in Adenosine Research (Gerlach, E. & Becker, B. F., eds.), pp. 275–285, Springer-Verlag, Berlin and Heidelberg