Response of glucose disposal to hyperinsulinaemia in human hypothyroidism and hyperthyroidism

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ABSTRACT

We have examined insulin action on glucose metabolism in six hypothyroid patients before and after regular thyroid hormone treatment, and in six healthy volunteers before and after transient induction of moderate hyperthyroidism. Insulin was infused under euglycaemic and eukalaemic clamps. An appropriate amino acid infusion was used to blunt insulin-induced decreases in amino acid levels. Glucose kinetics were assessed using a primed continuous infusion of [6,6-2H2]glucose. The results showed that basal plasma insulin and glucose levels (i.e. before infusion) were similar in each case. Despite similar insulin infusion rates, the plateau value of insulin was lower after thyroid treatment in both hypothyroid patients and healthy volunteers. The rate of exogenous glucose needed to maintain plasma glucose at a steady-state level was increased by thyroid hormone in hypothyroid patients (\(P < 0.05\)), but not in healthy volunteers. Thyroid treatment resulted in a significant increase in basal glucose disposal in both groups (\(P < 0.05\)). Insulin, in conjunction with glucose and amino acids, significantly stimulated glucose disposal (\(P < 0.05\)) under all conditions. The incremental increase in glucose disposal after infusion tended to be higher following thyroid hormone treatment, but this was not statistically significant. However, the ratio of the incremental increase in glucose disposal to the increase in plasma insulin was significantly improved after thyroid hormone treatment in hypothyroid patients (\(P < 0.05\)). It was also increased in healthy volunteers, but not significantly. We conclude that thyroid hormones improve the ability of insulin to stimulate glucose disposal related to insulinemia. This phenomenon may be highly sensitive, because it was only apparent at low thyroid hormone levels.

INTRODUCTION

Thyroid hormone status is important for glucose homeostasis. Thyrotoxic subjects are characterized by elevated glucose production and glycogenolysis (see [1] for a review). Glucose turnover and oxidation rates are increased, whereas non-oxidative glucose turnover has been estimated as being unchanged [2], decreased [3] or increased [4]. In contrast, glucose production has more often been shown to be decreased in hypothyroidism [3,5,6], whereas glucose utilization is reduced [5]. Glucose recycling at the level of three-carbon compounds (i.e. the Cori cycle and the glucose alanine cycle) and futile cycles between glucose and glucose 6-phosphate were partly inactivated by the induction of hypothyroidism [5,7].

Impaired glucose tolerance has long been recognized as a frequent complication of hyperthyroidism. This alteration may involve changes in both insulin secretion and degradation in humans (see [2] for a review). Moreover,
using the regular euglycaemic hyperinsulinaemic clamp, it was demonstrated that there is an impairment in the insulin-induced suppression of glucose production in hyperthyroid patients [2,8]. At low insulin levels, insulin-stimulated glucose disposal is usually unaffected [2,8–11], whereas it has been reported to be decreased [2], unchange in [8,10,12,13] or even increased [9,11,14,15] at high insulin levels. Other researchers reported that thyroid hormones blunted the insulin-induced increases in the total distribution volume of the exchanging pool of glucose, possibly reflecting an acceleration of intracellular glucose degradation [10].

Based on these clamp studies, the effects of thyroid hormones on insulin action in humans appear rather inconsistent. Part of these discrepancies may be related to patient heterogeneity and variations in thyroid hormone treatment (see discussion in [4]). Indeed, the actions of thyroid hormones are strongly dose-dependent. In addition, the interaction with insulin action depends on the prevailing glucose concentration [9]. All studies have been performed in patients with clinical thyrotoxicosis and in thyroid hormone-treated healthy volunteers. We hypothesized that investigations over a wider range of thyroid hormone status by including hypothyroid patients would be beneficial for increasing our understanding of the effects of thyroid hormones on insulin action in humans. In rats, thyroid hormone deficiency has been associated with a dramatic decrease in the responsiveness of glucose uptake, lactate formation and glycogen synthesis to insulin in skeletal muscle [16–19]. In contrast, treatment of hypothyroid rats with 3,3'-5-triiodothyronine (T3) stimulated basal, and to some extent insulin-stimulated, glucose uptake in skeletal muscle [20]. This induction was shown to be due primarily to an increase in GLUT4 protein expression [21].

Thus we decided to clarify the effects of thyroid hormones on the action of insulin on glucose metabolism in vivo using the euglycaemic potassium insulin clamp in two separate experiments. One experiment was performed in hypothyroid patients before and after regular thyroid hormone treatment, and the other in normal subjects under controlled experimental hyperthyroidism. In the same experiments we also analysed the effects of insulin on protein metabolism in vivo [22,23]. Because amino acid supply is crucial for the effects of insulin on protein synthesis and degradation [24], the insulin-induced decrease in amino acid levels was blunted by concomitant amino acid infusion [22,23].

Patients (five women and one man) were studied in both the hypothyroid and euthyroid states during study I. The hypothyroid state was assessed either before thyroid hormone treatment for primary hypothyroidism (n = 2) related to Hashimoto’s thyroiditis or after a 6 week period of thyroid hormone withdrawal in order to perform post-operative scinti-scans for thyroid carcinoma (n = 4). All patients had undetectable plasma thyroglobulin levels and a negative scinti-scan. As expected, plasma thyroxine (T4) concentrations were lower in the latter group of patients. The euthyroid state was achieved by regular treatment with T3 for 5 months, which is the most widely used method in Europe. This treatment comprised a dose of 153 ± 17 μg of T3 (Levothyrox; Merck-Lipha, Lyon, France) taken orally every morning (mean 2.1 ± 0.2 μg · kg⁻¹; range 1.3–2.5 μg · kg⁻¹). Based on hormone assays, the patients displayed frank hypothyroidism before treatment. After 5 months of hormone treatment, plasma free T4 and T3 concentrations had increased substantially, while thyroid-stimulating hormone (TSH; thyrotrophic hormone) levels had decreased (Table 1). The euthyroid patients had a lowered TSH level compared with the normal range. A consequence of the regular treatment of hypothyroidism after thyroidectomy for cancer is always a slightly decreased level of TSH [22]. This is the result of the negative feedback of T4 treatment. Treatment resulted in a significant decrease in body weight (−2.5 ± 0.2 kg; P < 0.001). The resting heart rate increased with treatment (P < 0.05), and clinical symptoms of hypothyroidism were resolved (Table 1).

Healthy volunteers (study II) were studied in the control state and again after induction of an experimental hyperthyroid state. Younger adults than in the hypothyroid group were selected, to minimize the risk of cardiac side effects of hyperthyroidism. In addition, spontaneous hyperthyroidism commonly occurs in young adults. Hyperthyroidism was induced by the daily intake of 2 μg · kg⁻¹ T4 (one oral dose each morning) for 4 weeks; this treatment was continued for another 2 weeks in conjunction with a total daily intake of 1 μg · kg⁻¹ T3 (Cynomel; Merrel Dow, Levallois Perret, France), taken over three doses, to induce hyperthyroidism more rapidly. Hormone assays demonstrated expected values for healthy young adults in the control state (Table 1). After 4 weeks of hormone intake, plasma free T4 and T3 concentrations showed a clear-cut increase, while TSH had decreased. After 6 weeks, plasma T4 was frankly increased, while the elevation in T3 was maintained and TSH became undetectable. Thus the intake of thyroid hormones clearly induced biological hyperthyroidism. However, this induced hyperthyroidism was moderate compared with spontaneous pathological hyperthyroidism. Plasma free T4 concentrations were around the top of the normal range, and free T3 was twice the normal value. Clinical effects were also limited compared with

**METHODS**

**Subjects**

Experiments were performed in six patients (study I) and six healthy volunteers (study II).
spontaneous hyperthyroidism. There was a trend for a decrease in body weight (−1.1 ± 0.7 kg; not significant). However, a significant increase in resting heart rate was noted compared with the control state (P < 0.05). Most subjects mentioned increased appetite. Other side effects were limited to moderate nervousness (n = 4), hyperdefecation (n = 1) and insomnia (n = 1); in contrast, paradoxical hypsomnia was noted by one patient.

The study protocols were approved by the local Ethics Committee (Comité Consultatif pour la Protection des Personnes en Recherche Biomédicale d’Auvergne), and each subject gave written informed consent for the study.

**Materials**

[6,6-²H₂]Glucose (purchased from Mass Trace, Woburn, MA, U.S.A.), 99% pure and rigorously tested for chemical purity, was dissolved aseptically in water (final concentration 219.8 mmol·L⁻¹). The solution was then passed through a 0.2 μm filter into vials that were subsequently sealed under sterile conditions. A beet-derived glucose solution (100 g·L⁻¹) was obtained from Braun (St Gallen, Germany). A commercially available amino acid solution (Priméne 5; Baxter, Maurepas, France) was used to compensate for the decrease in amino acid levels that occurs after insulin infusion [22,23]. It contained the following l-amino acids (μmol·L⁻¹): l-leucine, 38.17; l-isoleucine, 25.57; l-valine, 32.48; l-lysine, 37.67; l-methionine, 8.05; l-phenylalanine, 12.73; l-threonine, 15.55; l-tryptophan, 4.90; l-alanine, 44.94; l-arginine, 24.14; l-aspartic acid, 22.56; l-cysteine, 10.16 (chlorhydrate); l-glutamic acid, 34.01; glycine, 26.67; taurine, 2.40; l-histidine, 12.26; l-proline, 13.05; l-serine, 19.05; l-tyrosine, 2.49; l-ornithine, 8.56.

**Experimental protocol**

The four groups (hypothyroid and euthyroid states in study I; control and hyperthyroid states in study II) were studied in the post-absorptive state (12 h overnight fast). At 08.00 hours a sampling catheter (Venflon 2, 20 G; Viggo, Helsingborg, Sweden) was inserted in a retrograde manner into a dorsal hand vein. Another catheter was placed in a contralateral forearm vein and used for infusions.

Each experiment consisted of a 180 min basal period followed by a 140 min hyperinsulinaemic euglycaemic clamp (Figure 1). To determine glucose kinetics, [6,6-²H₂]Glucose was injected intravenously at a priming dose (22.62 ± 0.62 μmol·kg⁻¹ in 7 ml of fluid) at the beginning of the control period, and was then infused continuously at a constant rate (0.50 ± 0.01 μmol·min⁻¹·kg⁻¹) for 320 min. Hyperinsulinaemia was induced by a primed infusion (31.8 ± 1.0 pmol·kg⁻¹ in 15 ml of saline) continuous infusion (6.5 ± 0.3 pmol·min⁻¹·kg⁻¹) of human regular insulin (Actrapid HM; Novo, Copenhagen, Denmark) using a syringe pump (Precidor; Infors AG, Basel, Switzerland). Plasma glucose concentration was maintained at the basal level by intravenous infusion of beet glucose using a peristaltic pump (Vip 2; Beckton Dickinson, St Etienne de St Geoirs, France). The infusion rate was adjusted every 3–5 min for the first 45 min and every 7–10 min thereafter, based on rapid blood glucose concentration measurements (within 1 min after blood sampling). To blunt insulin-induced hypoaminoacidemia, amino acids were infused concomitantly using another peristaltic pump at a constant rate of 1.00 ± 0.01 ml·min⁻¹. KCl (Aguettant, Lyon, France) was administered with the amino acid mixture at a rate of 67 μmol·min⁻¹ to prevent insulin-induced hypokalaemia. Arterialized venous blood was taken from the dorsal hand catheter during the basal and the hyperinsulinaemic periods (Figure 1) after placing the forearm and hand in a heating box (60 °C). Blood samples were collected in heparinized tubes and centrifuged at 4 °C, and the resulting plasma was stored at −20 °C for subsequent analyses.
Contacting and others

Figure 1 Study protocol
All subjects underwent a 320 min period of [6,6-2H2]glucose infusion, which comprised a 180 min basal period (glucose infusion alone) and then a 140 min period of a hyperinsulinaemic euglycaemic clamp. Amino acids and potassium were infused concomitantly to blunt insulin-induced hypoaminoacidaemia and hypokalaemia respectively. At time zero, a primed constant infusion of [6,6-2H2]glucose was begun, which was continued throughout the whole experiment to determine glucose kinetics during both the basal and the hyperinsulinaemic periods. Blood sampling is indicated by the arrows.

Metabolite assays
Glucose was measured in whole blood obtained during the infusions by the glucose oxidase procedure using a glucose analyser (Glucometer IV; Bayer Diagnostics, Puteaux, France). Additional assays (see Table 2) were performed with plasma samples using the enzymic kit D-glucose oxidase D peroxidase (Unimate 7 Gluc amino-4-antipyrine 073-6740; Roche, Neuilly sur Seine, France) on a Cobas analyser (Cobas Mira Diagnostic Systems, Neuilly sur Seine, France). Plasma potassium was assayed by flame emission (PHF 90 apparatus with K filter; ISA Biologie, Pouilly en Auxois, France).

Hormone assays
Plasma insulin was determined by homologous RIA using a commercial kit (INSI-PR; Cis Bio International, Gif sur Yvette, France), with intra- and inter-assay coefficients of variation of approx. 5% and 10% respectively. Plasma free T3 and free T4 concentrations were also measured by RIA (FT3 Amerlex M and FT4 Amerlex MAB; Kodak, Clinical Diagnostics, Amersham, U.K.), as was TSH (RIA Gnost-hTSH, OCPL; Behringwerke AG, Marburg, Germany).

Analysis of [6,6-2H2]glucose enrichment
Glucose was extracted by sequential anion and cation exchange chromatography, and derivatives were obtained by boroacetylation [25]. Plasma [6,6-2H2]glucose enrichment was analysed by GC-MS (mass-selective detector 5972, coupled to a gas chromatograph 5890 series II; Hewlett Packard, Les Ullis, France). The ions at m/z 297 (unlabelled glucose) and m/z 299 ([6,6-2H2]glucose) were selectively monitored for the calculation of isotopic enrichment using a standard curve prepared using glucose solutions of known enrichment. Glucose kinetics were obtained [25] using samples taken during the final 40 min of the basal period (at 130, 150 and 170 min) and of the hyperinsulinaemic euglycaemic clamp (at 260, 280, 300 and 320 min). [6,6-2H2]Glucose enrichments and concentrations were at near steady state in plasma (see the Results section).

The total glucose turnover rate (Q; mg·min⁻¹·kg⁻¹) is determined by the equation:

$$Q = (F \times IE_{\text{inf}}) / IE_n$$

where $F$ is the [6,6-2H2]glucose infusion rate (mg·min⁻¹·kg⁻¹), $IE_{\text{inf}}$ is the isotopic enrichment of the infusate (i.e. 99 mol% excess) and $IE_n$ (also in mol% excess) is the [6,6-2H2]glucose enrichment in plasma.

The model generates the equation:

$$Q = F + I + R_a = R_d$$

where $I$ is the rate of intravenous infusion of unlabelled glucose (zero in the basal state), $R_a$ is the rate of appearance of endogenous glucose in plasma and $R_d$ is the rate of glucose disposal from plasma (all in mg·min⁻¹·kg⁻¹). Knowing $Q$, $F$ and $I$, $R_a$ and $R_d$ can be determined.

Statistical analysis
All data are expressed as means±S.E.M. In each experiment, Student’s two-tailed t tests for paired data were
used for comparisons between the two groups and
between the basal and insulin-stimulated periods. Welch’s t test was used when S.D.s were significantly
different between groups. P ≤ 0.05 was considered to be
significant.

RESULTS

Plasma insulin levels
Plasma insulin levels during the basal period did not
change either after the normalization of thyroid hormone
levels in patients or after the induction of hyperthyroidism in healthy volunteers (Table 2). Combined
infusion of insulin, glucose, amino acids and potassium
resulted in a stable hyperinsulinaemic state between 230 and 320 min. Despite similar insulin infusion rates, the
plateau value of insulin was lower after thyroid hormone
treatment in both groups. It was higher (P < 0.05) in the
untreated hypothyroid patients than after normalization
of thyroid hormone levels. It was lower after induction of
hyperthyroidism than in control group.

Plasma potassium and free amino acids
The mean kalaemia over the hyperinsulinaemic period
was kept at normal levels (normal range 3.5–5.0 mol · l⁻¹)
in the hypothyroid and euthyroid (study I), and the
control and hyperthyroid (study II) states (3.69 ± 0.02;
3.36 ± 0.02; 3.88 ± 0.03 and 4.14 ± 0.04 mol · l⁻¹ respectively).

Plasma free amino acid levels have been described in
detail elsewhere (see Table 3 in [22] and Figure 2 in [23]).
Briefly, the normalization of thyroid hormone levels in
patients did not alter basal plasma free amino acids. An
exception was serine, the concentration of which was
raised in the hypothyroid state. The induction of hyper-
thyroidism in healthy volunteers always resulted in
increases in plasma free valine, threonine and glutamine
in the basal period. There were also (not always during
the basal period) occasional increases in plasma free
phenylalanine, tyrosine, lysine, histidine, leucine and
isoleucine. In contrast, there were always decreases in
aspartic acid and serine, and sometimes in glutamic acid
and glycine.

Following infusion, the plasma free concentrations of
lysine, leucine, isoleucine, histidine, arginine, tryptophan,
valine, phenylalanine, methionine and alanine were
slightly increased. However, concentrations of valine and
alanine in euthyroid patients (study I), methionine in
patients (study I; both hypothyroid and euthyroid states)
and phenylalanine in volunteers (study II; both control
and hyperthyroid states) were maintained at their basal
levels. In contrast, modest but significant decreases in
tyrosine (all conditions), threonine (except in hypo-
thyroid patients) and branched-chain amino acids in the
last 60 min of infusion in volunteers (control and hyper-
thyroid states) were observed.

Plasma glucose concentrations and glucose
infusion rates
Basal plasma glucose concentrations during the basal
period did not change either after normalization of
thyroid hormone levels in patients or after induction
of hyperthyroidism in healthy volunteers (Table 2). This
basal level was maintained during hyperinsulinaemia.
The mean coefficients of variation during the hyper-
insulinaemic period were 6.0%, 7.2%, 12.5% and 5.3% for
the hypothyroid, euthyroid (study I), control and
hyperthyroid (study II) states respectively (Table 2).

The rate of exogenous glucose infusion was started at
1.35 ± 0.12 mg · min⁻¹ · kg⁻¹. It was increased gradually
and remained constant thereafter. The mean coefficients of variation for the exogenous rate of glucose infusion during the final 30 min of infusion were 4.8%, 2.6%, 3.1% and 2.1% for the hypothyroid, euthyroid (study I), control and hyperthyroid (study II) states respectively. The mean steady-state rate of exogenous glucose infusion was lower in the untreated hypothyroid patients than after the normalization of thyroid hormone levels (4.30 ± 1.08 compared with 6.31 ± 1.26 mg·min⁻¹·kg⁻¹; \( P < 0.05 \)). The induction of hyperthyroidism in normal volunteers did not alter the glucose infusion rate (control, 7.51 ± 0.39 mg·min⁻¹·kg⁻¹; hyperthyroidism, 7.84 ± 0.27 mg·min⁻¹·kg⁻¹).

### Plasma glucose 6,6-\( ^2 \)H\(_2 \) enrichment

In all states, 6,6-\( ^2 \)H\(_2 \) enrichment of plasma glucose was measured in samples taken during the final 40 min of the basal period and during the final 60 min of insulin infusion. Steady-state 6,6-\( ^2 \)H\(_2 \) enrichment was achieved during the observed periods (Table 3).

The normalization of thyroid hormone levels in patients resulted in a significant decrease in 6,6-\( ^2 \)H\(_2 \) enrichment during the basal period (\( P < 0.05 \)). The induction of hyperthyroidism in normal volunteers did not have any effect.

## Whole-body glucose kinetics

Both normalization of thyroid hormone levels in hypothyroid patients and induction of hyperthyroidism in healthy volunteers resulted in a significant increase in basal glucose disposal (\( P < 0.05 \)). Infusion of insulin, in conjunction with glucose and amino acids, always increased glucose disposal significantly (\( P < 0.05 \), Table 4). The incremental increase above basal tended to be improved after normalization of thyroid hormone levels in patients, but this was not statistically significant (2.61 ± 0.97 compared with 0.84 ± 0.23 mg·min⁻¹·kg⁻¹; \( P < 0.15 \)). This was also the case after induction of hyperthyroidism in healthy volunteers (4.67 ± 0.83 compared with 3.08 ± 0.30 mg·min⁻¹·kg⁻¹). With both normalization of thyroid hormones and induction of hyperthyroidism, these incremental increases in glucose disposal were obtained with smaller increases in plasma insulin, suggesting increased insulin action. Accordingly, the ratio of the absolute increase in

### Table 3 6,6-\( ^2 \)H\(_2 \) enrichment of plasma glucose as a function of time in the hypothyroid, euthyroid and hyperthyroid states

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Study I: patients</th>
<th>Study II: healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothyroid</td>
<td>Euthyroid</td>
</tr>
<tr>
<td>Basal</td>
<td>4.09 ± 0.18 (4.5%)</td>
<td>3.85 ± 0.19 (6.0%)</td>
</tr>
<tr>
<td>Under clamp</td>
<td>2.96 ± 0.21 (4.6%)</td>
<td>2.22 ± 0.38 (5.6%)</td>
</tr>
<tr>
<td>Decrease from basal</td>
<td>1.13 ± 0.25 ‡</td>
<td>1.63 ± 0.37 ‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Hyperthyroid</td>
</tr>
<tr>
<td>Basal</td>
<td>3.39 ± 0.09 (4.3%)</td>
<td>3.21 ± 0.11 (4.2%)</td>
</tr>
<tr>
<td>Under clamp</td>
<td>1.66 ± 0.10 †</td>
<td>1.34 ± 0.16 †</td>
</tr>
<tr>
<td>Decrease from basal</td>
<td>1.70 ± 0.09 †</td>
<td>1.91 ± 0.17 †</td>
</tr>
</tbody>
</table>

Enrichment values are means ± S.E.M. for six subjects. Coefficients of variation (given in parentheses) were calculated for each subject during the basal and insulin infusion periods. Significance of differences: †\( P < 0.05 \) compared with euthyroid state during the same period; \( P < 0.05 \) compared with basal period in the same state; \( P < 0.05 \) compared with zero.

### Table 4 Glucose disposal rate before and during a euglycaemic euaminoacidaemic hyperinsulinaemic clamp in the hypothyroid, euthyroid and hyperthyroid states

<table>
<thead>
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<th>Study II: healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypothyroid</td>
<td>Euthyroid</td>
</tr>
<tr>
<td>Basal</td>
<td>1.05 ± 0.12</td>
<td>2.31 ± 0.14 ‡</td>
</tr>
<tr>
<td>Under clamp</td>
<td>2.49 ± 0.34 †</td>
<td>4.92 ± 1.08 ‡</td>
</tr>
<tr>
<td>Incremental increase above basal</td>
<td>0.84 ± 0.23 $\dagger$</td>
<td>2.61 ± 0.97 $\dagger$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Hyperthyroid</td>
</tr>
<tr>
<td>Basal</td>
<td>2.63 ± 0.08</td>
<td>2.94 ± 0.04 †</td>
</tr>
<tr>
<td>Under clamp</td>
<td>5.71 ± 0.28 †</td>
<td>7.61 ± 0.85 †</td>
</tr>
<tr>
<td>Incremental increase above basal</td>
<td>3.08 ± 0.30 $\dagger$</td>
<td>4.67 ± 0.83 $\dagger$</td>
</tr>
</tbody>
</table>

Glucose disposal (mg·min⁻¹·kg⁻¹) values are means ± S.E.M. for six subjects. Significance of differences: †\( P < 0.05 \) compared with hypothyroid state during the same period; \( P < 0.05 \) compared with control state during the same period; \( P < 0.05 \) compared with basal period in the same state; \( P < 0.05 \) compared with zero.
glucose disposal to the increase in plasma insulin was markedly improved in patients with normalized hormone levels compared with hypothyroid patients (4.8 ± 1.8 and 1.8 ± 0.8 mg glucose · min⁻¹ · kg⁻¹/nmol insulin · l⁻¹ respectively; P < 0.05). The induction of hyperthyroidism in healthy volunteers also increased this index, but not significantly (14.1 ± 2.8 compared with 7.6 ± 0.9 mg glucose · min⁻¹ · kg⁻¹/nmol insulin · l⁻¹; P = 0.08). Endogenous glucose appearance was completely inhibited by insulin infusion in all groups.

**DISCUSSION**

Our results clearly show that glucose metabolism in humans is dependent on thyroid status. Hypothyroidism induced post-absorptive decreases in both glucose disposal and endogenous glucose appearance, and impaired the ability of insulin to stimulate glucose disposal related to insulinemia. These alterations were corrected by regular thyroid hormone therapy. In contrast, induction of hyperthyroidism in healthy volunteers increased basal glucose disposal, but did not significantly improve insulin-stimulated glucose disposal.

Several studies have examined body composition in hypothyroidism and hyperthyroidism. The most important feature of hypothyroidism is an increase in body fat mass, which occurs even during mild hypothyroidism [26]. An increase in extracellular water has also been reported. Body cell mass is unchanged or decreased in hypothyroid patients. In hyperthyroidism, a decrease in body cell mass occurs in most studies [26–28], whereas body fat mass is decreased [28] or does not change [27]. In our studies, the weight loss observed on thyroid hormone replacement or after induction of hyperthyroidism may be partly due to a decrease in fat mass and a decrease in body cell mass respectively. The increase in the glucose disposal/body weight ratio after treatment of hypothyroidism or after induction of hyperthyroidism may be a reflection of the decrease in body weight. This increase would be artifactual if glucose disposal did not change. However, when expressed in absolute terms, the increase in glucose disposal with thyroid treatment was significant [hypothyroid, 138.9 ± 14.0 mg/min; euthyroid, 164.2 ± 8.5 mg/min (P < 0.05); control, 190.91 ± 6.3 mg/min; hyperthyroid, 210.0 ± 6.3 mg/min (P < 0.05)].

Futile cycles are important in metabolic regulation and thermogenesis. Substrate cycling between glucose and glucose 6-phosphate, and between fructose 6-phosphate and fructose 1,6-phosphate, is increased in the hyperthyroid state and decreased in the hypothyroid state. Since heat production related to ATP dissipation occurs during the operation of substrate cycling, it is conceivable that energy waste is increased [7,29]. These metabolic adaptations could partly explain why hypothyroid patients gain weight and hyperthyroid subjects tend to lose weight. An absence of weight change in hyperthyroid subjects is probably due to an increase in food intake.

Despite the same rate of insulin infusion, plasma insulin concentrations were lower in the hyperthyroid than in the control state in volunteers, and higher in the hypothyroid state than after normalization of thyroid hormone levels in patients. Endogenous insulin production during insulin infusion could be inhibited to a greater extent in hyperthyroidism. However, it is more likely that exogenous insulin is degraded to a greater extent in hyperthyroidism: several studies have shown an increase in insulin half-life (+20%) associated with increased thyroid hormone levels during a euglycaemic hyperinsulinaemic clamp [2,13].

Thyroid hormone treatment increases glucose disposal in healthy volunteers [2,8]. Splanchnic tissues and skeletal muscle both participate in this increase in glucose utilization [15,20,30,31]. Tracer studies in humans have also shown that the appearance of endogenous glucose is increased in hyperthyroidism [2,8,15,31]. Less data are available on glucose metabolism in hypothyroidism than in hyperthyroidism. The increase in glucose disposal after normalization of thyroid hormone levels in hypothyroid patients is consistent with the observed difference in glucose utilization between the hypothyroid and euthyroid states in rats [5].

The normalization of thyroid hormone levels in hypothyroid patients significantly improved the index of insulin action on glucose disposal, i.e. the ratio of the absolute increase in glucose disposal to the increase in plasma insulin. No similar studies have been performed previously in patients with hypothyroidism. However, the results agree with those from studies at the tissue level in rats [16–18,32]. The index of insulin action was also increased following the induction of hyperthyroidism in healthy volunteers, but this increase was not statistically significant. In accordance with our results, no clear conclusion about the effects of thyroid hormones on insulin responsiveness has been drawn from studies on clinical and experimental hyperthyroidism. Many studies have failed to demonstrate any change in insulin action [2,8–10,12,13], whereas an increase in insulin action has sometimes been observed [9,11,14,15]. Our present study, which included hypothyroid patients, allows to clarify further the effects of thyroid hormone in humans. Moreover, we may speculate that the dose–response curve of this effect is more significant in the low hormone level range than at high levels. Interestingly, our results on glucose disposal agree with those observed previously for proteolysis [22,23]. Similarities in the effects of insulin on whole-body glucose disposal and on proteolysis have already been noted: hypothyroidism impaired the anti-proteolytic effect of insulin [22], whereas hyperthyroidism increased this effect [23]. Moreover, insulin-induced hypoaminoacidaemia was more significant in the hyperthyroid than in the euthyroid state [14,33].
Our experimental approach did not allow us to fully determine the mechanism of the interaction between thyroid hormone status and glucose metabolism. A major characteristic of thyroid hormones is the multiplicity of cellular processes and functions they regulate in every type of tissue. Chronic thyroid hormone replacement no doubt resulted in a variety of metabolic changes (e.g. stimulation of catabolic and oxidative pathways) that could alter glucose metabolism. Thyroid hormones also modulate glucose metabolism by direct actions, which include the synthesis of key regulatory enzymes and alterations in other regulatory hormones, such as catecholamines, glucagon and insulin. As far as insulin action is concerned, it has been demonstrated that the GLUT4 gene contains a \( T_3 \)-responsive element. Accordingly, GLUT4 expression is increased in hyperthyroidism, whereas it is decreased in hypothyroidism [21,34]. Thyroid hormone-dependent insulin action has also been reported for pyruvate kinase, aldolase B and hepatic glucokinase [35,36]. Moreover, thyroid hormones regulate \( Ca^{2+} \)-ATPase activity, which can interact with insulin receptor substrate 1 (a key component in insulin signalling).

In conclusion, thyroid hormone replacement corrected the alteration in the ability of insulin to stimulate glucose disposal in hypothyroid patients. Transient thyroid hormone excess also tended to improve this action of insulin in healthy volunteers (although this effect was not statistically significant). This suggests that the phenomenon may be highly sensitive and only apparent at low thyroid hormone levels. Finally, our study renders more consistent previous results concerning the effects of thyroid hormone status on glucose metabolism in humans. There were similarities in the effects of thyroid hormones on the ability of insulin to stimulate glucose disposal and to inhibit proteolysis (see [22,23]).

ACKNOWLEDGMENTS

This study was supported by grants from the French Ministère de l’Education Nationale (Programme Hospitalier de Recherche Clinique), Région Auvergne and Lipha Santé Lyon. We thank D. Bonin and H. Lafarge for literature requisitions, S. Corny and A. Arvouet for preparing and testing stable isotopes, Dr S. Brionnet, G. Dutot and Baxter France for providing the amino acid mixture, and M. Balage for critical comments.

REFERENCES

Thyroid hormones and glucose disposal


Received 11 June 2002; accepted 10 October 2002