

# Insulin Sensitivity and Exogenous Insulin Clearance in Graves' Disease

## Measurement by the Glucose Clamp Technique and Continuous Indirect Calorimetry

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### SUMMARY

Insulin sensitivity was measured in a group of seven thyrotoxic patients and in a group of seven normal subjects by means of the glucose clamp technique. Infusion of insulin at a rate of  $0.80 \pm 0.05$  mU/kg · min in the hyperthyroid patients and of  $0.55 \pm 0.04$  mU/kg · min in the control group was performed to obtain a steady-state plasma insulin concentration of approximately 50  $\mu$ U/ml. Substrate oxidation rates were measured in the postabsorptive state and during the 2 h of the clamp by means of continuous indirect calorimetry.

In the postabsorptive state, hyperthyroid patients presented a preferential oxidation of lipids. During the period 60–120 min of the clamp, mean plasma glucose ( $92 \pm 2$  versus  $93 \pm 2$  mg/dl), insulin ( $50 \pm 5$  versus  $58 \pm 3$   $\mu$ U/ml), and total glucose metabolism ( $5.8 \pm 0.7$  versus  $6.1 \pm 0.3$  mg/kg · min) were similar in the hyperthyroid patients and the control subjects. The rate of glucose oxidation was higher in hyperthyroid patients than in control subjects ( $4.3 \pm 0.5$  versus  $2.2 \pm 0.2$  mg/kg · min,  $P < 0.001$ ), while that of lipid oxidation was similar in both groups ( $0.6 \pm 0.2$  versus control  $0.7 \pm 0.1$  mg/kg · min). The calculated metabolic clearance rate of insulin was markedly higher in the hyperthyroid patients ( $1144 \pm 132$  ml/min) than in the normal subjects ( $812 \pm 56$  ml/min,  $P < 0.025$ ).

It is concluded that insulin sensitivity is not altered in the thyrotoxic state. The major route of insulin-stimulated glucose disposal in the hyperthyroid patients appears to be glucose oxidation. *DIABETES* 1986; 35:178–81.

Impaired glucose tolerance is a common finding in patients with hyperthyroidism.<sup>1–6</sup> Moreover, thyrotoxicosis has been reported to worsen a preexistent diabetes.<sup>7</sup> Different mechanisms, including a resistance to insulin action,<sup>2</sup> a preferential oxidation of fatty acids,<sup>4</sup> and an increase in gastric emptying rate,<sup>5</sup> have been put forward to explain this alteration of glucose tolerance.

In a previous study, we observed that thyrotoxic patients

presented an important increase in resting metabolic rate and in lipid oxidation rate in the postabsorptive state as well as a marked elevation of circulating free fatty acids (FFA).<sup>6</sup> A moderate impairment of glucose tolerance was observed after administration of a 100-g glucose load. Glucose oxidation was markedly increased when compared with values observed in a control group of normal subjects, and both lipid oxidation and FFA concentrations were suppressed to control values during the 3 h after the load. These findings make a defect of oxidative glucose metabolism or an increased FFA concentration unlikely to be the cause of impaired glucose intolerance in thyrotoxicosis. We had therefore postulated a defect in nonoxidative glucose disposal in Graves' disease.<sup>6</sup>

In the present study, insulin sensitivity was determined in a group of seven thyrotoxic patients and in a group of normal volunteers by means of the glucose clamp technique<sup>8</sup> in combination with continuous indirect calorimetry. The metabolic clearance rate of insulin during insulin infusion was also evaluated.

### MATERIALS AND METHODS

**Subjects.** Six women and one man with Graves' disease were selected to participate in the study. The diagnosis of hyperthyroidism was established on clinical grounds and by conventional thyroid function tests. All patients were newly diagnosed hyperthyroid subjects with diffuse goiter demonstrating uniform increased uptake of <sup>131</sup>I-scan and elevated serum total T4 levels ( $228 \pm 16$  nmol/L; normal range: 60–150 nmol/L) and serum total T3 levels ( $7.7 \pm 0.9$  nmol/L; normal range: 1.5–3.3 nmol/L). Their mean age was  $38 \pm 4$  yr (range 20–50 yr). Their mean body weight was  $98 \pm 5\%$  of ideal body weight (range 82–125%) according to the Met-

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TABLE 1  
Plasma glucose and insulin concentration and total glucose metabolism during the hyperinsulinemic, euglycemic clamp

	Hyperthyroid patients (N = 7)	Control group (N = 7)
Insulin infusion rate (mU/kg · min)	1.0	0.5
MCR (ml/min)	1144 ± 132 (P < 0.025)	812 ± 56
Steady-state plasma glucose (mg/dl)	92 ± 2 (NS)	93 ± 2
Steady-state plasma insulin (μU/ml)	50 ± 5 (NS)	58 ± 3
Total glucose uptake (mg/kg · min)	5.8 ± 0.7 (NS)	6.1 ± 0.3

All values represent the mean ± SEM for the 60–120-min time period. Comparison of means was done using the *t*-test for unpaired data.

ropolitan Life Insurance Tables, 1959. No subject had any personal or family history of diabetes, and no patients were taking any drug. All patients were studied on the third or fourth day after diagnosis, before any antithyroid therapy. A group of seven euthyroid volunteers (one woman and six men) were studied under the same conditions; their mean age was 25 ± 1 yr (range 23–29 yr), and their mean weight was similar to that of hyperthyroid patient group (67 ± 3 versus 61 ± 3 kg); their mean body weight was 97 ± 2% of ideal body weight (range 90–106%).

The protocol was submitted to review and accepted by the institutional ethical committee of the Department of Internal Medicine, Lausanne University Hospital. All patients gave their informed consent before participating in the study.

**Experimental protocol.** All normal and hyperthyroid subjects received a weight-maintenance diet containing at least 250–300 g carbohydrate per day for 2 or 3 days before each study and were studied after an overnight fast. All studies were in the recumbent position at 8:00 a.m. A Teflon catheter was inserted into an antecubital vein for the infusion of all test substances. A second catheter was inserted into an antecubital vein of the other arm for blood sampling, and was kept patent with a slow infusion of isotonic saline. Sixty minutes before beginning the insulin clamp studies, continuous respiratory exchange measurements were begun and continued throughout the duration of the experimental protocol.

After a 60-min equilibration period, a primed-continuous infusion of crystalline porcine insulin (Actrapid, Novo, Copenhagen, Denmark) was administered. The priming dose was performed according to DeFronzo et al.:<sup>8</sup> 800 mU/m<sup>2</sup> of insulin was administered over 10 min to hyperthyroid patients and 400 mU/m<sup>2</sup> to normal subjects. An aliquot of the solution of crystalline porcine insulin infused was removed for determination of insulin concentration. Insulin was infused at a rate of 0.55 ± 0.04 mU/kg · min in the euthyroid and at a rate of 0.80 ± 0.05 mU/kg · min in the hyperthyroid subjects to achieve comparable steady-state insulin concentrations (see below). The plasma glucose concentration was maintained constant at a level of 93 ± 2 mg/dl by determination of the plasma glucose concentration every 5 min and periodically adjusting a variable 20% glucose solution based on a negative feedback principle.<sup>8</sup> The glucose clamp lasted 120 min.

During the 120-min control period and throughout the 2-h insulin clamp study, substrate utilization rates were deter-

mined by computerized open-circuit indirect calorimetry as described in detail elsewhere.<sup>9</sup>

**Analytic procedures.** Plasma glucose concentration was determined in duplicate by the glucose-oxidase method on a Beckman glucose analyzer II (Beckman Instruments, Inc., Fullerton, California). Total serum T4 and total serum T3 were measured by automated radioimmunoassay ARIA II (Becton-Dickinson, Mountain View, California). Plasma immunoreactive insulin was determined by radioimmunoassay according to Herbert et al.<sup>10</sup> Plasma free fatty acids (FFA) were extracted using the method of Dole and Meinertz<sup>11</sup> and determined according to Heindel et al.<sup>12</sup> Urinary nitrogen was measured by the method of Kjeldahl.<sup>13</sup>

**Data analysis.** The glucose oxidation rate was calculated from calorimetric measurements for 30 min in the basal state and throughout the 120-min insulin clamp period. Steady-state plasma glucose and insulin concentrations during the glucose clamp are the mean of the values obtained from 60 to 120 min. Glucose and lipid oxidation rates are the mean of the 30-min basal and of the 60–120-min period of the clamp. Nonoxidative glucose disposal was calculated by subtracting the amount of glucose oxidized during the 60–120-min period of the clamp from the total glucose infused during the same time period.

The metabolic clearance rate (MCR) of infused insulin was computed from data on the euglycemic clamp<sup>8</sup> and was obtained as follows: MCR (ml/min) = [insulin infusion rate (μU/ml)]/[steady-state plasma insulin – basal insulin (μU/ml)]. Comparison of means was performed using the *t*-test for unpaired data.

## RESULTS

All results in the text are expressed as mean ± SEM.

The mean fasting plasma glucose concentration was not significantly different in the two groups (hyperthyroid 96.5 ± 3 versus control 93 ± 2 mg/dl). During the last 60 min of the euglycemic insulin clamp, the steady-state plasma glucose values were similar (hyperthyroid 92 ± 2 versus control 93 ± 2 mg/dl) with coefficients of variation of 1.9 ± 0.2% and 2.7 ± 1.0%, respectively (NS). The fasting plasma insulin concentrations were similar (hyperthyroid 12.0 ± 2 versus control 12.6 ± 2 μU/ml). The steady-state plasma insulin levels during the last 60 min of the clamp were 50 ± 5 and 58 ± 3 μU/ml in hyperthyroid and control groups, respectively (NS), with coefficients of variation of 4.0 ± 0.7% and 3.3 ± 0.4% (NS) (Table 1). The metabolic insulin clearance rate was higher in the hyperthyroid group: 1144 ± 132 versus 812 ± 56 ml/min in the control group, P < 0.025.

TABLE 2  
Glucose and lipid oxidation rates during the hyperinsulinemic, euglycemic clamp

	Glucose oxidation		Lipid oxidation	
	Basal (mg/kg · min)	Postinsulin (mg/kg · min)	Basal (mg/kg · min)	Postinsulin (mg/kg · min)
Hyperthyroid patients	1.2 ± 0.2	4.3 ± 0.5†	1.8 ± 0.1*	0.6 ± 0.2
Control group	1.0 ± 0.3	2.2 ± 0.2	1.2 ± 0.1	0.7 ± 0.1

All values represent the mean ± SEM for the basal or 60–120-min time period. Hyperthyroid as compared with control: \*P < 0.005, †P < 0.001.

Basal glucose oxidation was similar in the two groups ( $1.2 \pm 0.2$  and  $1.0 \pm 0.3$  mg/kg · min in the hyperthyroid and control groups, respectively). During the 60–120-min period of the clamp, total glucose metabolism (glucose infusion rate required to maintain euglycemia) was  $5.8 \pm 0.7$  and  $6.1 \pm 0.3$  mg/kg · min (NS) in the hyperthyroid and control groups, respectively (Table 1). Total glucose oxidation was markedly higher in the hyperthyroid patients ( $4.3 \pm 0.5$  mg/kg · min) than in the euthyroid subjects ( $2.2 \pm 0.2$  mg/kg · min,  $P < 0.001$ ) (Table 2); conversely, nonoxidative glucose disposal was significantly decreased in the hyperthyroid patients ( $1.5 \pm 0.6$  mg/kg · min) when compared with the control group ( $3.9 \pm 0.3$  mg/kg · min,  $P < 0.001$ ).

The mean fasting plasma FFA concentrations were significantly elevated in the hyperthyroid group:  $699 \pm 46$  versus  $511 \pm 60$   $\mu$ mol/L in the controls,  $P < 0.05$ . FFA levels were suppressed to control values during the last 60 min of the clamp ( $154 \pm 25$  versus control  $177 \pm 8$   $\mu$ mol/L, NS). In the postabsorptive state, the lipid oxidation rate was higher in the hyperthyroid than in the control group:  $1.8 \pm 0.1$  versus  $1.2 \pm 0.1$  mg/kg · min,  $P < 0.005$ . Lipid oxidation rates were similar in both groups during the last hour of the clamp ( $0.6 \pm 0.2$  and  $0.7 \pm 0.1$  mg/kg · min, NS) (Table 2).

## DISCUSSION

In the present study, insulin sensitivity (assessed by the ratio of total glucose metabolism over steady-state plasma insulin concentration during the hyperinsulinemic, euglycemic clamp) was found to be basically normal in a group of thyrotoxic patients (Table 1). Our hyperthyroid patients were somewhat older than the control subjects, and insulin sensitivity has been reported to be decreased in elderly people.<sup>14</sup> Therefore, it can be assumed that insulin sensitivity is in no case decreased in hyperthyroidism. Insulin sensitivity has been described as either normal<sup>15</sup> or decreased<sup>2</sup> by other authors. In the glucose clamp technique, the use of "M" as a measurement of total body glucose metabolism assumes that basal hepatic glucose production is completely suppressed by the infusion of glucose and insulin.<sup>8</sup> Recently, several authors have demonstrated a lower degree of suppression of hepatic glucose production after insulin and glucose infusion in experimental hyperthyroidism.<sup>16,17</sup> For this reason, and because of the fact that the plasma insulin concentrations attained were moderate, it is possible that total glucose metabolism was somewhat underestimated in our hyperthyroid patients. With this possibility in mind, it can be assumed that insulin sensitivity might possibly be increased, but certainly not decreased in our thyrotoxic patients.

In the present study, the insulin clamp technique was combined with indirect calorimetry to measure glucose uptake and the two major routes of glucose disposal, namely, glucose oxidation and glucose storage. Postabsorptive glucose oxidation was found to be unchanged in hyperthyroid patients. In the postabsorptive state, glucose oxidation mainly depends on non-insulin-dependent tissues.<sup>18</sup> Thyroid hormones have been shown to enhance insulin action at the receptor and the postreceptor level,<sup>19</sup> possibly explaining why fasting glucose oxidation was unchanged in hyperthyroid patients. It appears from our results that the preferential route of glucose disposal during hyperinsulinism was oxida-

tion in our hyperthyroid patients. In fact, total insulin-stimulated glucose oxidation was strongly elevated in the hyperthyroid group when compared with the controls, with plasma insulin levels similar in both groups. This may reflect a direct stimulatory effect of thyroid hormones on glucose oxidation as has been observed *in vitro*.<sup>19–21</sup> Whether the predominance of glucose oxidation over nonoxidative glucose disposal persists at higher insulin levels due to this direct stimulatory effect of thyroid hormones remains to be answered. Previous studies have shown that the main part of intravenously infused glucose is taken up by peripheral tissues under normal conditions,<sup>22,23</sup> primarily by muscle.<sup>24</sup> Glycogen probably represents the major form of glucose storage after insulin infusion.<sup>22,25</sup> This preferential oxidation of glucose in the postprandial state in thyrotoxic patients may account for the decreased glycogen content in muscle of hyperthyroid animals.<sup>26</sup>

Although lipid oxidation was strongly elevated in the postabsorptive state in hyperthyroid patients, we observed that the insulin-glucose infusion induced a rapid fall of both FFA levels and lipid oxidation to control values. These findings demonstrate that insulin has a normal antilipolytic effect in hyperthyroidism. Moreover, it suggests that in thyrotoxicosis, elevated fasting FFA concentrations do not produce a state of insulin resistance as has been observed during exogenous FFA administration.<sup>27</sup>

During the constant infusion of insulin, the metabolic clearance rate (MCR) of insulin can be fairly well evaluated. In the present study, a clearly increased metabolic clearance rate of insulin was demonstrated in hyperthyroid patients. This is in agreement with other studies: hyperthyroidism in animals has been shown to be accompanied by a decreased insulin half-life;<sup>28</sup> in other studies, increased insulin degradation was observed in human hyperthyroidism.<sup>29,30</sup> However, the clearance rate of radiolabeled insulin was reported elsewhere to be similar in hyperthyroidism and in the normal state.<sup>31</sup>

In conclusion, this study demonstrates that insulin sensitivity is normal in hyperthyroidism. Glucose oxidation seems to be the major route of glucose disposal in Graves' disease, but further studies of glucose disposal at supraphysiologic insulin levels are required to examine the pathways of glucose storage in thyrotoxicosis. An increased metabolic clearance rate of insulin was observed, and could be a major cause of the increase in insulin requirement by insulin-dependent diabetic patients with Graves' disease.<sup>7</sup>

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