

Insulin Action in Adipose Tissue and Muscle in Hypothyroidism

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Background: Although insulin resistance in thyroid hormone excess is well documented, information on insulin action in hypothyroidism is limited.

Methods: To investigate this, a meal was given to 11 hypothyroid (HO; aged 45 ± 3 yr) and 10 euthyroid subjects (EU; aged 42 ± 4 yr). Blood was withdrawn for 360 min from veins (V) draining the anterior abdominal sc adipose tissue and the forearm and from the radial artery (A). Blood flow (BF) in adipose tissue was measured with ^{133}Xe and in forearm with strain-gauge plethysmography. Tissue glucose uptake was calculated as $(A-V)_{\text{glucose}}(\text{BF})$, lipoprotein lipase as $(A-V)_{\text{Triglycerides}}(\text{BF})$, and lipolysis as $[(V-A)_{\text{glycerol}}(\text{BF})]/\text{lipoprotein lipase}$.

Results: The HO group had higher glucose and insulin levels than the EU group ($P < 0.05$). In HO vs. EU after meal ingestion (area under curve 0–360 min): 1) BF (1290 ± 79 vs. 1579 ± 106 ml per 100 ml

tissue in forearm and 706 ± 105 vs. 1340 ± 144 ml per 100 ml tissue in adipose tissue) and glucose uptake (464 ± 74 vs. 850 ± 155 μmol per 100 ml tissue in forearm and 208 ± 42 vs. 406 ± 47 μmol per 100 ml tissue in adipose tissue) were decreased ($P < 0.05$), but fractional glucose uptake was similar (28 ± 6 vs. $33 \pm 6\%$ per minute in forearm and 17 ± 4 vs. $14 \pm 3\%$ per minute in adipose tissue); 2) suppression of lipolysis by insulin was similar; and 3) plasma triglycerides were elevated (489 ± 91 vs. 264 ± 36 nmol/liter-min, $P < 0.05$), whereas adipose tissue lipoprotein lipase (42 ± 11 vs. 80 ± 21 μmol per 100 ml tissue) and triglyceride clearance (45 ± 10 vs. 109 ± 21 ml per 100 ml tissue) were decreased in HO ($P < 0.05$).

Conclusions: In hypothyroidism: 1) glucose uptake in muscle and adipose tissue is resistant to insulin; 2) suppression of lipolysis by insulin is not impaired; and 3) hypertriglyceridemia is due to decreased clearance by the adipose tissue. (*J Clin Endocrinol Metab* 91: 4930–4937, 2006)

IT IS WELL established that thyroid hormones affect insulin action (1). Although thyroid hormone excess has been consistently found to induce insulin resistance (1, 2), there are only a few studies examining insulin action in thyroid hormone deprivation in humans, with conflicting results.

Glucose regulation by insulin depends on the suppression of endogenous glucose production and the stimulation of peripheral glucose disposal. Hepatic glucose production is decreased (3, 4) or unchanged (5) in hypothyroidism, but there is little information on the effects of insulin in peripheral tissues and, in particular, *in vivo*.

Whole-body sensitivity of glucose disposal to insulin in hypothyroid patients has been examined by euglycemic-hyperinsulinemic clamps (6, 7) and iv or oral administration of glucose (8–10) and found to be normal (6, 8), increased (10), or decreased (7, 9). In the forearm muscles of hypothyroid patients, the sensitivity of glucose disposal to insulin was found to be normal (6), whereas in adipocytes isolated from

hypothyroid patients and examined *in vitro*, the sensitivity of glucose oxidation to insulin was normal (11) and that of glucose transport was decreased (12). In rats made hypothyroid, the responsiveness of glucose disposal to insulin was decreased in muscle (13–15) but increased in adipocytes (16).

Finally, there are no studies in hypothyroidism to examine the effects of insulin on lipid metabolism *in vivo*. Lipoprotein lipase (LPL) activity in hypothyroid patients has been estimated in plasma after iv injections of triglyceride emulsion and heparin and found to be decreased (17–22), whereas the activity of this enzyme in adipose tissue biopsies from hypothyroid patients was either normal (19) or decreased (21).

This study was undertaken in patients with hypothyroidism to investigate insulin action on glucose disposal and lipolysis. This was performed after the consumption of a mixed meal to create a metabolic environment, permitting the interaction of insulin and substrates to be investigated under conditions as close to physiological as possible.

Subjects and Methods

Subjects

Eleven female patients with primary hypothyroidism [HO; aged 45 ± 3 yr, body mass index (BMI) 24 ± 1 kg/m², T_3 43 ± 10 ng/dl (0.66 ± 0.1 nmol/liter), T_4 1.6 ± 0.4 μU /dl, TSH 72 ± 10 μU /ml (range 16–100 μU /ml); three were in the postmenopausal state] were studied before initiation of treatment, and compared with 10 female euthyroid subjects

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Abbreviations: A, Artery; AUC, area under curve; BMI, body mass index; EU, euthyroid; HO, hypothyroid; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; V, veins; VLDL, very low-density lipoprotein.

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[EU; aged 42 ± 4 yr, BMI 24 ± 1 kg/m², T₃ 114 ± 9 ng/dl (1.8 ± 0.1 nmol/liter), T₄ 9 ± 1 μU/dl, TSH 1 ± 0.08 μU/ml; two were in the postmenopausal state). Body composition was not assessed in our study (23); the percentage of fat was found to be significantly higher in hypothyroid subjects when compared with healthy controls matched for sex, age, height, and weight (24). However, this does not underestimate the value of our findings because in lean females, the depot of visceral adipose tissue is expected to be small (25, 26). The subjects were not taking any medications before or during the study. The study was approved by the hospital ethics committee, and subjects gave informed consent.

Experimental protocol

All subjects were admitted to the hospital at 0700 h after an overnight fast and had the radial artery (A) and two veins (Vs), a superficial abdominal and a forearm deep vein, catheterized (27). All experiments were undertaken in a temperature-controlled room at 23°C.

A meal was given (730 kcal, 50% carbohydrate, 40% fat, 10% protein) at least 1 h after catheter insertion and consumed within 20 min (28).

Blood samples were withdrawn from the three sites before the meal (at –30 and 0 min) and at 30- to 60-min intervals for 360 min thereafter for measurements of insulin and glucagon (RIA; Linco Research, St. Charles, MO), leptin, and TNF-α (ELISA; R&D Systems, Oxon, UK), adiponectin (RIA; DRG Diagnostics, Marburg, Germany), visfatin (ELISA; Phoenix Pharmaceuticals, Belmont, CA), glucose (Yellow Springs Instruments, Yellow Springs, OH), glycerol, triglycerides (corrected for free glycerol), and nonesterified fatty acids (NEFAs; Roche Diagnostics, Mannheim, Germany). The NEFA assay was validated by measuring dilutions of palmitate and found to be accurate for concentrations of 50 μmol/liter. No inhibitor of lipolysis was added in the tubes used for measurement of NEFAs. After collection of blood, the tubes were put into ice; spinning, separation of plasma, and freezing at –20°C was always less than 30 min from collection. With this procedure, the amount of lipolysis in the blood specimens has been shown to be insignificant (29).

Tissue blood flow was measured immediately before each blood sample (27, 28). In the adipose tissue, ¹³³Xe dissolved in sterile saline (4 MBq; DuPont, MDS Nordion, Fleurus, Belgium) was injected into the sc adipose tissue of the anterior abdominal wall within the drainage area of the cannulated abdominal vein (but on the opposite side), about 8 cm from the midline and 5 cm below the level of the umbilicus. The duration of the injection and the withdrawal of the needle were 2 min and 30 sec, respectively. The injection was given at least 45 min before the first measurement and the washout analyzed with a scintillation detector (Oakfield Instruments, Oxford, UK). During measurements, the subjects remained still. These manipulations were necessary to increase the accuracy and precision of adipose tissue blood flow measurements (27). In recent studies, ¹³³Xe washout was found to be higher in the upper areas of the abdominal anterior wall, compared with lower areas (30, 31); moreover, these rates showed significant variation among different subjects and within the same subject on different occasions (32).

Although the AV-difference technique has allowed unique insights into the metabolic fluxes across adipose tissue (33), the limitations should be borne in mind when considering the results. For instance, it is not possible to separate skin drainage from that of adipose tissue. However, the contribution of skin metabolism is minor (34) and human skin takes little part in lipid metabolism (35). Moreover, the concentrations of glucose, glycerol, and lactate reported in the adipose tissue venous effluent are very similar to those in sc adipose tissue interstitial fluid measured by microdialysis (27, 36). These findings suggest that the composition of the blood in the sc abdominal veins is dominated by the metabolic activity of the adipose tissue surrounding them and that skin metabolism had no major influence on our results.

The forearm blood flow was measured immediately after sampling by mercury strain-gauge plethysmography (Hokanson, Bellevue, WA). Two minutes before taking an antecubital sample, a cuff was inflated to a pressure of 220 mm Hg around the wrist for 2 min to exclude the hand circulation from the forearm preparation and prevent the wrist perfusers from draining blood from the superficial venous system (skin and sc adipose tissue) into the deep venous system (27, 37). In addition, a cool fan was used over the forearm for 10 min before measurements to minimize contamination with skin blood (27). With these manipulations,

the contribution of skin and sc adipose tissue blood flow to muscle blood flow in the forearm is small and the variability of forearm blood flow measurements is reduced (38, 39).

Calculations (27, 40)

The values obtained from the two preprandial samples were averaged to give a zero time value. The plasma levels of metabolites were converted to whole blood by using fractional hematocrit.

In the fasting state, insulin sensitivity was estimated by the homeostasis model assessment index (41). In the postprandial state, insulin sensitivity was estimated by the Belfiore index (42) and the Matsuda index (43).

Glucose uptake from muscle or adipose tissue was calculated as: $(A-V)_{\text{glucose}}(\text{blood flow})$, and the fractional glucose extraction as: $(A-V)_{\text{glucose}}/A_{\text{glucose}}$ (this calculation is independent of blood flow).

The net release of NEFAs from the adipose tissue was calculated as $(V-A)_{\text{NEFA}}$ and multiplied by blood flow to give absolute values.

Reesterification of NEFAs within the adipose tissue was calculated on the assumption that hydrolysis of each triglyceride molecule releases one molecule of glycerol and three molecules of NEFAs as follows: $3[(V-A)_{\text{glycerol}}(\text{blood flow})] - [(V-A)_{\text{NEFA}}(\text{blood flow})]$.

Triglyceride clearance across the adipose tissue was calculated as: $[(A-V)_{\text{triglycerides}}(\text{blood flow})]/(A_{\text{triglycerides}})$.

The relative rates of LPL activity and adipose tissue lipolysis were calculated as follows: $\text{LPL} = (A-V)_{\text{triglycerides}}(\text{blood flow})$, and adipose tissue lipolysis = $[(V-A)_{\text{glycerol}}(\text{blood flow})] - \text{LPL}$ (expressed in glycerol concentration units).

The rates of LPL activity and adipose tissue lipolysis were calculated on the basis of the following assumptions (40): 1) extraction of triglycerides from blood represents the activity of LPL; 2) there is no significant reuse of glycerol or oxidation of fatty acids within the tissue; and 3) both LPL activity and adipose tissue lipolysis release glycerol mole for mole with hydrolysis of triglycerides. This involves the assumption that there is no release of partial hydrolysis products of triglycerides (mono- and diacylglycerol) from the tissue.

Statistical analysis

Results are presented as mean \pm SEM of plasma levels or integrated postprandial responses [areas under curve (AUC), calculated with the trapezoid rule from the start of the meal to 360 min (AUC_{0-360})]. Differences between groups were tested with the Mann-Whitney test. Differences within groups were tested with paired Student's *t* test. A repeated-measures ANOVA for ranked measurements was applied to evaluate differences between groups across time, after adjusting for BMI and age (23). Grouping variable was considered as fixed and group-to-time interaction was evaluated; no random effects factors were used. *Post hoc* analysis was applied to test for the group effect on the investigated variables at specific time points. The Bonferroni rule was applied to account for the inflation in type I error. All hypotheses tested were two sided and the calculated *P* values were exact. All statistical calculations were performed in SPSS (version 12; SPSS Inc., Chicago, IL).

Results

Arterial levels of hormones and metabolites

Fasting plasma glucose, insulin, and NEFA levels were not altered by hypothyroidism, whereas fasting triglycerides were increased ($P = 0.03$). Fasting plasma glucagon levels in HO (94 ± 7 pg/ml) were higher than those in EU (72 ± 3 pg/ml, $P = 0.04$).

In HO, fasting arterial and abdominal venous levels of adiponectin were lower, whereas those of leptin were higher than those in EU (Table 1). Fasting arterial and abdominal venous levels of TNF-α, resistin, and visfatin were similar in both groups (Table 1).

After the meal, plasma glucose levels were higher at 120 min ($P = 0.02$) and insulin levels at 90 and 120 min ($P = 0.03$) in HO *vs.* EU. Integrative (AUC_{0-360}) plasma glucose and

TABLE 1. Postabsorptive arterial and abdominal venous levels of leptin, TNF- α , adiponectin, resistin, and visfatin in HO and EU subjects

| | Arterial | | Abdominal venous | |
|---------------------------|---------------------------|---------------|-------------------------------|----------------------------|
| | HO | EU | HO | EU |
| Leptin (pg/ml) | 11 \pm 1.7 ^a | 5.3 \pm 0.7 | 15.1 \pm 2.9 ^{a,b} | 6.9 \pm 0.9 ^b |
| TNF- α (pg/ml) | 1.6 \pm 0.2 | 1.5 \pm 0.2 | 1.4 \pm 0.08 | 1.7 \pm 0.3 |
| Adiponectin (μ g/ml) | 12 \pm 2 ^c | 22 \pm 3 | 10.8 \pm 2 ^a | 18 \pm 1.9 |
| Resistin (ng/ml) | 3.5 \pm 0.3 | 2.9 \pm 0.3 | 3.3 \pm 0.4 | 2.8 \pm 0.2 |
| Visfatin (ng/ml) | 7.6 \pm 0.2 | 6.3 \pm 0.9 | 8.5 \pm 0.8 | 7.2 \pm 0.9 |

Results are presented as mean + SEM.
^a Statistical significant differences between HO and EU subjects, $P = 0.04$.
^b Statistical significant differences between arterial and abdominal venous values, $P = 0.02$.
^c Statistical significant differences between HO and EU subjects, $P = 0.03$.

insulin levels were higher in HO *vs.* EU (2295 \pm 159 *vs.* 2063 \pm 36 mM \cdot min, $P = 0.001$ for glucose and 15 \pm 2 *vs.* 10 \pm 0.7 U/liter \cdot min, $P = 0.04$ for insulin) (Fig. 1, A and B). Integrative (AUC_{0–360}) plasma glucagon levels were similar in HO (31 \pm 3ng/ml \cdot min) and EU (26 \pm 2 ng/ml \cdot min).
Postprandial changes in plasma NEFAs showed a similar pattern in both HO and EU (Fig. 1C).
Postprandial plasma triglycerides increased steadily in both groups and by 240 min reached values 1.5- to 2-fold

higher than their fasting levels; in HO, these levels were all significantly higher than those of EU ($P = 0.02$) (Fig. 1D).
The homeostasis model assessment index was not different in the two groups (1.4 \pm 0.3 and 1.1 \pm 0.1 in HO and EU, respectively), suggesting a normal sensitivity to insulin in the fasting state. In contrast, the Belfiore index in HO was significantly lower than the comparison value of one (0.87 \pm 0.08, $P = 0.002$), and the Matsuda index was lower in HO *vs.* EU (5.7 \pm 0.8 *vs.* 8.1 \pm 0.66, $P = 0.04$); both indices suggested

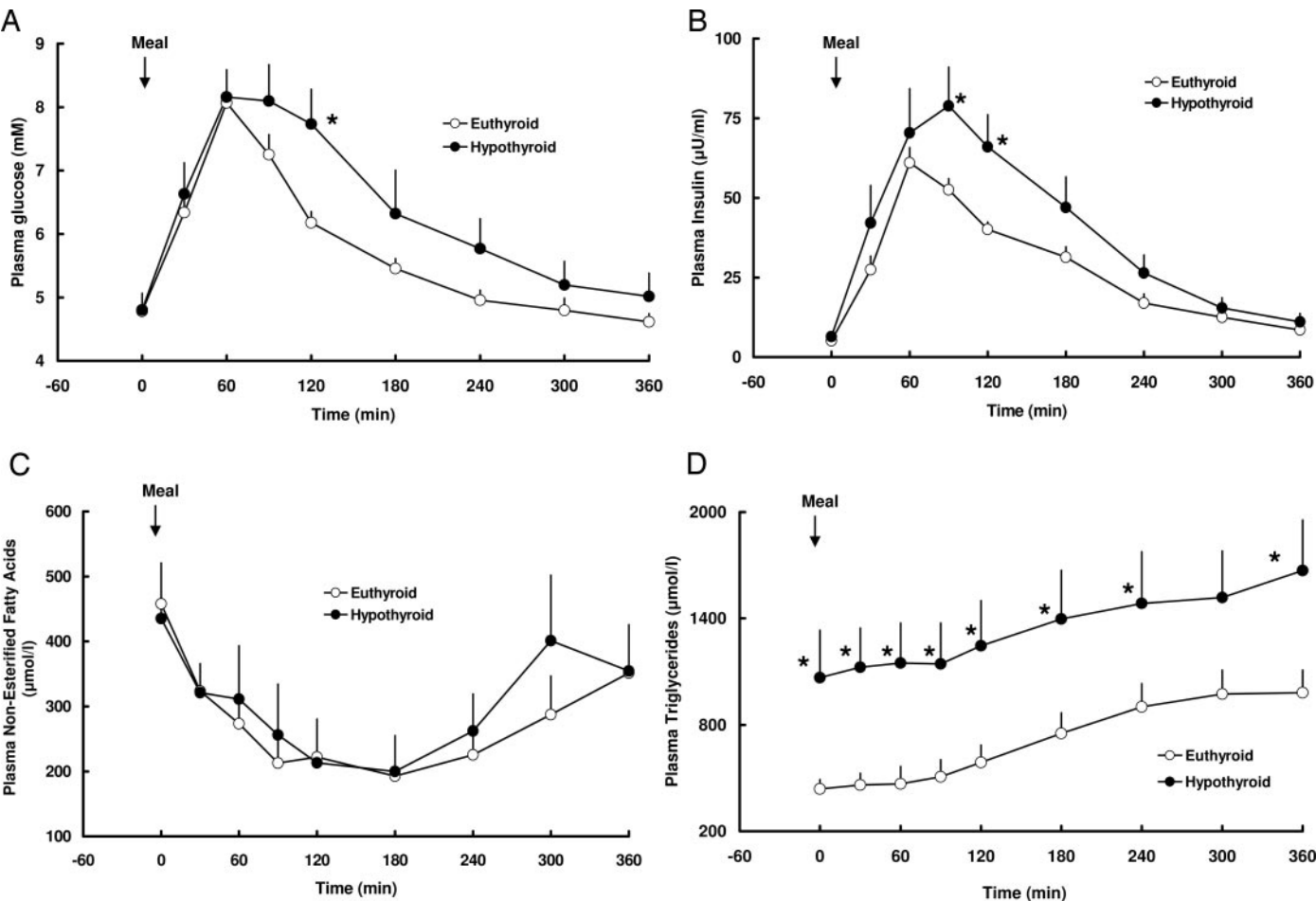


FIG. 1. Arterial plasma glucose (A), insulin (B, multiply by 6 to convert to picomoles per liter), NEFA (C), and triglyceride (D) levels in EU and HO subjects after a meal (p_{overall} between groups: 0.04 for insulin, 0.52 for glucose, 0.74 for NEFAs, and 0.024 for triglycerides). *, $P < 0.05$ from *post hoc* analysis using Bonferroni rule.

the presence of insulin resistance in the postprandial state. In the calculation of the Belfiore index, the mean of values of EU are considered equal to 1; values lower than 1 indicate insulin resistance (41–43).

Blood flow

In EU, forearm and adipose tissue blood flow increased by about 1.5- to 2-fold at 90 min and then progressively returned to baseline by the end of the experiment.

In HO, fasting blood flow rates in the forearm and adipose tissue were not different from those of EU; however, after the meal, blood flow did not increase in either tissue resulting in significantly lower rates at 60–90 min in the forearm ($P = 0.007$) and 60–180 min in the adipose tissue ($P = 0.0001$) (Figs. 2A and 3A).

Glucose uptake

In the fasting state, the net glucose uptake into the forearm and adipose tissue was similar in both groups.

In HO, glucose uptake after the meal was significantly lower at 60 and 90 min in the forearm ($P = 0.02$) (Fig. 2B) and at 60 min in the adipose tissue ($P = 0.015$) (Fig. 3B). The integrative postprandial glucose uptake in both tissues was

significantly lower in HO *vs.* EU [AUC_{0-360} 464 ± 74 *vs.* 850 ± 155 μmol per 100 ml tissue in the forearm ($P = 0.03$) and 208 ± 42 *vs.* 406 ± 47 μmol per 100 ml tissue in the adipose tissue ($P = 0.03$)]. In contrast, the fractional extraction of glucose in the forearm and adipose tissue in HO was similar to that of EU at all time points after the meal (Figs. 2C and 3C).

Lipid fluxes

Fasting net release of NEFAs from the adipose tissue in HO (551 ± 83 nmol/min per 100 ml tissue) was not different from that in EU (406 ± 74 nmol/min per 100 ml tissue); these rates were suppressed to the same extent in both groups after the meal (AUC_{0-360} 23 ± 8 and 30 ± 9 μmol per 100 ml tissue in HO and EU, respectively). The suppression of adipose tissue lipolysis by insulin after the meal was also similar in both groups (AUC_{0-360} 14 ± 6 and 19 ± 9 μmol per 100 ml tissue in HO and EU, respectively).

There were no differences in adipose tissue NEFA re-esterification rates between HO and EU (fasting 82 ± 30 and 92 ± 17 nmol/min per 100 ml tissue, respectively, and AUC_{0-360} 149 ± 37 and 140 ± 52 μmol per 100 ml tissue, respectively).

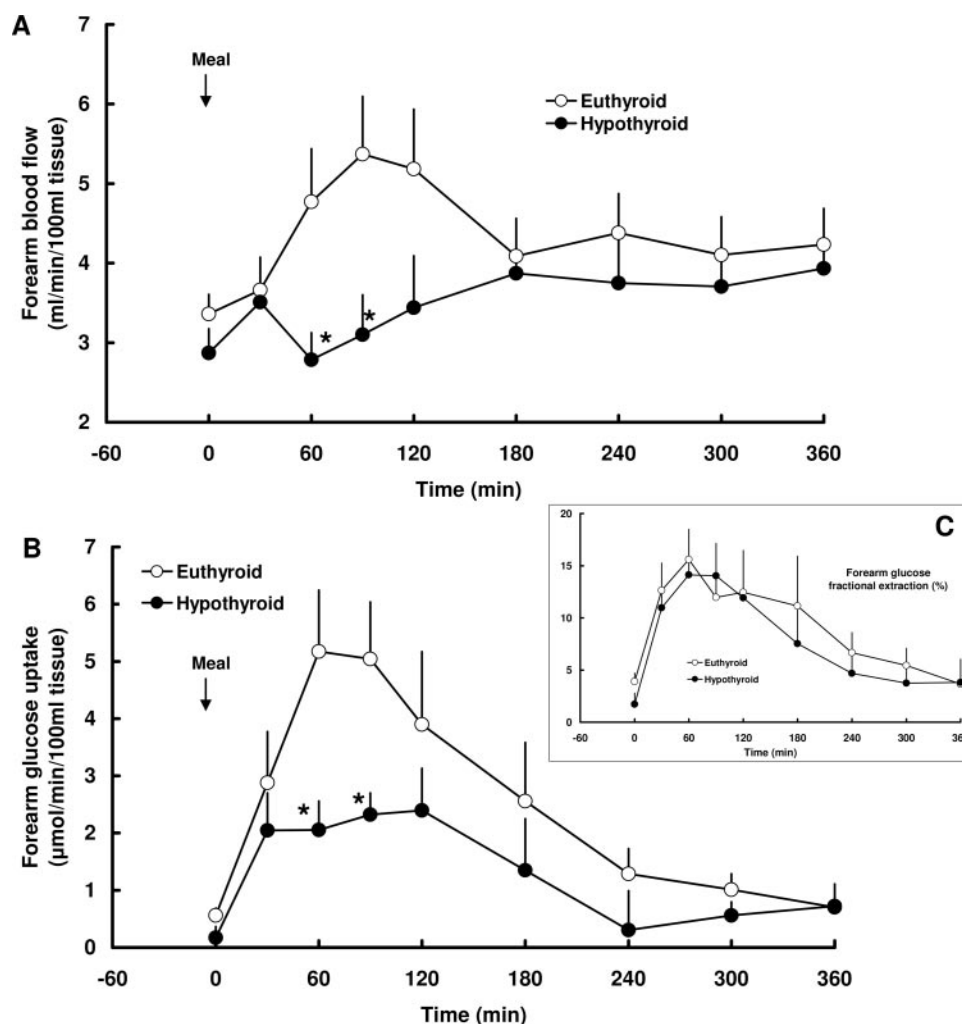


FIG. 2. Forearm blood flow (A), glucose uptake (B), and fractional glucose uptake (C) in EU and HO subjects after a meal (p_{overall} between groups: 0.021 for blood flow, 0.023 for glucose uptake, and 0.59 for fractional glucose uptake). *, $P < 0.05$ from *post hoc* analysis using Bonferroni rule.

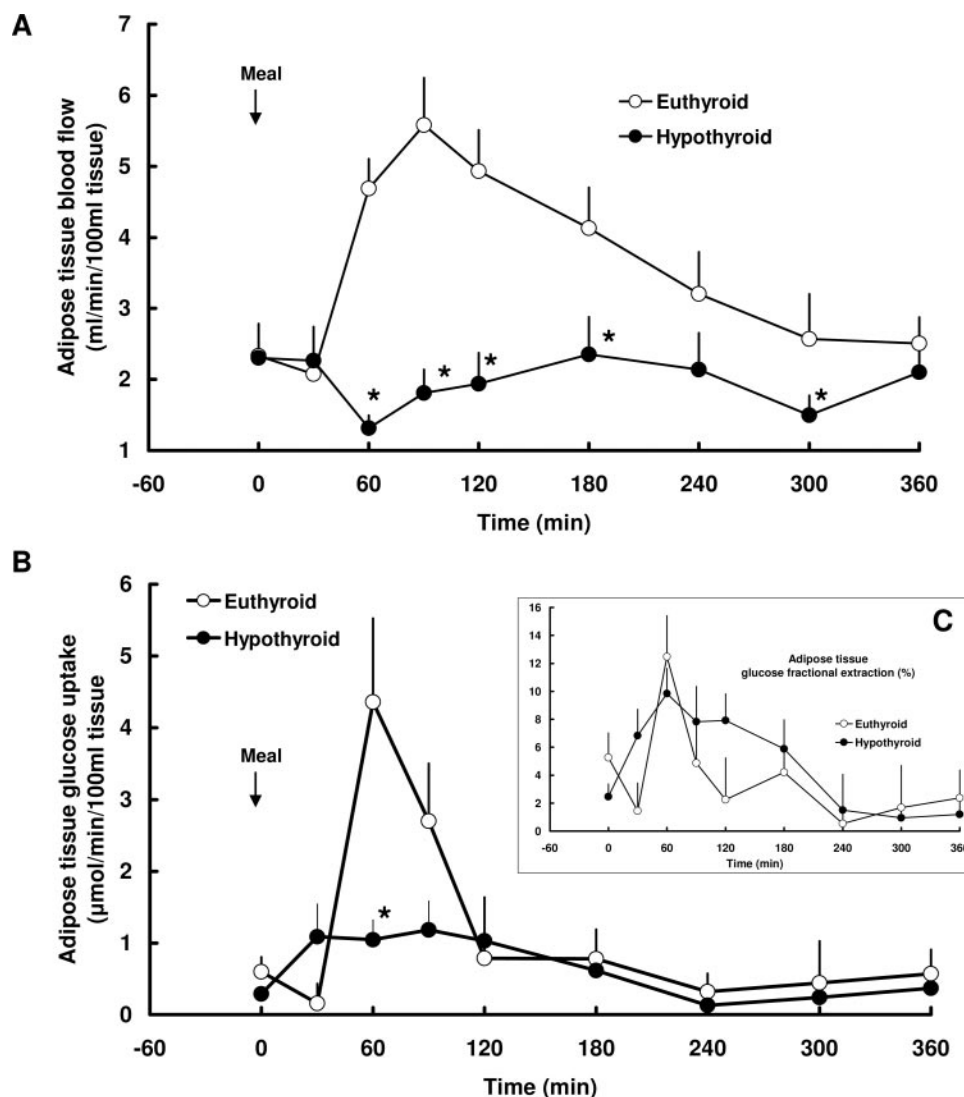


FIG. 3. Adipose tissue blood flow (A), glucose uptake (B), and fractional glucose uptake (C) in EU and HD subjects after a meal (p_{overall} between groups: 0.0001 for blood flow, 0.03 for glucose uptake, and 0.69 for fractional glucose uptake). *, $P < 0.05$ from *post hoc* analysis using Bonferroni rule.

Differences were observed in triglyceride clearance rates across the adipose tissue (AUC_{0-360} 45 ± 10 ml per 100 ml tissue in HO *vs.* 109 ± 21 ml per 100 ml tissue in EU, $P = 0.01$) but not in the forearm (AUC_{0-360} 55 ± 14 ml per 100 ml tissue in HO *vs.* 79 ± 10 ml per 100 ml tissue in EU, $P = 0.21$) after adjusting for BMI and age.

In HO, values of LPL activity in the adipose tissue [fasting 27 ± 7 nmol glycerol per minute per 100 ml tissue and postprandial (AUC_{0-360}) 42 ± 11 μmol per 100 ml tissue] were also significantly different from respective values in EU [fasting 40 ± 10 nmol glycerol per minute per 100 ml tissue and postprandial (AUC_{0-360}) 80 ± 21 μmol per 100 ml tissue, $P = 0.04$] after adjusting for BMI and age of the subjects. In the forearm, values of LPL activity in HO [fasting 14 ± 8 nmol glycerol per minute per 100 ml tissue and postprandial (AUC_{0-360}) 48 ± 10 μmol per 100 ml tissue] were not significantly different from those in EU [fasting 28 ± 7 nmol glycerol per minute per 100 ml tissue and postprandial (AUC_{0-360}) 42 ± 9 μmol per 100 ml tissue, $P = 0.99$].

Venoarterial differences of circulating triglyceride levels across the adipose tissue were not significantly different

between HO (AUC_{0-360} 30 ± 10 mmol/liter·min) and EU (AUC_{0-360} 20 ± 8 mmol/liter·min).

Discussion

Glucose uptake

Despite the increase in plasma insulin levels in HO, net glucose uptake in the forearm muscles and adipose tissue was not increased after the meal, suggesting the presence of insulin resistance.

These results are in agreement with experiments performed in muscle of hypothyroid rats (13–15) and adipose tissue segments isolated from patients with hypothyroidism (12), showing that rates of glucose transport did not respond when insulin was increased within the physiological range. They also correspond well with observations in hypothyroid subjects using euglycemic-hyperinsulinemic clamps (7), showing resistance of whole-body glucose disposal to plasma insulin levels, similar to those obtained in our HO after the meal.

Our results are not in agreement with a previous report

that measured insulin-stimulated glucose disposal in the forearm of hypothyroid patients during euglycemic-hyperinsulinemic clamps: these rates remained unaltered after treatment with thyroxine, implying that hypothyroidism had no effect on insulin action in muscle (6). The results of this study are, however, difficult to interpret because plasma insulin levels during the clamp were about 20% higher before treatment of hypothyroidism than after treatment [most likely due to differences in clearance rates (2)], and no euthyroid group was included for comparison.

The effect of insulin on blood flow is an important component of its stimulation of glucose uptake (44); impairment of this mechanism in insulin-sensitive tissues may partly account for insulin resistance in obesity and type 2 diabetes (45). In our study, forearm and adipose tissue blood flow and glucose disposal rates in HO were significantly decreased at 60–90 min after the meal when plasma insulin levels reached their peak. To examine the possibility that the decreases in blood flow were responsible for the defect in insulin-stimulated glucose disposal in the forearm and adipose tissue, we calculated fractional glucose extraction: this was not impaired, suggesting that the resistance of glucose disposal to insulin in both tissues in HO could be accounted for by the impairment of the vasodilatory response. The increase of blood flow by insulin in muscle is direct, whereas in adipose tissue this effect is indirect through a stimulation of the sympathetic system (44–46). Our results may therefore be explained by reports demonstrating that hypothyroidism impairs flow-mediated vasodilatation (a marker of endothelial dysfunction) in muscle (47, 48) and decreases sympathetic responses in the adipose tissue (49, 50).

Lipid metabolism

In the euthyroid state, the buffering of NEFA flux by insulin is regulated mainly by the suppression of lipolysis and NEFA release from the adipose tissue via a decrease in the activity of hormone-sensitive lipase and adipose triglyceride lipase (33, 51, 52).

In our study, rates of NEFA release from the adipose tissue of HO and adipose tissue lipolysis were suppressed after the meal to the same extent as in EU, suggesting that the response of hormone-sensitive lipase and adipose triglyceride lipase to insulin was not impaired (plasma insulin levels were similar in both groups from 0 to 60 min). These results correspond favorably with observations made in adipocytes isolated from patients with hypothyroidism and incubated *in vitro*: at levels of insulin within the physiological range (5–50 μ U/ml), the suppression of glycerol release (used as a measure of lipolysis) was similar with that of euthyroid cells (11).

Changes in plasma triglycerides have never been examined after meal ingestion in hypothyroidism: these levels were elevated in the fasting state and continued to rise after the meal (Fig. 1D), suggesting a lower triglyceride turnover (17). The plasma triglyceride concentration is the net result of several metabolic processes including: 1) endogenous synthesis of the very low-density lipoprotein (VLDL; the carrier for triglycerides of endogenous origin) by the liver and secretion into plasma, and 2) clearance from plasma, which depends on the activities of LPL and hepatic triglyceride

lipase and tissue blood flow (33, 51). In the fasting state, the activity of hepatic triglyceride lipase is higher than that of LPL; in the postprandial state, the increase in plasma insulin stimulates the activity of LPL, whereas that of hepatic triglyceride lipase remains unaltered (53).

Our results show that the increase in plasma triglycerides in HO was secondary to a decreased rate of removal by the adipose tissue because both fasting and postprandial LPL activities were significantly decreased in the presence of hyperinsulinemia. Because the venoarterial differences of plasma triglycerides across the adipose tissue were similar in the two groups, low LPL activity in HO may be due, at least in part, to the decrease in the blood flow rates. However, establishment of this suggestion would require experiments with direct measurements of LPL activity in the adipose tissue. Previous studies in patients with hypothyroidism using iv injections of triglyceride emulsions and heparin suggested that the removal of both endogenous and exogenous triglycerides was reduced (17–22); however, this method measures only an average systemic lipase activity and cannot detect changes in LPL activity that may occur in individual tissues (53). In rat adipose tissue, thyroid hormones have been shown to regulate LPL posttranscriptionally, resulting in changes in both synthetic rate and activity (54). Direct measurements of LPL in adipose tissue biopsies in patients with hypothyroidism have been done in two studies: the total activity of LPL was found to be normal, but the activated form of this enzyme (measured *in vitro* as the heparin-elutable enzyme) was either normal (19) or decreased (21).

Although not investigated in our study, the possibility that triglyceride increases in HO could be accounted for, at least in part, by a lower rate of triglyceride removal by the liver cannot be excluded. This is supported by studies in humans suggesting that hypothyroidism decelerates the capacity of the liver for removal of remnants of chylomicrons and VLDL due to a decreased activity of hepatic triglyceride lipase (20, 22).

Could increased VLDL-triglyceride production by the liver account for the elevated plasma triglyceride levels? This is suggested by experiments in rats made hypothyroid, showing that the output of VLDL-triglycerides from the isolated perfused liver was increased (55). However, in patients with hypothyroidism, hepatic VLDL-triglyceride synthesis and release were found to be normal (55).

Finally, it is likely that a delay in meal absorption may be responsible, at least in part, for the late increases of plasma triglyceride levels after the meal in HO. Hypothyroidism has been shown to delay gastric emptying and meal absorption rates (56).

Although overnight NEFA kinetics were not investigated in our study (57), it is unlikely that these were different between HO and EU: in HO, fasting plasma NEFA levels and NEFA fluxes in the adipose tissue (output and reesterification) and adipose tissue lipolysis were all similar to those in EU.

To support insulin resistance in HO, we measured parameters related to adipose tissue that could be responsible for the decrease in insulin sensitivity in the postprandial state (see *Results* and Table 1) (58–61). In HO, plasma levels of leptin (62–65) were increased, whereas those of adiponectin

(62, 64) were decreased. In muscle and adipocytes, adiponectin has been found to stimulate glucose use by activating AMP-activated protein kinase, whereas leptin to reduce insulin-stimulated glucose uptake (66, 67). In recent reports, plasma adiponectin levels were found to correlate positively with endothelium-dependent vasodilatation (68), an effect that was independent of that on insulin sensitivity (69), and in insulin-resistant subjects with type 2 diabetes or obesity, low adiponectin levels were closely linked to decreases in tissue blood flow rates (70). These results may explain, at least in part, low blood flow rates and insulin resistance in the forearm and adipose tissue in HO in our study.

Concluding remarks

Our study showed that in hypothyroidism: 1) glucose uptake in muscle and adipose tissue is resistant to insulin; 2) the suppression of lipolysis by insulin is not impaired; 3) plasma levels of triglycerides are elevated due to decreased clearance by the adipose tissue; 4) a major finding to explain most of the metabolic defects is the decrease in adipose tissue blood flow rates.

These results, taken together with our recently published data on hyperthyroidism (71), suggest that thyroid hormone excess and deprivation do not make a consistent story: in hypothyroidism the decrease of blood flow in adipose tissue and muscle may be considered as part of the pathogenetic mechanism of insulin resistance explaining most of the metabolic defects in these tissues; in contrast, in hyperthyroidism the increase of blood flow seems to correct the intrinsic metabolic defects in muscle and adipose tissue (71). Moreover, in hypothyroidism the targets of insulin action are not uniformly impaired: glucose uptake and proteolysis (72) are resistant to insulin, but lipolysis is not; the latter may be necessary to relieve tissues from the burden of NEFA surplus after meals. In contrast, in hyperthyroidism all three targets are resistant to insulin (1, 71).

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The authors have nothing to declare.

References

- Dimitriadis G, Raptis SA 2001 Thyroid hormone excess and glucose intolerance. *Exp Clin Endocrinol Diabetes* 109(Suppl 2):S225–S239
- Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, Haymond M, Gerich J 1985 Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans. *Am J Physiol* 248:E593–E601
- Okajima F, Ue M 1979 Metabolism of glucose in hyper- and hypothyroid rats *in vivo*. *Biochem J* 182:565–575
- McCulloch AJ, Nosadini R, Pernet A, Piniewska M, Cook DB, Clark F, Johnston DG, Alberti KGMM 1983 Glucose turnover and indices of recycling in thyrotoxicosis and primary thyroid failure. *Clin Sci* 64:41–47
- Muller MJ, Mitchinson PE, Paschen U, Seitz HJ 1988 Glucoregulatory function of glucagon in hypo-, eu-, and hyperthyroid miniature pigs. *Diabetologia* 31:368–374
- Harris PE, Walker M, Clark F, Home PD, Alberti KGMM 1993 Forearm muscle metabolism in primary hypothyroidism. *Eur J Clin Invest* 23:585–588
- Rochon C, Tauveron I, Dejax C, Benoit P, Capitan P, Fabricio A, Berry C, Champedon C, Thieblot P, Grizard J 2003 Response of glucose disposal to hyperinsulinemia in human hypothyroidism and hyperthyroidism. *Clin Sci* 104:7–15
- Lamberg BA 1965 Glucose metabolism in thyroid disease. *Acta Med Scand* 178:351–362
- Andreani D, Menzinger G, Falluca F, Aliberti G, Tamburano G, Cassano C 1970 Insulin levels in thyrotoxicosis and primary myxoedema: response to intravenous glucose and glucagon. *Diabetologia* 6:1–7
- Jackson IMD, Prentice CRM, McKiddie MT 1970 The effect of hypothyroidism on glucose tolerance and insulin metabolism. *J Endocrinol* 47:257–258
- Arner P, Bolinder J, Wennlund A, Ostman J 1984 Influence of thyroid hormone level on insulin action in human adipose tissue. *Diabetes* 33:369–375
- Pedersen O, Richelsen B, Bak J, Arnfred J, Weeke J, Schmitz O 1988 Characterization of the insulin resistance of glucose utilization in adipocytes from patients with hyper- and hypothyroidism. *Acta Endocrinol* 119:228–234
- Dimitriadis G, Leighton B, Parry-Billings M, West D, Newsholme E 1989 Effects of hypothyroidism on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat. *Biochem J* 257:369–373
- Dimitriadis G, Parry-Billings M, Bevan S, Leighton B, Krause U, Piva T, Tegos K, Challiss RAJ, Wegener G, Newsholme EA 1997 The effects of insulin on transport and metabolism of glucose in skeletal muscle from hyperthyroid and hypothyroid rats. *Eur J Clin Invest* 27:475–483
- Cettour-Rose P, Theander-Carrillo C, Asensio C, Klein M, Visser TJ, Burger AG, Meier CA, Rohrer-Jeanraud F 2005 Hypothyroidism in rats decreases peripheral glucose utilization, a defect partially corrected by central leptin infusion. *Diabetologia* 48:624–633
- Correze C, Krug E, Verhaegen M, Nunez J 1979 Regulation of lipogenesis in adipocytes: Independent effects of thyroid hormones, cyclic-AMP and insulin on the uptake of deoxy-D-glucose. *Biochim Biophys Acta* 574:164–172
- Nikkila E, Kekki M 1972 Plasma triglyceride metabolism in thyroid disease. *J Clin Invest* 51:2103–2114
- Tulloch B, Lewis B, Russell-Fraser T 1973 Triglyceride metabolism in thyroid disease. *Lancet* 1:391–394
- Persson B 1973 Lipoprotein lipase activity of human adipose tissue in health and in some diseases with hyperlipidaemia as a common feature. *Acta Med Scand* 193:457–462
- Krauss RM, Levy RI, Fredrickson DS 1974 Selective measurement of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. *J Clin Invest* 54:1107–1124
- Pykalisto O, Goldberg AP, Brunzell JD 1976 Reversal of decreased human adipose tissue lipoprotein lipase and hypertriglyceridemia after treatment of hypothyroidism. *J Clin Endocrinol Metab* 43:591–600
- Abrams J, Grundy S, Ginsberg H 1981 Metabolism of plasma triglycerides in hypothyroidism and hyperthyroidism. *J Lipid Res* 22:307–322
- Gallagher D, Visse M, Sepulveda D, Pierson RN, Harris T, Heymsfield SB 1996 How useful is body mass index for comparison of body fatness across age, sex and ethnic groups? *Am J Epidemiol* 143:228–239
- Wolf M, Weigert A, Kreymann G 1996 Body composition and energy expenditure in thyroidectomized patients during short-term hypothyroidism and thyrotropin-suppressive thyroxine therapy. *Eur J Endocrinol* 134:168–173
- Hoffstedt J, Arvidsson E, Sjolin E, Wahlen K, Arner P 2004 Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. *J Clin Endocrinol Metab* 89:1391–1396
- Miles JM, Jensen MD 2005 Visceral adiposity is not causally related to insulin resistance. *Diabetes Care* 28:2326–2328
- Coppack S, Fisher R, Gibbons G, Frayn K 1990 Postprandial substrate deposition in human forearm and adipose tissue *in vivo*. *Clin Sci* 79:339–348
- Dimitriadis G, Boutati E, Lambadiari V, Mitrou P, Maratou E, Brunel P, Raptis SA 2004 Restoration of early insulin secretion after a meal in type 2 diabetes: effects on lipid and glucose metabolism. *Eur J Clin Invest* 34:490–497
- Beysen C, Belcher AK, Karpe F, Fielding BA, Herrera E, Frayn KN 2003 Novel experimental protocol to increase specific plasma nonesterified fatty acids in humans. *Am J Physiol* 284:E18–E24
- Simonsen L, Enevoldsen LH, Bulow J 2003 Determination of adipose tissue blood flow with local ^{133}Xe clearance. Evaluation of a new labelling technique. *Clin Physiol Funct Imaging* 23:320–323
- Ardilouze JL, Karpe F, Currie JM, Frayn KN 2004 Subcutaneous adipose tissue blood flow varies between superior and inferior levels of the anterior abdominal wall. *Int J Obes* 28:228–233
- Summers LKM, Samra JS, Humphreys SM, Morris RJ, Frayn KN 1996 Subcutaneous abdominal adipose tissue blood flow: variation within and between subjects and relationship to obesity. *Clin Sci* 91:679–683
- Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW 2003 Integrative physiology of human adipose tissue. *Int J Obes* 27:875–888
- Frayn KN, Coppack SW, Humphreys SM, Whyte PL 1989 Metabolic characteristics of human adipose tissue *in vivo*. *Clin Sci* 76:509–516
- Johnson JA, Fusaro RM 1972 The role of the skin in carbohydrate metabolism. In: Levine R, Luft R, eds. *Advances in metabolic disorders*. Vol 6. New York: Academic Press; 1–55

36. Summers LKM, Arner P, Ilic V, Clark ML, Humphreys SM, Frayn KN 1998 Adipose tissue metabolism in the postprandial period: microdialysis and arteriovenous techniques compared. *Am J Physiol* 274:E651–E655
37. Elia M 1991 The inter-organ flux of substrates in fed and fasted man, as indicated by arterio-venous balance studies. *Nutrition Res Rev* 4:3–31
38. Butler PC, Home PD 1987 the measurement of metabolic exchange across muscle beds. *Baillieres Clin Endocrinol Metab* 1:863–878
39. Blaak EE, van Baak MA, Kemerink GJ, Pakbiers MTW, Heidendal GAK, Saris WHM 1994 Total forearm blood flow as an indicator of skeletal muscle blood flow: effect of subcutaneous adipose tissue blood flow. *Clin Sci* 87:559–566
40. Frayn K, Shadid S, Hamrani R, Humphreys S, Clark M, Fielding B, Boland O, Coppack S 1994 Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 266:E308–E317
41. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R 1985 Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations. *Diabetologia* 28:412–419
42. Belfiore F, Iannello S, Volpicelli G 1998 Insulin sensitivity indices calculated from basal and OGTT-induced insulin, glucose and FFA levels. *Mol Genet Metab* 63:134–141
43. Matsuda M, DeFronzo R 1999 Insulin sensitivity indices obtained from oral glucose tolerance testing. *Diabetes Care* 22:1462–1470
44. Baron A 1994 Hemodynamic actions of insulin. *Am J Physiol* 267:E187–E202
45. Baron AD, Laakso M, Brechtel G, Hoit B, Watt C, Edelman SV 1990 reduced postprandial skeletal muscle blood flow contributes to glucose intolerance in human obesity. *J Clin Endocrinol Metab* 70:1525–1533
46. Karpe F, Fielding B, Ardilouze JL, Ilic V, Macdonald I, Frayn K 2002 Effects of insulin on adipose tissue blood flow in man. *J Physiol* 540.3:1087–1093
47. Wennlund A, Linde B 1984 Influence of hyper- and hypothyroidism on subcutaneous adipose tissue blood flow in man. *J Clin Endocrinol Metab* 59:258–262
48. Lekakis J, Papamichael C, Alevizaki M, Pipingos G, Marafelia P, Mantzos J, Stamatelopoulos S, Koutras DA 1997 Flow-mediated, endothelium-dependent vasodilatation is impaired in subjects with hypothyroidism, borderline hypothyroidism and high-normal serum thyrotropin levels. *Thyroid* 7:411–414
49. Wahrenberg H, Wennlund A, Arner P 1994 Adrenergic regulation of lipolysis in fat cells from hyperthyroid and hypothyroid subjects. *J Clin Endocrinol Metab* 78:898–903
50. Haluzik M, Nedvikova J, Bartak V, Dostalova I, Vlcek P, Racek P, Taus M, Svacina S, Alesci S, Pacak K 2003 Effects of hypo- and hyperthyroidism on noradrenergic activity and glycerol concentrations in human subcutaneous abdominal adipose tissue assessed with microdialysis. *J Clin Endocrinol Metab* 88:5605–5608
51. Coppack SW, Jensen MD, Miles JM 1994 The *in vivo* regulation of lipolysis in humans. *J Lipid Res* 35:177–193
52. Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R 2004 Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 306:1383–1386
53. Coppack SW, Yost TJ, Fisher RM, Eckel RH, Miles JM 1996 Periprandial systemic and regional lipase activity in normal humans. *Am J Physiol Endocrinol Metab* 270:E718–E722
54. Saffari B, Ong JM, Kern PA 1992 Regulation of adipose tissue lipoprotein lipase gene-expression by thyroid hormones in rats. *J Lipid Res* 33:241–249
55. Heimberg M, Olubadewo J, Wilcox H 1985 Plasma lipoproteins and regulation of hepatic metabolism of fatty acids in altered thyroid states. *Endocr Rev* 6:590–607
56. Kahraman H, Kaya N, Demircali A, Bernay I, Tanyeri F 1997 Gastric emptying time in patients with primary hypothyroidism. *Eur J Gastroenterol Hepatol* 9:901–904
57. Miles JM, Wooldridge D, Grellner WJ, Windsor S, Isley WL, Klein S, Harris WS 2003 Nocturnal and postprandial free fatty acid kinetics in normal and type 2 diabetic patients. *Diabetes* 52:675–681
58. Liljenquist JE, Bomboy JD, Lewis SB, Sinclair-Smith BC, Felts PW, Lacy WW, Crofford OB, Liddle GW 1974 Effects of glucagon on lipolysis and ketogenesis in normal and diabetic man. *J Clin Invest* 53:190–197
59. Richter WO, Robl H, Schwandt P 1989 Human glucagon and vasoactive intestinal polypeptide stimulate free fatty acid release from human adipose tissue *in vitro*. *Peptides* 10:333–335
60. Gravholt CH, Moller N, Jensen MD, Christiansen JS, Schmitz O 2001 Physiological levels of glucagon do not influence lipolysis in abdominal adipose tissue as assessed by microdialysis. *J Clin Endocrinol Metab* 86:2085–2089
61. Koerner A, Kratzsch J, Kiess W 2005 Adipocytokines: leptin—the classical, resistin—the controversial, adiponectin—the promising, and more to come. *Best Pract Res Clin Endocrinol Metab* 19:525–546
62. Botella-Carretero JL, Alvarez-Blasco F, Sancho J, Escobar-Morreale HF 2006 Effects of thyroid hormones on serum levels of adipokines as studied in patients with differentiated thyroid carcinoma during thyroxine withdrawal. *Thyroid* 16:397–402
63. Valcavi R, Zini M, Peino R, Casanueva F, Diequez C 1997 Influence of thyroid status on serum immunoreactive leptin levels. *J Clin Endocrinol Metab* 82:1632–1634
64. Iglesias P, Alvarez Fidalgo P, Codoceo R, Diez JJ 2003 Serum concentrations of adipocytokines in patients with hyperthyroidism and hypothyroidism before and after control of thyroid function. *Clin Endocrinol (Oxf)* 59:621–629
65. Sesmilo G, Casamitjana R, Halperin I, Gomis R, Villardell E 1998 Role of thyroid hormones on serum leptin levels. *Eur J Endocrinol* 139:428–430
66. Yamacuchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda N, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T 2002 Adiponectin stimulates glucose utilization and fatty acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8:1288–1295
67. Ceddia RB, Koistinen HA, Zierath JR, Sweeney G 2002 Analysis of paradoxical observations on the association between leptin and insulin resistance. *FASEB J* 16:1163–1176
68. Shimabukuro M, Higa N, Asahi T, Oshiro Y, Takasu N, Tagawa T, Ueda S, Shimomura I, Funahashi T, Matsuzawa Y 2003 Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *J Clin Endocrinol Metab* 88:3236–3240
69. Fernandez-Real JM, Castro A, Vazquez G, Casamitjana R, Lopez-Bermejo A, Penarroja G, Ricart W 2004 Adiponectin is associated with vascular function independent of insulin sensitivity. *Diabetes Care* 27:739–745
70. Ouchi N, Mitsuru O, Kihara S, Funahashi T, Nakamura T, Nagaretani H, Kumada M, Ohashi K, Okamoto Y, Nishizawa H, Kishida K, Maeda N, Nagasawa A, Kobayashi H, Hiraoka H, Komai N, Kaibe M, Rakugi H, Ogiwara T, Matsuzawa Y 2003 Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension* 42:231–234
71. Dimitriadis G, Mitrou P, Lambadiari V, Boutati E, Maratou E, Koukkou E, Tzanela M, Thalassinou N, Raptis SA 2006 Glucose and lipid fluxes in the adipose tissue after meal ingestion in hyperthyroidism. *J Clin Endocrinol Metab* 91:1112–1118
72. Rochon C, Tauveron I, Dejax C, Benoit P, Capitan P, Bayle G, Prugnaud J, Fabricio A, Berry C, Champredon C, Thieblot P, Grizard J 2000 Response of leucine metabolism to hyperinsulinemia in hypothyroid patients before and after thyroxine replacement. *J Clin Endocrinol Metab* 85:697–706