Thermogenic Effect of Slight Hyperglycemia During a Lipid Infusion

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Resistance to the glucoregulatory action of insulin is a common finding in obesity and may affect thermogenesis. In 13 healthy subjects, we studied the influence of acute insulin resistance induced by a lipid infusion on thermogenesis without any glucose load (n = 4) or during a euglycemic-hyperinsulinemic clamp (n = 5) and an oral glucose tolerance test (OGTT, n = 8). When substrates were not administered at the same time, the energy cost of storage was significantly ($P < .05$) lower for lipids (3.9% ± 0.9%) than for glucose (11.9% ± 0.5% during the clamp and 14.9% ± 4.0% during the OGTT, NS). The lipid infusion decreased glucose storage during the clamp (control, 3.99 ± 0.40 mg · kg^{-1} · min^{-1}; lipid infusion, 0.92 ± 0.39; $P < .05$) but increased it during the OGTT (control, 1.76 ± 0.22 mg · kg^{-1} · min^{-1}; lipid infusion, 2.94 ± 0.27; $P < .05$). Infused lipids were stored more (clamp, 3.31 ± 0.16; OGTT, 2.65 ± 0.11 mg · kg^{-1} · min^{-1}; $P < .01$) and oxidized less (clamp, 0.64 ± 0.21; OGTT, 1.02 ± 0.09 mg · kg^{-1} · min^{-1}; $P < .05$) during the clamp than during the OGTT. When lipids were infused, the energy cost of substrate storage was lower during the clamp versus the OGTT (clamp, 3.2% ± 0.8%; OGTT, 7.3% ± 1.0%; $P < .05$). This effect was attributed to a lipid-induced impairment of glucose tolerance, which overcomes the inhibitory effect of lipid infusion on glucose storage observed in euglycemia. A slight elevation of plasma glucose in response to a lipid infusion impairs thermogenesis by redirecting the storage of substrates from lipids to glucose, which has a higher energy cost.

RESISTANCE to the glucoregulatory action of insulin is a well-known characteristic of obesity.[1] As demonstrated by the euglycemic clamp technique, stimulation of oxidative and nonoxidative glucose disposal and suppression of endogenous glucose production are less efficient in obese versus lean subjects.[2] The mechanism for this insulin resistance is unclear, but a metabolic origin involving the Randle cycle has been proposed.[3,4] The main experimental arguments for this hypothesis are the increased availability of lipid substrates in obese subjects as shown by higher circulating free fatty acid levels and higher rates of lipid oxidation,[5] the relation between these lipid abnormalities and the insulin resistance of glucose metabolism in obese subjects,[6] and the transient insulin resistance induced by lipid infusion in normal subjects.[7]

On the other hand, it has been suggested that insulin resistance upsets energy balance by reducing glucose-induced thermogenesis (GIT).[8] Although diet-induced thermogenesis is a minor part (~10%) of total thermogenesis, a slight defect could affect body weight over a period of years. Acheson et al[9] have shown that the major part of GIT (~70%, the obligatory component) is related to glucose storage, which is an insulin-sensitive, energy-requiring process, with the remaining 30% being β-adrenergically mediated. Both components are normally stimulated during a clamp procedure in obese subjects if the rates of glucose uptake and storage are matched to controls (with twice the plasma insulin level), and thus the lower GIT in obesity has been attributed to lower insulin-stimulated glucose storage.[8] However, reduced GIT is not a consistent finding in obese subjects,[10,11] and may be present in the absence of any alteration in nonoxidative glucose metabolism.[12] These discrepancies could be accounted for by differences in the regulation of GIT in the conditions of the hyperinsulinemic-euglycemic clamp and the oral glucose tolerance test (OGTT),[12] or by the marked variations in glucose metabolism in obese subjects.

To assess the influence of the insulin resistance of glucose metabolism on thermogenesis, we performed indirect calorimetry in normal subjects twice, either during a saline infusion (control) or a lipid infusion (Ivélip) to create an acute insulin-resistant state. Stimulation of glucose metabolism was obtained by the OGTT (n = 8) or the hyperinsulinemic-euglycemic clamp (n = 5), with an insulin level similar to the insulin peak during the OGTT. The specific effect of the lipid infusion on thermogenesis was evaluated from the energy production rate (EPR) during the lipid infusion alone.

SUBJECTS AND METHODS

Subjects

Thirteen healthy subjects (seven men and six women) were studied. Five (age, 24 ± 1 years; body weight, 63.0 ± 4.4 kg; height, 1.73 ± 0.03 m; body mass index, 21.2 ± 0.5 kg/m^2) underwent the clamp study (with/without lipid infusion). Eight (age, 23 ± 2 years; body weight, 63.0 ± 3.2 kg; height, 1.70 ± 0.02 m; body mass index, 21.8 ± 0.4 kg; all NS *v* subjects of the clamp study) underwent the OGTT study (with/without lipid infusion), four of whom were also infused with lipids without OGTT.

They were normal healthy volunteers. None had a family history of diabetes or used any drugs. They were not chronic alcohol users, and they were asked not to drink any alcoholic beverage 24 hours before the tests. They were asked to continue their normal diet before and between the tests. Their body weight was stable for 3 months before the first test and did not significantly change between tests. Each subject provided written consent for the study after being informed about its nature, purpose, and potential risks. The protocol was approved by the ethical committee of Edouard Herriot Hospital (Lyon, France).

Materials

D-[6,6-$^2H_2$]glucose (99% atom% excess) was obtained from the Commissariat à l'Energie Atomique (Gif-sur-Yvette, France). It was verified to be sterile and pyrogen-free, and was dissolved in sterile normal saline solution before administration. The triglyceride emulsion

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(Ivélip 20%) was obtained from Cernep Synthélabo (Montargis, France). Gas exchange was measured on a Deltatrac metabolic monitor (Datex, Champagne au Mont D'Or, France). A calibration procedure for oxygen and carbon dioxide was performed with a reference gas before and after each test to ensure that no deviation of the measurement occurred.

Experimental Protocols

All subjects were studied in the postabsorptive state after a 12-hour overnight fast at 9 AM. A retrograde catheter was inserted in a dorsal hand vein and kept in a hot blanket (55°C) to collect arterialized venous blood. Another forearm vein was catheterized in the contralateral arm to infuse D-[6,6-$^2H_2$]glucose and either saline or Ivélip 20% (0.015 $mL \cdot kg^{-1} \cdot min^{-1}$). Boden et al[7] have shown that it requires 3 to 4 hours to suppress insulin-stimulated, nonoxidative glucose disposal during a euglycemic clamp. Since the main objective of the lipid infusion in our tests was to create an insulin-resistant state of nonoxidative glucose disposal, it had to be commenced prior to the insulin stimulation and was prolonged until the end of it. For the clamps, the lipid infusion was started at the end of the study of the postabsorptive state (time 150 minutes) and the clamp began 4 hours later (total duration, 6 hours of lipid infusion); for the OGTTs, it was started during the postabsorptive state 90 minutes before ingestion of the oral load (total duration, 7 hours of lipid infusion).

Influence of lipid infusion on EPR (n = 4). After 30 minutes for postabsorptive measurement of respiratory exchange, lipids were infused for a period of 420 minutes.

Influence of lipid infusion on thermogenesis during euglycemic-hyperinsulinemic clamp (n = 5). The study began with a 150-minute basal period to evaluate the EPR and nonoxidative glucose disposal in the postabsorptive state. A primed (3.2 $mg \cdot kg^{-1}$) continuous infusion (0.04 $mg \cdot kg^{-1} \cdot min^{-1}$) of D-[6,6-$^2H_2$]glucose was initiated. At time 150 minutes, the lipid infusion began and continued for 360 minutes until the end of the test (time 510 minutes); 240 minutes later at time 390 minutes, the subjects received another bolus of D-[6,6-$^2H_2$]glucose (6.4 $mg \cdot kg^{-1}$) and the rate of tracer infusion was increased to 0.12 $mg \cdot kg^{-1} \cdot min^{-1}$, designed to avoid underestimation of the glucose turnover rate during the clamp.[13] Simultaneously, insulin was infused (4.2 $pmol \cdot kg^{-1} \cdot min^{-1}$ for 120 minutes, primed according to Rizza et al[14]), and any decrease in blood glucose was prevented by infusion of glucose via the euglycemic clamp system.[15] The rate of insulin infusion was chosen to produce moderate hyperinsulinemia comparable to that observed during the OGTT. Substrate oxidation and storage and the EPR were determined over the last 30 minutes of the postabsorptive and clamp periods. During these 30 minutes, gas exchange was measured continuously in the Deltatrac metabolic monitor, and blood samples were withdrawn every 10 minutes for determination of glucose, insulin, and C-peptide levels and isotopic enrichment. Urine samples were collected at the end of both study periods to measure the nitrogen excretion rate.

Influence of lipid infusion on thermogenesis during an OGTT (n = 8). A priming dose of D-[6,6-$^2H_2$]glucose (6 $mg \cdot kg^{-1}$) was administered and then D-[6,6-$^2H_2$]glucose was infused at a constant rate (0.06 $mg \cdot kg^{-1} \cdot min^{-1}$) for 450 minutes. Thirty minutes later, saline (control tests) or Ivélip (Ivélip tests) was infused at a constant rate for 420 minutes until the end of the test. The first 120 minutes of the tests were allocated for isotopic equilibration and measurement of the postabsorptive glucose turnover rate. A dose of 1 $g \cdot kg^{-1}$ glucose diluted in water (30 $g \cdot 100\ mL^{-1}$) was then ingested. Blood samples were drawn every 30 minutes for the determination of metabolites, hormones, and isotopic enrichment. Gas exchange was continuously measured in the Deltatrac metabolic monitor, and urine samples were collected at 0, 120, and 450 minutes.

Calculations

Total glucose and lipid oxidation (GOx and LOx) and the EPR were calculated for 30-minute intervals from gas exchange measurements using the equations proposed by Ferrannini.[16] Substrate storage rates were calculated as follows: for lipids, Lstor = Linfused − (LOx during infusion − LOx before infusion); for glucose, Gstor = RdT − GOx, where RdT is the tracer-derived rate of glucose disappearance calculated from Steele's equation for a non–steady-state[17] during the OGTT as previously used by others[18,19] and the steady-state equation in the postabsorptive state. During the clamp, despite the higher tracer infusion rate, we calculated erroneously low values for the glucose turnover rate, as indicated by values less than the glucose infusion rate (GIR). In a recent report from Hother-Nielsen et al[13] using the constant specific activity technique to avoid this model error, the relative error for the Rd due to the use of a conventional isotope dilution technique was about 6% at similar insulin infusion rates. Because we used a higher tracer infusion rate during the clamp, the error should be smaller in our calculation than with the conventional approach. In accordance with Nosadini et al,[20] we therefore assumed total suppression of endogenous glucose production, and calculated Gstor = GIR − GOx.

The effect of substrate administration on thermogenesis was evaluated three ways: (1) increment in EPR above baseline (ΔEPR), which is the difference between the EPR before and after administration of the substrate as expressed by Blaak et al[21]; (2) substrate-induced thermogenesis (SIT), which is the ΔEPR divided by the energy content of the substrate load[9,12] (the energy content was 3.74 $kcal \cdot g^{-1}$ for glucose and 2 $kcal \cdot mL^{-1}$ for lipid infusion); and (3) energy cost of storage, which is the ΔEPR divided by the energy content of glucose and lipids stored as proposed by Thiebaud et al.[22,23]

Analytical Procedures

Plasma glucose concentrations were determined enzymatically.[24] Urinary nitrogen was determined by the Kjeldahl method. Insulin and C-peptide levels were measured by radioimmunoassay. Plasma D-[6,6-$^2H_2$]glucose enrichment was measured by ion-monitoring gas chromatography–mass spectrometry (5971A-MSD; Hewlett Packard, Paris, France) as described by Bier et al.[25]

Statistical Analysis

Results are shown as the mean ± SEM. Comparisons between tests were performed by one-way ANOVA, and when differences were observed, protected *t* tests were used to locate the differences. When comparisons concerned tests performed in the same subjects, one-way ANOVA for repeated measurements was performed. Results were considered significant at a P level less than .05.

RESULTS

Effect of Lipid Infusion on EPR

Plasma glucose (time 0 minutes, 4.6 ± 0.1 $mmol \cdot L^{-1}$; time 450 minutes, 4.7 ± 0.1; NS) and insulin (time 0 minutes, 44.7 ± 9.3 $pmol \cdot L^{-1}$; time 450 minutes, 44.7 ± 9.3; NS) levels did not change during the lipid infusion. GOx declined progressively during the test from 1.22 ± 0.29 to 0.40 ± 0.22 $mg \cdot kg^{-1} \cdot min^{-1}$, with significantly lower values at all times after 180 minutes ($P < .05$). LOx increased progressively during the test from 0.71 ± 0.08 to 1.20 ± 0.22 $mg \cdot kg^{-1} \cdot min^{-1}$, with significantly higher values after 210 minutes ($P < .05$). Lstor declined progressively during the test from 2.96 ± 0.09 to 2.52 ± 0.21 $mg \cdot kg^{-1} \cdot min^{-1}$, with significantly lower values after 150 minutes ($P < .05$). The mean Lstor for the 420 minutes of lipid infusion was 2.64 ± 0.08

mg · kg^{-1} · min^{-1}. The EPR increased progressively during the test from 15.1 ± 1.2 to 16.4 ± 1.5 cal · kg^{-1} · min^{-1}, with significantly higher values after 90 minutes ($P < .05$). The mean ΔEPR during the 420 minutes of lipid infusion was 0.9 ± 0.2 cal · kg^{-1} · min^{-1}. Mean lipid-induced thermogenesis during the 420 minutes of lipid infusion was 3.0% ± 0.6%. The energy cost for lipid storage was 3.9% ± 0.9%.

Hyperinsulinemic-Euglycemic Clamp

Postabsorptive glucose and insulin levels were identical before the clamps with either saline or Ivélip infusions. During the clamps, plasma glucose was maintained at the postabsorptive level and the insulin level was elevated to the same extent during control and Ivélip tests (control, 268 ± 14 pmol · L^{-1}; Ivélip, 260 ± 10 pmol · L^{-1}; NS). During control clamps, LOx decreased ($P < .05$ *v* postabsorptive state) and GOx, Gstor, and EPR increased ($P < .01$; $P < .05$; $P < .01$, respectively, *v* postabsorptive state). The only significant changes during the clamp under lipid infusion were the increases in the EPR ($P < .05$ *v* postabsorptive state) and GOx ($P < .05$). Compared with the control clamps, Gstor, GOx, and the effects on thermogenesis were significantly lower during the clamp under lipid infusion, while LOx was higher (Table 1).

OGTTs

Plasma glucose levels did not change before the test (time 0 to +30 minutes) or after 90 minutes of lipid infusion prior to the oral load. The lipid infusion induced a slight deterioration in glucose tolerance as indicated by significantly higher values from time +240 to +300 minutes ($P < .05$). Insulin levels were also slightly higher during the lipid infusion, with significant differences from time +300 to +360 minutes ($P < .05$; Fig 1). Before the oral glucose was ingested, LOx increased slightly from 0.70 ± 0.06 to 0.78 ± 0.08 mg · kg^{-1} · min^{-1} (NS) in the control tests and from 0.72 ± 0.6 to 0.90 ± 0.09 ($P = .051$) in the Ivélip tests. After ingestion of the oral load, the transient decline in LOx after saline infusion was prevented by the lipid infusion (Fig 2). GOx increased during the OGTT (control, from 0.97 ± 0.16 at 90 to 120 minutes to a mean value during 330 minutes of OGTT of 1.95 ± 0.05 mg · kg^{-1} · min^{-1}, $P < .01$; Ivélip, from 0.84 ± 0.10 to 1.22 ± 0.10 mg · kg^{-1} · min^{-1} for the same time interval, $P < .05$). However, values were significantly lower with Ivélip ($P < .001$). Gstor also increased significantly during the OGTT (control, from 1.26 ± 0.11 at time 90 to 120 minutes to a mean value during 330 minutes of OGTT of 1.76 ± 0.22 mg · kg^{-1} · min^{-1}, $P < .05$; Ivélip, from 1.42 ± 0.11 to 2.94 ± 0.27 mg · kg^{-1} · min^{-1} over the same time intervals, $P < .001$). The lipid infusion had a marked stimulatory influence on this parameter. The EPR did not differ between control and Ivélip tests in the postabsorptive state (time 0 to 30 minutes; control, 15.9 ± 0.6 cal · kg^{-1} · min^{-1}; Ivélip, 16.0 ± 0.8; NS) or before the ingestion of glucose (time 90 to 120 minutes: control, 16.1 ± 0.6 cal · kg^{-1} · min^{-1}; Ivélip, 16.4 ± 0.7; NS). The ΔEPR was higher during Ivélip infusions, but because much more energy was delivered, SIT and the energy cost of storage were lower than during control OGTTs (Table 2 and Figs 1 and 2).

Table 1. Influence of Lipid Infusion on Substrate Oxidation and Storage (mg · kg^{-1} · min^{-1}) and EPR (cal · kg^{-1} · min^{-1}) During the Hyperinsulinemic-Euglycemic Clamp

Parameter	Control	Ivélip	*P*
Postabsorptive			
LOx	0.87 ± 0.09	0.91 ± 0.11	NS
GOx	1.35 ± 0.20	1.09 ± 0.42	NS
Gstor	1.11 ± 0.35	1.32 ± 0.52	NS
EPR	16.6 ± 0.5	17.0 ± 0.4	NS
Clamp			
LOx	0.15 ± 0.08	0.64 ± 0.21	<.05
GOx	3.83 ± 0.39	2.39 ± 0.62	<.01
Gstor	3.99 ± 0.40	0.91 ± 0.39	<.01
EPR	18.3 ± 0.5	18.1 ± 0.3	NS
Effect on thermogenesis			
ΔEPR	1.7 ± 0.2	1.1 ± 0.3	<.05
SIT	6.4% ± 0.4%	2.9% ± 0.9%	<.05
Energy cost of substrate storage	11.9% ± 0.5%	3.2% ± 0.8%	<.01

NOTE. Results are the mean ± SEM for the last 30 minutes of the postabsorptive and clamp periods.

Table 2. Influence of Lipid Infusion on Substrate Oxidation and Storage (mg · kg^{-1} · min^{-1}) and EPR (cal · kg^{-1} · min^{-1}) During the OGTT

Parameter	Control	Ivélip	*P*
Before glucose ingestion			
LOx	0.78 ± 0.08	0.90 ± 0.09	NS
GOx	0.97 ± 0.16	0.84 ± 0.10	NS
Gstor	1.26 ± 0.11	1.42 ± 0.11	NS
EPR	16.1 ± 0.6	16.4 ± 0.7	NS
OGTT			
LOx	0.70 ± 0.10	1.29 ± 0.12	<.001
GOx	1.95 ± 0.05	1.22 ± 0.10	<.001
Gstor	1.76 ± 0.22	2.94 ± 0.27	<.001
EPR	17.0 ± 0.7	18.4 ± 0.8	<.05
Effect on thermogenesis			
ΔEPR	0.9 ± 0.2	2.0 ± 0.3	<.05
SIT	9.3% ± 1.8%	5.2% ± 0.8%	NS
Energy cost of substrate storage	16.3% ± 3.7%	5.8% ± 0.9%	<.05

NOTE. Results are the mean ± SEM for the 30 minutes before ingestion of glucose and 330 minutes after ingestion of glucose.

Comparison Between Clamp and OGTT Experiments

Since plasma insulin levels between 180 and 210 minutes were similar to those during the clamps and comparable glucose storage rates were observed in the two conditions of insulin stimulation in control tests, we could compare SIT and the cost of substrate storage between the clamp and this period of the OGTT (Table 3).

During the control tests, LOx was suppressed less and GOx was stimulated less by the OGTT than by the clamp, although there was no influence on glucose storage and thermogenesis. The cost of glucose storage was comparable in the OGTT and clamp conditions (14.9% ± 4.0% *v* 11.9% ± 0.5%, NS).

With the lipid infusion, plasma glucose was significantly higher during the OGTT versus the clamp. LOx was suppressed less and GOx was stimulated less by the OGTT than by the clamp. By contrast, the OGTT led to higher glucose storage and lower lipid storage. The lipid infusion led to higher ΔEE

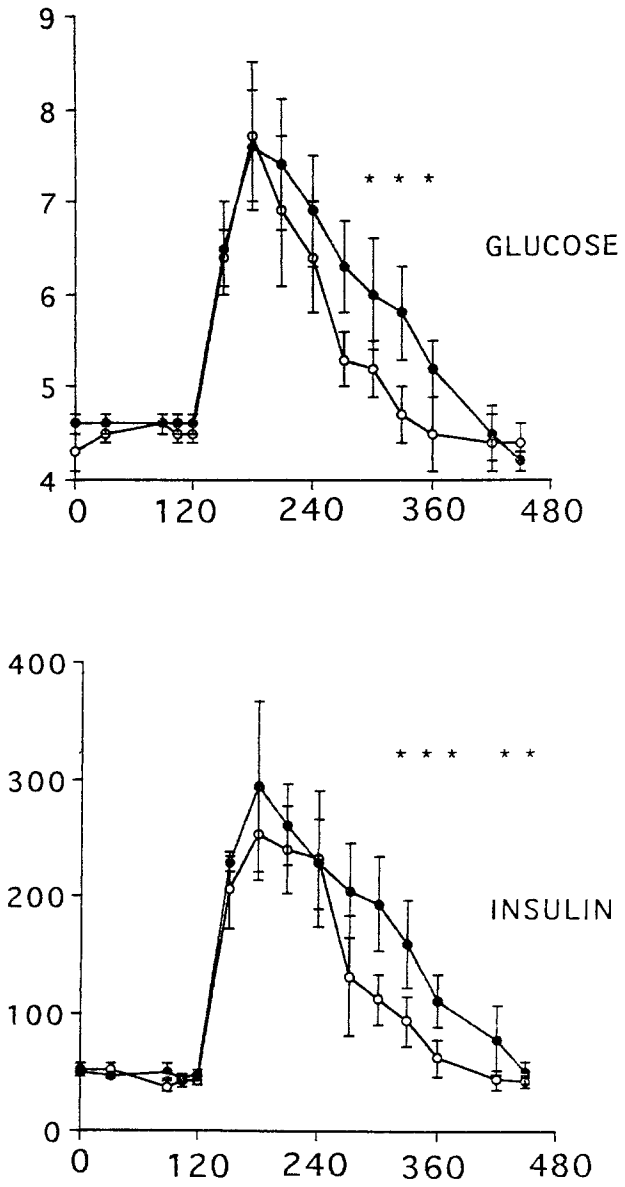

Fig 1. Time course of plasma glucose (mmol · L^{-1}) and insulin (pmol · L^{-1}) levels during the OGTT without (○) or with (●) lipid infusion. *$P < .05$, saline *v* lvélip tests.

(3.0 ± 0.3 cal · kg^{-1} · min^{-1} *v* 1.1 ± 0.2, $P < .01$), nonsignificantly higher SIT (4.9% ± 0.7% *v* 2.9% ± 0.9%, NS), and a higher cost of substrate storage (7.3% ± 1.0% *v* 3.2% ± 0.8%, $P < .05$) during the OGTT versus the clamp.

DISCUSSION

In this study, we evaluated the influence of experimental insulin resistance on thermogenesis in 13 healthy subjects. Acute insulin resistance was induced by a lipid infusion, which had a slight proper thermogenic effect (energy cost for lipid storage during lipid infusion alone, 3.9% ± 0.9%). The energy cost of glucose storage was significantly ($P < .05$) higher both during a euglycemic clamp and an OGTT (clamp, 11.9% ± 0.5%; OGTT, 16.3% ± 3.7%). During the clamp, the lipid infusion produced a state of insulin resistance as shown by lower GOx and glucose storage, and the EPR increased less than with the control clamps. Under the conditions of the OGTT, the lipid infusion also produced a state of insulin resistance as shown by higher plasma glucose and insulin levels and lower GOx. However, by contrast, glucose storage was not affected by this insulin resistance. Indeed, it was higher and the EPR increased more as compared with control OGTTs. Subjects

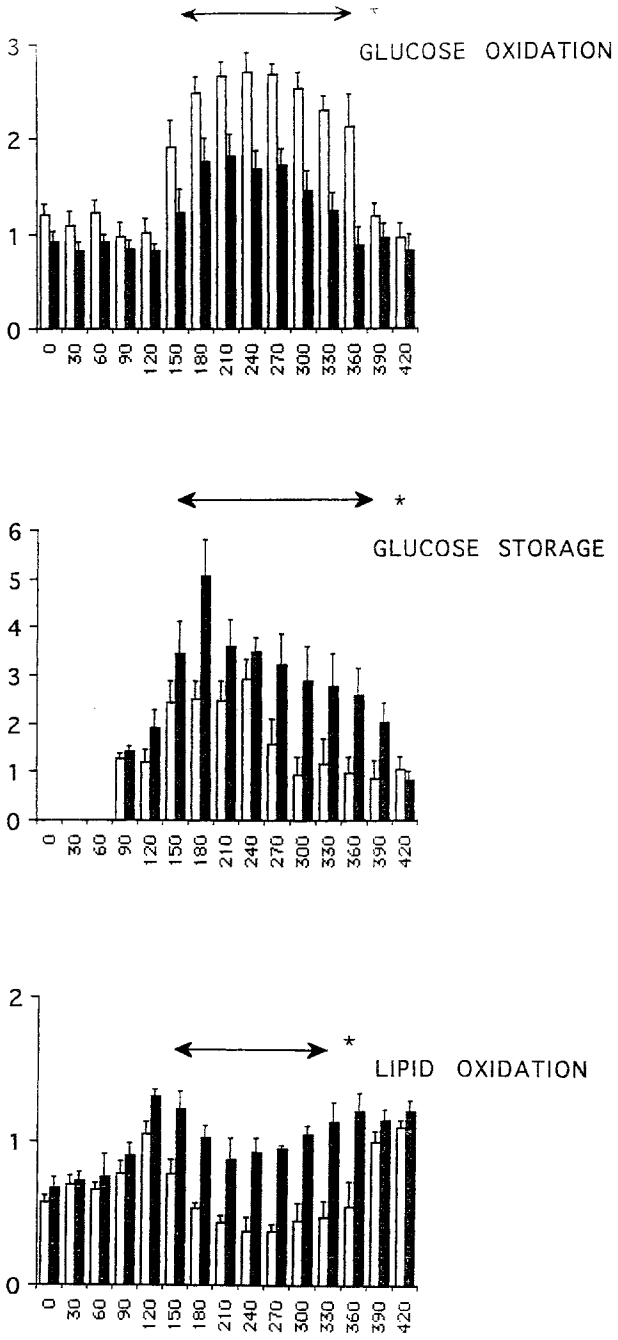

Fig 2. Time course of GOx, glucose storage, and LOx (mg · kg^{-1} · min^{-1}) during the OGTT without (□) or with (■) lipid infusion. *$P < .05$, saline *v* lvélip tests.

Table 3. Influence of the Type of Glucose Challenge (hyperinsulinemic-euglycemic clamp or OGTT) on Substrate Oxidation and Storage (mg · kg^{-1} · min^{-1}) and EPR (cal · kg^{-1} · min^{-1}) With or Without Lipid Infusion

Parameter	Clamp	OGTT (180-210 min)	P
Control			
Plasma insulin	268 ± 14	240 ± 38	NS
Plasma glucose	5.0 ± 0.1	6.9 ± 0.8	NS
LOx	0.15 ± 0.08	0.54 ± 0.05	<.01
GOx	3.83 ± 0.39	2.48 ± 0.17	<.01
Gstor	3.99 ± 0.40	3.53 ± 0.37	NS
ΔEE	1.7 ± 0.2	1.7 ± 0.2	NS
GIT	6.4% ± 0.4%	8.4% ± 1.8%	NS
Energy cost of glucose storage	11.9% ± 0.5%	14.9% ± 4.0%	NS
Ivélip			
Plasma insulin	260 ± 10	294 ± 73	NS
Plasma glucose	5.0 ± 0.1	7.4 ± 0.7	<.05
LOx	0.64 ± 0.21	1.02 ± 0.09	<.05
GOx	2.39 ± 0.62	1.75 ± 0.26	NS
Gstor	0.91 ± 0.39	5.05 ± 0.78	<.01
Lstor	3.31 ± 0.16	2.65 ± 0.11	<.01
ΔEE	1.1 ± 0.2	3.0 ± 0.3	<.01
SIT	2.9% ± 0.9%	4.9% ± 0.7%	NS
Energy cost of substrate storage	3.2% ± 0.8%	7.3% ± 1.0%	<.05

NOTE. To compare challenges of similar magnitude, the clamp was moderately hyperinsulinemic, and its results were compared to those obtained at the insulinemic peak during the OGTT (time 180-210 minutes).

who underwent the clamp and OGTT experiments were not the same, but their characteristics were similar. Therefore, similar plasma insulin levels allowed comparison between the clamp and the OGTT (from 60 to 90 minutes after oral glucose ingestion). Without lipids, the rate of glucose storage and the energy cost did not differ significantly. With the lipid infusion, more glucose and less lipids were stored during the OGTT, leading to a higher energy cost of substrate storage. We think this effect on substrate storage partitioning and thermogenesis can be attributed to the moderate glucose intolerance induced by the lipid infusion.

The low energy cost of lipid ingestion is a well-known fact.[26,27] Thiebaud et al,[22] who infused lipids as we did, also found an energy cost of lipid storage at 4%. Again in accordance with our results, the effects of glucose on thermogenesis are higher when studied by euglycemic clamps (about 12%[22,23,28]) or by OGTTs (8.9% ± 1.5% and 8.0% ± 0.7% expressed as GIT[12,21]). To our knowledge, results from both techniques have not been compared. In the small number of subjects we studied, glucose storage did not cost significantly more after oral ingestion than during the clamp, showing that ingestion, digestion, and absorption of glucose do not use much energy. However, this does not preclude that glucose storage is regulated the same way in the two conditions, as suggested by Laville et al.[12]

Under conditions of euglycemia, lipid infusions are well known to decrease insulin-stimulated GOx.[5,7] Insulin-stimulated nonoxidative glucose disposal is also inhibited, as we observed, if the lipid infusion has a duration of 3 to 4 hours as shown by Boden et al.[7] To our knowledge, the only previous report about thermogenesis in such conditions is from Thiebaud et al,[22] who found an intermediate energy cost for substrate storage: 9% with combined lipid-glucose infusion versus 12% with glucose alone. Nine percent is much higher than our estimate (3.3%), although this difference probably stems from differences in the experimental conditions: the insulin plateau was higher (63, 167, and 410 μU · mL^{-1}) in their tests and lipids were only infused for 210 minutes, so glucose storage was stimulated despite the lipid infusion. In our clamp situation, we attempted to reproduce a physiological level of hyperinsulinemia (~40 μU · mL^{-1}) to allow comparison to the OGTT and to produce a marked insulin resistance of glucose storage to evaluate its influence on thermogenesis. We succeeded in abolishing the insulin stimulation of glucose storage, and therefore, the low SIT was not unexpected. This indicates that the insulin resistance of glucose metabolism may help to perpetuate obesity by reducing diet-induced thermogenesis.

However, in normal life, the diet does not induce thermogenesis in euglycemic conditions, but in a variably hyperglycemic postprandial state, which is better mimicked by an OGTT. The effect of a lipid infusion on thermogenesis has not been reported in such conditions, although indications exist concerning the influence on glucose storage. A lipid infusion does not inhibit nonoxidative glucose disposal, calculated as the difference between ingested glucose and suprabasal glucose oxidation.[29,30] In a recent study combining a tracer infusion and indirect calorimetry as we did, Kruszynska et al[31] indeed found that nonoxidative glucose disposal was increased by a lipid-heparin infusion 30 minutes before the oral glucose was ingested. We confirm this with a duration of lipid infusion sufficient to produce an inhibitory effect during a euglycemic clamp. Because total suppression of endogenous glucose production had to be assumed during the clamp, glucose storage (calculated as GIR − GOx, which is slightly less than Rd − GOx with a perfect evaluation of Rd) has been slightly underestimated during the clamp. In the report from Hother-Nielsen et al[13] using the hot-infusion technique to avoid the model error of the conventional isotope-dilution technique, residual endogenous glucose production was about 10 mg/m^2/min (or 0.2 mg/kg/min) with insulin infusion rates similar to ours. The underestimation due to model error may also concern the clamps with lipid infusion; however, it was even weaker during these tests, as reflected by slightly positive values for endogenous glucose production (data not shown). Obviously, a 0.2-mg/kg/min model error does not change the fact that glucose storage was similar during the clamp and the OGTT without lipid infusion and different with the lipid infusion (clamp, 0.91 ± 0.39 mg/kg/min; OGTT, 5.05 ± 0.78; $P < .01$). Because the energy cost of storage is much higher for glucose than for lipids, the fact that lipid infusion leads to a higher ΔEPR during the OGTT is therefore not unexpected. As a consequence, our main finding is the different effect of the lipid infusion on thermogenesis between the clamp and the OGTT conditions. In both cases, lipid infusion caused the overall SIT and energy cost of substrate storage to be lower than when glucose was the main substrate. However, the comparison between clamps and OGTTs demonstrates that it did not occur the same way in both

conditions. The OGTT produced a redirection of substrate storage, with higher glucose and lower lipid storage, which contributed to a significantly higher cost of substrate storage (Table 3).

The occurrence of de novo lipogenesis, which is an energy-requiring process, also could have explained the higher ΔEPR during OGTTs under lipid infusion; however, this hypothesis is excluded because the respiratory quotient was never greater than 1 during these tests. Gluconeogenesis also requires energy, and may not be influenced by lipids to the same extent during a clamp and an OGTT. In both conditions, endogenous glucose production is suppressed less when lipids are infused.[32,33] However, a quantitative comparison of these effects would be speculative, because in both cases evaluation of endogenous glucose production is affected by methodological problems: model error as previously mentioned for clamps and the use of non–steady-state kinetics during OGTTs. We are not aware of any information about a different regulation of gluconeogenesis in conditions of clamps and OGTTs during a lipid infusion. Gluconeogenesis may therefore play a role in the different thermogenesis we observed, but available information arguing for this hypothesis is scanty.

The reasons for the different regulation of substrate storage—and hence thermogenesis—in the conditions of the euglycemic clamp and OGTT can be examined. This was not due to any difference in the duration of the lipid infusion (6 hours for the clamps and 7 hours for the OGTTs). Plasma insulin levels were not significantly higher 60 to 90 minutes after the start of the lipid infusion, although the difference reached significance in the following hours. Higher insulin concentrations could conceivably play a role in redirecting glucose to storage. This seems unlikely, because GOx tends to be more sensitive to moderate hyperinsulinemia than nonoxidative glucose disposal, as shown by a lower ED_{50} derived from dose-response curves in normal, obese, and diabetic subjects.[2] The effect of insulin may be different during clamps and OGTTs because the exposure of tissues is different: insulin levels are consistently high during the clamp, whereas they increase, peak, and decrease during the OGTT. However, an intrinsic thermogenic effect of insulin is considered negligible at physiological insulin levels.[33] The main mechanism was thus thought to be the higher plasma glucose level. Comparing the effect of a lipid infusion on glucose disposal during a euglycemic versus moderately hyperglycemic clamp, Felley et al[34] demonstrated that hyperglycemia predominantly stimulates nonoxidative glucose disposal. The lipid infusions in their experiments were short (210 minutes) and thus did not induce a significant effect on glucose storage; however, lipids clearly tended to inhibit it also in the conditions of the hyperglycemic clamp. The higher glucose storage during the OGTT under lipid infusion in our experience therefore was not due to a different influence of lipid in conditions of moderate hyperglycemia, but rather to the proper effect of a moderately ($+0.5$ mmol $\cdot L^{-1}$; Fig 1) higher glucose level on glucose storage.

In summary, acute lipid-induced insulin resistance affects thermogenesis, but this effect is conditioned by the effect on blood glucose: in euglycemia, thermogenesis is reduced, but not in the present of an increase, albeit small, in plasma glucose. With an increase in plasma glucose, more glucose and less lipids are stored, so the energy cost of substrate storage is higher. The thermogenic effect of slight hyperglycemia may explain previous conflicting results concerning diet-induced thermogenesis in obese subjects.

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