Respective Role of Plasma Nonesterified Fatty Acid Oxidation and Total Lipid Oxidation in Lipid-Induced Insulin Resistance

Martine Laville, Vincent Rigalleau, Jean-Paul Riou, and Michel Beylot

To investigate the respective role of nonesterified fatty acids (NEFA) oxidation and total lipid oxidation in lipid-induced insulin resistance, we measured the response of glucose metabolism to insulin in normal subjects without (control study) or with either heparin (heparin study) or triglycerides (TG) emulsion (Ivelip study) infusion. Three-step euglycemic–mild-hyperinsulinemic clamp studies were performed. Lipid and glucose metabolism were studied using indirect calorimetry and [6,6-$^2H_2$]glucose and [1-^{13}C]palmitate infusions. NEFA concentration and turnover and oxidation rates were decreased by insulin in the control study, but were maintained during the heparin and Ivelip infusion studies. Total lipid oxidation was decreased similarly in the control and heparin studies, but was increased during the Ivelip infusion. Stimulation of glucose oxidation and utilization by insulin was reduced in the Ivelip study, but not in the heparin study. Thus, peripheral insulin resistance was observed in the presence of a combined increase in total lipid and NEFA oxidation, but not during an isolated increase in NEFA oxidation. On the other hand, insulin-induced inhibition of glucose production was impaired in both the heparin and Ivelip studies. We conclude that total lipid oxidation is a major determinant of peripheral insulin resistance, whereas hepatic insulin resistance could be induced even by a moderate increase in NEFA availability.

SINCE THE DEMONSTRATION by Randle et al[1] that fatty acids in vitro inhibit glucose oxidation in heart muscle, it has been proposed that an excess of lipid substrates could play a role in the abnormalities of glucose metabolism in type II diabetes mellitus. To test this hypothesis, many studies[2-12] have used heparin or a combined triglycerides (TG) and heparin infusion as an experimental tool to mimic an increased lipid substrate availability. Most of these studies showed an impairment of insulin-stimulated glucose utilization and oxidation in the presence of high lipid concentrations.[2-7,11,12] Recently, Groop et al,[9] as well as Yki-Järvinen et al,[8] failed to demonstrate any alteration in insulin-mediated glucose disposal when the decrease in plasma nonesterified fatty acids (NEFA) during insulin infusion was prevented by heparin infusion. In addition, Capaldo et al,[10] using the forearm technique, showed that the maintenance of basal NEFA concentration did not affect insulin-stimulated glucose uptake, further questioning the importance of the so-called glucose–fatty acid cycle. However, it should be noted that NEFA oxidation and total lipid oxidation are not equivalent.[8,13] In the studies reported by Groop et al[9] and Yki-Järvinen et al,[8] little or no modifications of total lipid oxidation were observed despite significant alterations in NEFA metabolism. This suggests that increased plasma NEFA availability and oxidation are not sufficient by themselves to induce insulin resistance, but require a simultaneous increase in total lipid oxidation.

Therefore, the aim of the present study was to determine the respective roles of increased plasma NEFA oxidation and increased total lipid oxidation in the induction of insulin resistance. This was achieved by comparing the action of insulin on glucose metabolism in the absence (control test) and presence of either an increase in NEFA availability and oxidation without alteration in total lipid oxidation (observed during a heparin infusion) or a combined increase in NEFA and total lipid oxidation (observed during a TG emulsion infusion).

SUBJECTS AND METHODS

Subjects

Ten healthy nonsmoking subjects (five men, five women) with a mean ± SEM age of 24 ± 1 years and a body mass index of 21.2 ± 0.5 were studied. None had a personal or familial history of diabetes or obesity or were taking any medication. Their fasting blood glucose was 5.2 ± 0.2 mmol/L and fasting plasma insulin was 60 ± 6 pmol/L. All subjects were on their usual diet before the study. The purpose, nature, and potential risks of the study were carefully explained, and informed written consent was obtained from all subjects. This protocol was approved by the ethics committee of the "Hospices Civils de Lyon."

Materials

D[6,6-$^2H_2$]glucose (99% mole percent excess), $NaH^{13}CO_3$ (99% mole percent excess), and [1-^{13}C]palmitic acid (99% mole percent excess) were obtained from Eurisotop (Saint Aubin, France). Chemical and isotopic purity were confirmed by gas chromatography–mass spectrometry analysis. D[6,6-$^2H_2$]glucose and $NaH^{13}CO_3$ were dissolved in sterile water and sterilized by filtration through a 0.22-μm Millipore filter (Millipore, Bedford, MA). [1-^{13}C]palmitate was bound to albumin (Mérieux, Lyon, France) as previously described.[14] All infusates were checked for pyrogens by Limulus test before administration and were infused through a 0.22-μm Millipore filter. Insulin (Umuline Rapide; Lilly, St Cloud, France) was prepared in sterile isotonic sodium chloride containing 2% human serum albumin. Ivelip 20% (soya bean oil 20 g/100 mL, glycerol 2.5 g/100 mL) was obtained from Clintec (Amilly, France). Reagents for derivatization were from Pierce (Rockford, IL).

Experimental Protocol

Three different studies were performed: control (six subjects), heparin infusion (five subjects), and Ivelip infusion (five subjects).

From INSERM U197 Lyon; and the Department of Endocrinologie-Diabétologie et Nutrition, Hopital E. Herriot, Lyon, France.

Submitted April 13, 1994; accepted September 7, 1994.

Address reprint requests to Martine Laville, MD, INSERM U197, Faculté de médecine Alexis Carrel, Rue Guillaume Paradin, 69372 Lyon, Cedex 08.

0026-0495/95/4405-0013$03.00/0

During the control study, a three-step euglycemic-hyperinsulinemic clamp was performed. The other studies were similar except that heparin or Ivelip were infused at the same time that the insulin infusion began. One subject participated only in the control study, and three only in the heparin study. Four subjects participated in both control and Ivelip studies. Two subjects participated in all studies. An interval of more than 3 weeks was observed between the studies. All studies were performed in the postabsorptive state at least 12 hours after the last meal, and were initiated between 8 and 9 AM after at least 30 minutes of bed rest.

Control Study

Intravenous catheters were inserted into veins of one forearm for the different infusions. To obtain arterialized blood samples, another catheter was inserted in a retrograde manner in a vein of the opposite hand kept at 60°C in a warming blanket. The study began with a 150-minute basal period to determine basal glucose and NEFA turnover rates. After a bolus of $NaH^{13}CO_3$ (1.4 μmol/kg), a primed (17.6 μmol/kg)-continuous (0.22 μmol/kg · min) infusion of [6,6-$^2H_2$]glucose and a primed (0.4 μmol/kg)-continuous infusion (0.04 μmol/kg · min) of [1-^{13}C]palmitate were simultaneously initiated.

Euglycemic-hyperinsulinemic clamp. After the basal period, a primed-continuous insulin infusion was started. Insulin was infused at three successive rates (0.6, 1.2, and 4.2 pmol/kg · min for 2 hours each). Each insulin infusion rate was primed according to the method reported by Rizza et al.[15] Any decrease in blood glucose was prevented by infusion of glucose (beet glucose, Fandre, Luches, France) as described previously.[16] At the beginning of the higher insulin infusion rate (4.2 pmol/kg · min), subjects received another bolus of [6,6-$^2H_2$]glucose (35.2 μmol/kg) and the rate of the tracer infusion was increased to 0.66 μmol/kg · min.

Respiratory exchange measurements were used to estimate total glucose and lipid oxidation rates during the last hour of the basal period and of each different insulin infusion period. Measurements were performed with a computerized flow-through canopy gas analyzer system (Deltatrac metabolic monitor, Datex, Helsinski, Finland). Blood samples were drawn for determination of glucose, insulin, C-peptide, glucagon, NEFA, TG, glycerol, and ketone bodies concentrations and [6,6-$^2H_2$]glucose and [1-^{13}C]palmitate enrichment during the last 30 minutes of the basal period and at 60, 90, 100, 110, and 120 minutes of each insulin infusion period. Blood was collected in tubes maintained at 4°C and immediately centrifuged. Plasma was then stored at −20°C until assay.

^{13}C enrichment in CO_2 was determined in samples of expired gases as previously described.[17] Two samples were obtained before [1-^{13}C]palmitate infusion to determine basal $^{13}CO_2$ abundance. Then air samples were collected during the last 30 minutes of the basal period and of each insulin infusion period. Urine was collected in the basal state and during the clamp study for determination of nitrogen excretion.

Heparin Study

The heparin study was similar to the control study except that at the beginning of the insulin infusion subjects received a primed (200 U)-continuous (20 U/min) infusion of heparin. At the start of the second (1.2 pmol/kg · min) and the third (4.2 pmol/kg · min) insulin infusion period, subjects received another heparin bolus (200 U). At the beginning of the third insulin infusion period, the heparin infusion rate was increased to 35 U/min.

Ivelip Study

The Ivelip study was similar to the control study with one exception: at the beginning of the insulin infusion, subjects received an infusion of Ivelip (0.015 mL/kg · min).

Analytical Procedures

Plasma glucose, glycerol, and ketone bodies concentrations were determined in neutralized perchloric acid plasma extracts by enzymatic methods as previously described.[18] Plasma NEFA[19] and TG[20] levels were measured by enzymatic methods, and plasma insulin and glucagon levels by radioimmunoassay.[21,22] Urinary nitrogen was determined by chemiluminescence (Antek 703C, Sopares, Paris, France). Enrichment of plasma glucose and palmitate by tracers and the concentration of palmitate were determined by gas chromatography–mass spectrometry (5971 MSD, Hewlett-Packard, Palo Alto, CA) as previously described in detail.[14,16] ^{13}C enrichment of expired CO_2 was determined on a dual-inlet isotope ratio mass spectrometer (Sira 12, VG Instrument, Middlewich, UK) as previously described.[23]

Steady-state isotopic enrichments were obtained in each group for all insulin infusion rates, as shown in Fig 1. Thus, glucose turnover rates were determined using the steady-state equation.

Calculations

Glucose and palmitate turnover rates were calculated during the basal state and for 30 minutes at the end of each insulin infusion period. Endogenous glucose production (EGP) was calculated by subtracting the exogenous glucose infusion rate from the isotopically determined turnover rate. Negative values for EGP were observed only in the control study during the 4.2-pmol/kg · min insulin infusion rate. These negative values were observed despite an adaptation of the tracer infusion rate to the expected changes in glucose metabolism. According to Nosadini et al,[24] it was therefore

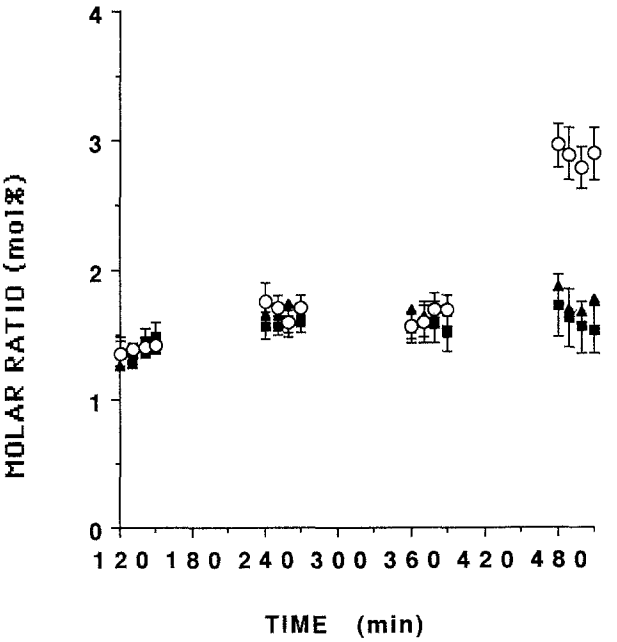

Fig 1. Evolution of isotopic enrichment during the basal period (120 to 150 minutes) and the last 30 minutes of the 3 insulin infusion rates (240 to 270, 360 to 390, and 480 to 510 minutes). (■) Control study (n = 6); (▲) heparin study (n = 5); (○) Ivelip study (n = 5). Mean ± SEM.

considered that EGP was totally suppressed and that glucose utilization was equal to the exogenous glucose infusion rate. The NEFA turnover rate was calculated as the product of the palmitate turnover rate and the ratio of NEFA to palmitate concentration. The $^{13}CO_2$ production rate was determined by multiplying $^{13}CO_2$ enrichment by the CO_2 production rate.

The plasma NEFA oxidation rate ([NEFA ox] μmol/kg · min) was calculated according to the following formula: NEFA ox = ($\dot{V}CO_2 \times {}^{13}CO_2$ APE × Rt NEFA)/(F × 0.8), where $\dot{V}CO_2$ is the amount of expired CO_2 (μmol CO_2/kg · min), $^{13}CO_2$ APE is the ^{13}C enrichment in expired CO_2 (atom percent), Rt NEFA is the NEFA turnover rate (μmol/kg · min), F is the [1-^{13}C]palmitate infusion rate (μmol/kg · min), and 0.8 is a correction factor for incomplete recovery of ^{13}C-bicarbonate.

Total glucose and lipid oxidation rates were estimated from gas exchange measurements.[25] Nonoxidative glucose metabolism during the last 30 minutes of each insulin infusion period was calculated as the difference between glucose utilization and glucose oxidation.

To compare total lipid oxidation and NEFA oxidation, total lipid oxidation was expressed as micromoles of equivalent palmitate per kilogram per minute. Milligrams per kilogram per minute of total lipid oxidation were converted to micromoles of equivalent palmitate per kilogram per minute using the molecular weight of palmitate (256 g) to convert milligrams to micromoles.

Statistical Analysis

Data are presented as the mean ± SEM. Within-study comparisons were performed with two-way ANOVA followed by the Scheffé protected *t* test to locate differences. Between-group comparisons were performed using one-way ANOVA followed by the Scheffé protected *t* test. *P* less than .05 was considered statistically significant.

RESULTS

Glucose Level and Hormonal Parameters

During all clamp studies, glucose was maintained at the basal level (Table 1). Plasma insulin concentration was comparable in all groups during the basal state and during the clamp study except for the last infusion rate. During this step, insulin concentration was lower in the heparin study than in the two others ($P < .01$). Glucagon was slightly decreased in the three groups, but this decrease was significant only during the last insulin infusion rate for heparin (98 ± 15 *v* 118 ± 14 ng/L, $P < .05$) and Ivelip (80 ± 15 *v* 125 ± 20 ng/L, $P < .05$) groups.

TG, NEFA, Glycerol, and Ketone Bodies Concentrations and NEFA Turnover Rate

In the control study, TG concentration was decreased during the last insulin infusion rate. During the heparin study, TG concentration was decreased at the beginning of heparin infusion and then remained constant, whereas it was increased by Ivelip infusion during all the Ivelip studies. NEFA concentration was decreased by insulin throughout the control study and was nearly indetectable during the last insulin infusion period. During heparin and Ivelip studies, NEFA concentration was maintained during the whole experiment at higher values than in the control study ($P < .001$). However, NEFA concentration was increased to a greater extent during the Ivelip study than during the heparin study ($P < .01$). Glycerol concentration was enhanced during the Ivelip study, whereas it was decreased during the control study and remained unchanged in the heparin study. Ketone bodies decreased throughout the experiment in the control and heparin studies, but were maintained close to baseline values during the Ivelip study. The NEFA turnover rate followed a similar NEFA concentration pattern in all experiments (Table 1).

NEFA Oxidation and Total Lipid Oxidation Rates

In the basal state, NEFA oxidation represented only 48% ± 7% of total lipid oxidation. The NEFA oxidation

Table 1. Metabolic Parameters During Control, Heparin, and Ivelip Studies

Insulin Infusion Rate (pmol/kg · min)	Plasma Insulin (pmol/L)	Plasma Glucose (mmol/L)	TG (mmol/L)	NEFA (μmol/L)	Glycerol (μmol/L)	Ketone Bodies (μmol/L)	NEFA Turnover (μmol/kg · min)
Basal							
C	69 ± 4	5.4 ± 0.2	0.40 ± 0.03	394 ± 50	43 ± 4	131 ± 32	5.5 ± 0.8
H	58 ± 15	4.7 ± 0.2	0.65 ± 0.11	537 ± 37	50 ± 8	175 ± 30	5.8 ± 1.4
IV	64 ± 7	5.4 ± 0.3	0.57 ± 0.14	536 ± 87	53 ± 14	250 ± 85	5.7 ± 1.0
0.6							
C	81 ± 3	4.8 ± 0.2	0.39 ± 0.03	211 ± 54‖	29 ± 4	82 ± 25	3.0 ± 0.5§
H	67 ± 8	4.5 ± 0.2	0.43 ± 0.05§	485 ± 69*	38 ± 5	118 ± 22‡	4.3 ± 0.7‡
IV	80 ± 8	5.0 ± 0.2	2.18 ± 0.47*‖	678 ± 107†	191 ± 26†‖	375 ± 115	7.1 ± 0.9*
1.2							
C	110 ± 6§	4.8 ± 0.2	0.36 ± 0.01	89 ± 19‖	18 ± 2‖	52 ± 6§	1.8 ± 0.4‖
H	95 ± 6§	4.5 ± 0.3	0.39 ± 0.03‡‖	398 ± 79†‡	35 ± 6	79 ± 7‡§	3.8 ± 0.5
IV	107 ± 12§	4.7 ± 0.2	2.05 ± 0.66*‖	708 ± 45†	221 ± 20†‖	572 ± 125*	6.5 ± 0.9†
4.2							
C	278 ± 6‖	4.8 ± 0.2	0.29 ± 0.01§	34 ± 9‖	15 ± 3‖	39 ± 4§	1.0 ± 0.2‖
H	207 ± 10‖	5.0 ± 0.2	0.30 ± 0.04‡‖	215 ± 45†‡§	35 ± 11	56 ± 6§	3.6 ± 1.4
IV	249 ± 14*‡‖	5.0 ± 0.2	2.60 ± 0.91*‖	456 ± 38†	220 ± 23†‖	302 ± 118*	6.6 ± 0.7†

Abbreviations: C, control (n = 6); H, heparin (n = 5); IV, Ivelip (n = 5).

*$P < .05$, †$P < .001$: H and IV *v* C.

‡$P < .05$, H *v* IV.

§$P < .05$, ‖$P < .01$: values during the clamp study *v* basal values.

rate had an evolution similar to that of NEFA concentration and turnover rate. NEFA oxidation was decreased by insulin in the control study, but was maintained at higher values during heparin and Ivelip studies. During Ivelip infusion, NEFA oxidation was increased as compared with basal values ($P < .01$). It was thus higher than during both the control and heparin studies ($P < .01$ *v* heparin study, $P < .001$ *v* control study). Total lipid oxidation showed a different evolution than NEFA oxidation, since it was similarly decreased by insulin during both heparin and control studies. In contrast, during Ivelip infusion, total lipid oxidation was higher than baseline values for the first and second insulin infusion rates ($P < .01$) and returned to initial values during the last insulin infusion period (Fig 2).

Glucose Metabolism

Glucose turnover rate. The glucose turnover rate was comparable in all groups in the basal state and was not modified by the first and second insulin infusion rates. During the third insulin infusion period, glucose utilization was increased. This increase was similar during the control and heparin studies, but was of a lower increment during the Ivelip study ($P < .01$ *v* control and heparin studies) (Fig 3).

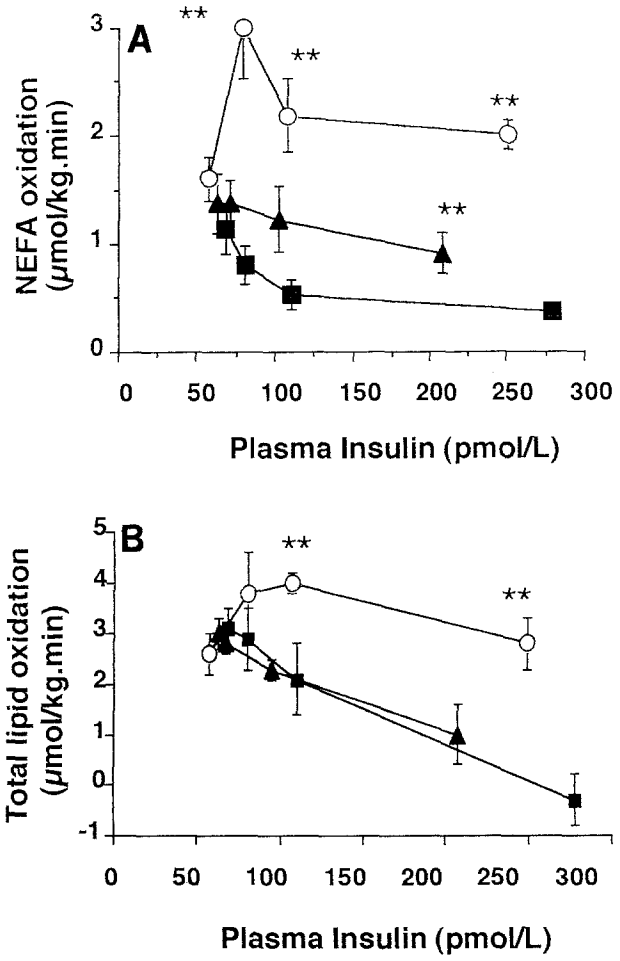

Fig 2. Evolution of plasma NEFA oxidation (A) and total lipid oxidation (B) rates during the basal period and the 3 insulin infusion rates (0.6, 1.2, and 4.2 pmol/kg · min) during a euglycemic insulin clamp. (■) Control study (n = 6); (▲) heparin study (n = 5); (○) Ivelip study (n = 5). Mean ± SEM. After significant ANOVA, protected *t* test is used to locate differences. For each insulin infusion rate, values obtained during the heparin or Ivelip studies are compared with those of the control study: *$P < .05$, **$P < .001$.

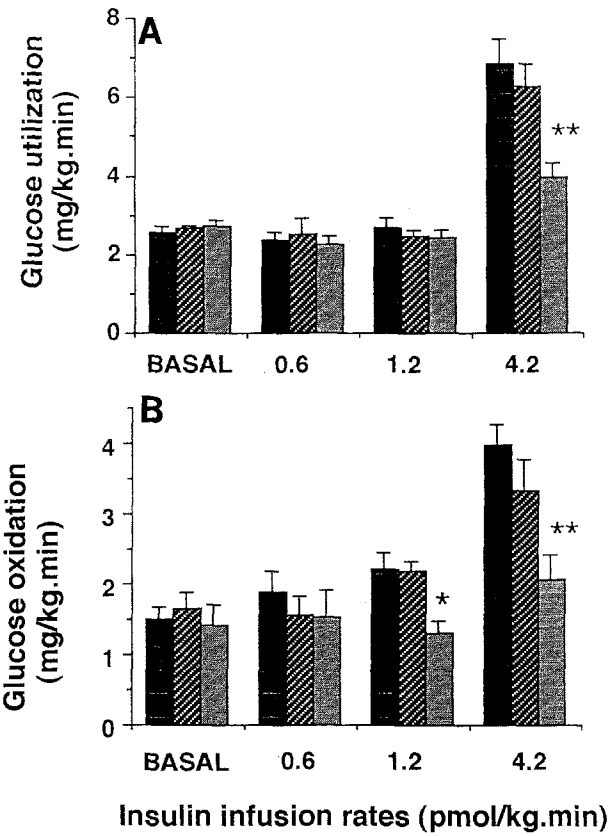

Fig 3. Insulin dose-response on glucose utilization (A) and glucose oxidation (B) rates during a 3-step euglycemic insulin clamp study performed without (■, control study) or with heparin (▨) or Ivelip (▩) infusions. Mean ± SEM. For each insulin infusion period, values obtained during heparin or Ivelip studies are compared with those of the control study: *$P < .05$, **$P < .001$.

Glucose oxidation. The basal rate of glucose oxidation was not different during all three studies. In the control study, glucose oxidation was increased during the second insulin infusion period ($P < .01$) and increased further at the end of the study ($P < .0001$). The glucose oxidation pattern was comparable during the heparin study. In contrast, during the Ivelip study, glucose oxidation was not significantly increased. Thus, these values were lower than values observed in the control and heparin studies during the second and third insulin infusion periods ($P < .01$) (Fig 3).

Nonoxidative glucose utilization. The nonoxidative glucose oxidation rate was comparable in all studies during the basal state (1.05 ± 0.25, 1.02 ± 0.23, and 0.98 ± 0.27 mg/kg · min for control, heparin, and Ivelip studies, respectively) and was not altered by the first and second insulin infusion periods. Nonoxidative glucose utilization was enhanced by the last insulin infusion rate in all groups.

However, this increase was of a lesser magnitude in the Ivelip study (1.89 ± 0.14) as compared with the control (3.74 ± 0.42) or heparin (2.91 ± 0.45 mg/kg · min) studies ($P < .01$).

EGP rate. In the basal state, hepatic glucose production was equal to the glucose turnover rate and was comparable in all groups. EGP was not decreased by the first insulin infusion rate. In the control study, glucose production was reduced by the second insulin infusion rate and completely abolished by the last insulin infusion rate. In contrast, during heparin and Ivelip studies, glucose production was barely decreased by the second insulin infusion rate and was decreased but not abolished during the last insulin infusion rate (Fig 4).

DISCUSSION

We showed in the present report that an increase in plasma NEFA oxidation did not impair insulin-mediated glucose utilization (heparin study) if it was not associated with an increase in total lipid oxidation (Ivelip study). Thus, these results could clarify the apparent discrepancies between previous studies[2-7,11,12] and the recent studies of Groop et al[9] and Yki-Järvinen et al.[8] The latter studies failed to demonstrate a lipid-induced peripheral insulin resistance, despite the maintenance of NEFA concentration and oxidation rate by heparin infusion. However, in these studies, little or no modification of total lipid oxidation was observed, a finding that is in agreement with the present report.

Our results confirm that plasma NEFA oxidation is only one component of total lipid oxidation (48% of total lipid oxidation in the basal state when using a factor of 0.8 for the incomplete collection of CO_2, 59% when using a factor of 0.65).[8,13] The results also show that the evolution of these two parameters could be dissociated, as observed in the heparin study. In the heparin study, NEFA oxidation during insulin infusion was maintained at a higher rate than during the control study, whereas total lipid oxidation was similarly decreased during control and heparin studies. As suggested by Yki-Järvinen et al,[8] the difference between NEFA oxidation and total lipid oxidation could be explained by a balance between intracellular and intravascular sources for lipid oxidation. An excess of plasma NEFA oxidation would be balanced by a decrease in the oxidation of intracellular lipids. This regulatory process would protect peripheral glucose metabolism against short time and/or moderate increase in circulating lipid substrate availability. Lipid-induced insulin resistance would appear only when this process is overcome by a large and/or prolonged increase in plasma lipid concentration, as observed in the Ivelip study. It should be emphasized that the lipid supply we realized during the Ivelip infusion (0.015 mL/kg · min of 20% Ivelip = 10 μmol/kg · min of fatty acids) is close to the fatty acid flux observed in non–insulin-dependent diabetes.[26] Thus, we show that a large increase in lipid substrate availability can induce peripheral insulin resistance. These data are in agreement with a role for lipid substrate in the pathogenesis of insulin resistance in non–insulin-dependent diabetes mellitus.

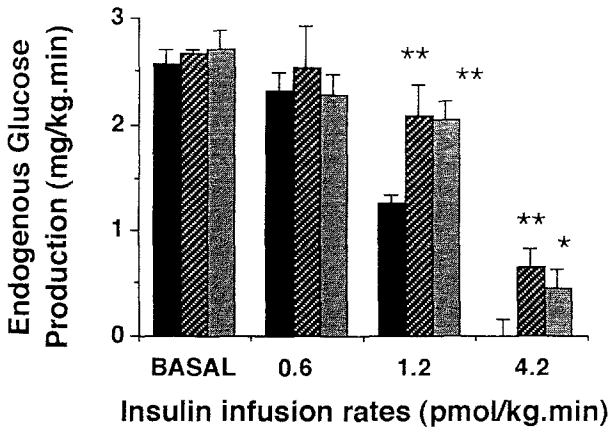

Fig 4. Insulin dose-response on EGP rates during a 3-step euglycemic insulin clamp study performed without (■, control study) or with heparin (▨) or Ivelip (▤) infusions. Mean ± SEM. For each insulin infusion rate, values obtained during heparin or Ivelip studies are compared with those of the control study: *$P < .05$, **$P < .001$.

The lipid-induced insulin resistance observed in the Ivelip study was characterized by a defect in both the oxidative and nonoxidative glucose pathway. A defect in glucose oxidation is almost a constant characteristic of lipid-induced insulin resistance,[3,5,6,12] opposite to the defect of the nonoxidative pathway, which is more rarely reported.[3,6,12] These discrepancies could be related to a time effect. Boden et al[11] demonstrated that glucose oxidation was promptly inhibited by lipid infusion, whereas a nonoxidative defect was observed after only 2 to 4 hours of lipid infusion. Since in our study the defect in glucose storage was observed during the last insulin infusion rate (ie, at least after a 4-hour lipid infusion), such a time effect appears possible. The small number of subjects in each group (five to six) must also be taken into account, since a larger sample could reveal significant differences that have not been shown in our study.

This study also demonstrates that hepatic insulin resistance could be induced by a moderate increase in NEFA turnover and oxidation rates, since identical abnormalities of EGP have been observed in the heparin and Ivelip studies. A lipid-induced decrease in hepatic insulin sensitivity was obvious during the second insulin infusion period, since EGP was reduced by 50% in controls but only by 22% to 25% in heparin and Ivelip studies, respectively ($P < .001$). This defect was also observed for the last insulin infusion rate, since EGP was totally suppressed in control but not in heparin and Ivelip studies. Compared with the amount of data concerning the action of lipids on peripheral glucose metabolism, few studies have examined their action on glucose production. One reason is that glucose production is very sensitive to insulin, and studies performed using insulin levels greater than 300 pmol/L were not able to demonstrate any effect of lipid infusion. Our data agree with those reported by Lee et al,[4] Chambrier et al,[7] and Saloranta et al[12] using either TG or TG plus heparin infusion, but are in disagreement with those reported by

Groop et al[9] and Yki-Järvinen et al,[8] who did not find any lipid-induced hepatic insulin resistance. The reasons for these discrepancies remain to be determined. A time effect has been suggested by Saloranta et al.[12] They report an alteration of insulin-induced inhibition of EGP when Intralipid plus heparin were pre-infused 120 minutes before insulin. In the present study, the effect on hepatic glucose production occurred during the second insulin infusion period, and these conditions approximated those of pre-infusion studies. The fact that a similar alteration in insulin-induced inhibition of glucose production was found in both the heparin and Ivelip studies suggests that a mild increase in NEFA supply to the liver is sufficient to have a significant effect on the sensitivity of EGP to insulin.

In conclusion, our data show that lipid-induced insulin resistance is observed only when total lipid oxidation is altered. This suggests that total lipid oxidation is a major determinant of lipid-induced insulin resistance. In contrast, hepatic insulin resistance was found even in the presence of a mild increase in NEFA supply, suggesting that the liver is more sensitive than peripheral tissues to lipid-induced insulin resistance.

REFERENCES

1. Randle PJ, Garland PB, Hales CN, et al: The glucose–fatty acid cycle, its role in insulin sensitivity and the metabolic disturbance of diabetes mellitus. Lancet 1:785-789, 1963
2. Balasse EO, Neef MA: Operation of the "glucose–fatty acid cycle" during experimental elevations of plasma free fatty acid levels in man. Eur J Clin Invest 4:247-252, 1974
3. Thiébaud D, De Fronzo RA, Jacot E, et al: Effect of long chain triglyceride infusion on glucose metabolism in man. Metabolism 31:1128-1136, 1982
4. Lee KU, Lee HK, Koh CS, et al: Artificial induction of vascular lipolysis by lipid-heparin infusion leads to insulin resistance in man. Diabetologia 31:285-290, 1988
5. Felley CP, Felley EM, Van Melle GD, et al: Impairment of glucose disposal by infusion of triglycerides in humans: Role of glycemia. Am J Physiol 256:747-752, 1989
6. Bonadonna RC, Zych K, Boni C, et al: Time dependence of the interaction between lipid and glucose in humans. Am J Physiol 257:E49-E56, 1989
7. Chambrier C, Picard S, Vidal H, et al: Interactions of glucagon and free fatty acids with insulin in control of glucose metabolism. Metabolism 39:976-984, 1990
8. Yki-Järvinen H, Puhakainen I, Saloranta C, et al: Demonstration of a novel feedback mechanism between FFA oxidation from intracellular and intravascular sources. Am J Physiol 260:E680-E689, 1991
9. Groop L, Bonnadonna RC, Shank M, et al: Role of fatty acids and insulin in determining free fatty acid and lipid oxidation in man. J Clin Invest 87:83-89, 1991
10. Capaldo B, Napoli R, Di Bonito P, et al: Dual mechanism of insulin action on human skeletal muscle: Identification of an indirect component not mediated by FFA. Am J Physiol 260:E389-E394, 1991
11. Boden G, Jadali F, White J, et al: Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest 88:960-966, 1991
12. Saloranta C, Koivisto V, Widen E, et al: Contribution of muscle and liver to glucose–fatty acid cycle in humans. Am J Physiol 264:E599-E605, 1993
13. Bonadonna RC, Groop LC, Zych K, et al: Dose-dependent effect of insulin on plasma free fatty acid turnover and oxidation in humans. Am J Physiol 259:E736-E750, 1990
14. Beylot M, Beaufrère B, Riou JP, et al: Effect of epinephrine on the relationship between nonesterified fatty acid availability and ketone body production in post absorptive man: Evidence for a hepatic antiketogenic effect of epinephrine. J Clin Endocrinol Metab 65:914-921, 1987
15. Rizza RA, Mandarino LJ, Gerich JE: Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 240:E630-E639, 1981
16. Laville M, Riou JP, Bougnères P, et al: Glucose metabolism in experimental hyperthyroidism: Intact in vivo sensitivity to insulin with abnormal binding and increased glucose turnover. J Clin Endocrinol Metab 58:960-964, 1984
17. Beaufrère B, Fournier V, Putet G, et al: Leucine kinetics in fed low–birth-weight infants: Importance of splanchnic tissues. Am J Physiol 263:E214-E220, 1992
18. Beylot M, Riou JP, Bienvenu F, et al: Increased ketonemia in hyperthyroidism. Evidence for a beta-adrenergic mechanism. Diabetologia 19:505-510, 1980
19. Laville M, Riou JP, Beylot M, et al: Measurement of free fatty acids by enzymatic method. Lyon Med 249:67-70, 1983
20. Wahlfeld AW: Triglyceride determination after enzymatic hydrolysis, in Bergmeyer HV (ed): Methods of Enzymatic Analysis. New York, NY, Academic, 1974, p 1831
21. Hales CM, Randle PJ: Immunoassay of insulin with antibody precipitate. Biochem J 88:137-148, 1963
22. Harris J, Faloona GR, Unger RH: Methods of hormone radioimmunoassay, in Joffe BM, Behrman HR (eds): San Diego, CA, Academic, 1979, pp 643-671
23. Guilluy R, Billion-Rey F, Pachiaudi C, et al: On-line purification and carbon-13 isotopic analysis of carbon dioxide in breath: Evaluation of on-line gas chromatography–isotope ratio mass spectrometry. Anal Chim Acta 259:193-202, 1992
24. Nosadini R, Del Prato S, Tiengo A, et al: Insulin resistance in Cushing's syndrome. J Clin Endocrinol Metab 57:529-536, 1983
25. Ferranini E: The theoretical bases of indirect calorimetry: A review. Metabolism 37:287-301, 1988
26. Groop LC, Saloranta C, Shank M, et al: The role of free fatty acid metabolism in the pathogenesis of insulin-resistance in obesity and noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 72:96-107, 1991