Effect of Obesity on Susceptibility to Fatty Acid–Induced Peripheral Tissue Insulin Resistance

Yolanta T. Kruszynska, Jerrold M. Olefsky, and Juan P. Frias

Elevation of plasma nonesterified fatty acid (NEFA) levels has been shown to impair the actions of insulin on peripheral glucose uptake and suppression of hepatic glucose output (HGO). These studies have been conducted almost exclusively in healthy, lean men. We therefore set out to test the hypothesis that obese subjects, because they are already insulin-resistant, are less susceptible than lean subjects to the inhibitory effects of elevated NEFA on insulin-stimulated glucose disposal. We studied 15 lean (11 men, 4 women; age, 45 ± 3 years [mean ± SE]; body mass index [BMI], 22.7 ± 0.6 kg/m^2) and 15 obese normal subjects (11 men, 4 women; 49 ± 3 years; 31.7 ± 1.0 kg/m^2). Each subject underwent two 5-hour 80-mU/m^2/min hyperinsulinemic euglycemic clamps with measurement of glucose kinetics (intravenous 3-^{3}H-glucose). Plasma NEFA levels were elevated in one study for 3 hours before and during the clamp (~1 mmol/L in both groups) by infusion of 20% Intralipid (60 mL/h) and heparin (900 U/h). The obese subjects had higher fasting insulin levels (9.1 ± 1.1 *v* 4.8 ± 0.6 mU/L, P < .005) and were insulin-resistant (glucose disposal rate [GDR] at the end of the control glucose clamps: obese, 7.96 ± 0.55, lean, 10.24 ± 0.35 mg/kg/min, *P* < .002). Contrary to our hypothesis, elevation of plasma NEFA had a similar effect in the lean and obese subjects, both in terms of the absolute reduction of insulin stimulated GDR in the lean (1.82 ± 0.36 mg/kg/min decrement) and obese subjects (2.03 ± 0.37 mg/kg/min decrement) and the overall percentage reduction in GDR (lean, 17.1% ± 3.1%; obese, 24.5% ± 4.2%; difference not significant [NS]). Suppression of HGO during the lipid clamps was also impaired to a similar extent in the 2 groups. Findings were similar for the 9 obese subjects with a BMI of 30 kg/m^2 or more. Combining the 2 groups, the NEFA induced reduction of insulin stimulated GDR did not correlate with BMI (*r* = 0.08, NS) or with insulin sensitivity (GDR) measured in the control study (*r* = 0.11, NS). In summary, the effect of a short term elevation of plasma NEFA levels on insulin stimulated GDR and suppression of HGO is comparable in lean and moderately obese subjects.

IT IS WELL ESTABLISHED that an elevation of plasma nonesterified fatty acid (NEFA) levels can impair tissue sensitivity to insulin.[1-5] The effect on insulin-stimulated peripheral glucose disposal is seen after plasma NEFA are increased and maintained at high physiological or supraphysiological levels for a few hours. While the mechanism is still not clearly defined, there is evidence that it is linked to a build up of fatty acyl coenzyme A (CoA) species within the muscle, which ultimately influences a number of steps in the insulin signaling pathway and impairs glucose transport.[6-10]

We noted a wide variation between individuals in the degree of peripheral tissue insulin resistance induced when plasma NEFA are elevated. This observation could be important in that it could also signify differences in susceptibility to the development of insulin resistance as a result of environmental/lifestyle factors such as content and composition of dietary fat. One possible explanation for the variation is that in subjects in whom insulin action is already compromised, for example, obese subjects, elevation of plasma NEFA would have relatively little further effect. Intramyocellular lipid levels are increased in obesity and are inversely related to whole body insulin sensitivity.[11,12] One might therefore postulate that because muscle fatty acyl CoA levels are already increased in obesity, experimental elevation of plasma NEFA would cause a smaller increase in muscle fatty acyl CoA content and so affect muscle insulin action to a lesser extent than in lean subjects.

To our knowledge only one study has specifically examined the effects of elevated NEFA in obesity.[13] In that study elevation of plasma NEFA levels by a 2-hour infusion of Intralipid (Fresenius Kabi Clayton, Clayton, NC) and heparin in 7 obese women led to an impairment of suppression of hepatic glucose output (HGO) but did not affect the peripheral glucose disposal rate (GDR). These findings fit the notion that experimental elevation of plasma NEFA levels may have a smaller impact on tissue insulin sensitivity in obese than in lean subjects. However, because women may be less susceptible to NEFA-induced insulin resistance,[14] the lack of effect of elevated NEFA concentrations on insulin-stimulated GDR may have been due to the fact that the study subjects were female.[13]

The aim of our study was to test the hypothesis that obese subjects, because they already have an impairment of insulin action, would be less susceptible than lean subjects to NEFA-induced insulin resistance. To test this hypothesis we measured insulin sensitivity in 15 lean and 15 obese subjects by the hyperinsulinemic euglycemic clamp under conditions of low and elevated plasma NEFA levels.

MATERIALS AND METHODS

Subjects

Fifteen lean normal subjects (11 men, 4 women; body mass index [BMI] ≤25 kg/m^2) and 15 obese normal subjects (11 men, 4 women; BMI ≥ 27 kg/m^2) matched for age were recruited. Their clinical characteristics are given in Table 1. Data on 9 of the men (7 lean, 2

From the Department of Endocrinology and Metabolism, University of California San Diego and the Whittier Institute for Diabetes, La Jolla, CA.

Submitted June 26, 2002; accepted August 28, 2002.

Supported by the Whittier Institute, National Institutes of Health Grant No. DK 33649, and General Clinical Research Center Grant No. MO1 RR00827.

Current address for J.P.F.: Barbara Davis Center, Univ. Colorado Health Sciences Center, 4200 E 9th Ave, Denver, CO 80262.

Address reprint requests to Yolanta Kruszynska, MRCP, PhD, Department of Endocrinology and Metabolism (9111G), VA Medical Center, 3350 La Jolla Village Dr, La Jolla, CA. 92093.

0026-0495/03/5202-0010$35.00/0

doi:10.1053/meta.2003.50029

Table 1. Clinical Characteristics of the Subjects Studied

	Lean (n = 15)	Obese (n = 15)
Gender	11 M, 4 F	11 M, 4 F
Age (yr)	45 ± 3	49 ± 3
Weight (kg)	68.9 ± 2.6	93.6 ± 2.8†
BMI (kg/m^2)	22.7 ± 0.6	31.7 ± 1.0†
Fasting plasma glucose (mmol/L)	5.0 ± 0.1	5.2 ± 0.1
Fasting plasma insulin (mU/L)	4.8 ± 0.6	9.1 ± 1.1*

NOTE. Values are mean ± SEM.
*$P < .005$ and †$P < .001$ *v* lean subjects.

obese) have been presented in a previous publication.[14] All of the women were postmenopausal and had not been on any hormone replacement. All subjects underwent a complete history, physical examination, and a 75-g oral glucose tolerance test prior to study. All had normal renal and hepatic function. Two of the subjects in the lean group and 2 of the obese subjects had impaired glucose tolerance (IGT).[15] Although fasting plasma glucose concentrations were not significantly different, the obese subjects had a nearly 2-fold increase in fasting plasma insulin levels (Table 1, $P < .005$). Each subject underwent two 5-hour 80-mU/m^2/min hyperinsulinemic euglycemic clamps: (1) a control study and (2) a study with elevated plasma NEFA levels. The experimental protocol was approved by the Committee on Human Investigation of the University of California, San Diego. Informed written consent was obtained from each subject.

Hyperinsulinemic Euglycemic Clamps

Glucose clamps were performed in the morning after a 10-hour overnight fast. At 3 AM, an 18-gauge cannula was inserted in an antecubital vein, and a constant infusion of [3-^{3}H]-glucose (0.25 μCi/min) (New England Nuclear, Boston, MA) was started. To elevate plasma NEFA levels, an infusion of 20% Intralipid (60 mL/h) and heparin (900 U/h) was started 3 hours prior to beginning one of the 5-hour glucose clamps, and was continued until the end of the glucose clamp study. Saline was administered instead of Intralipid and heparin in the control glucose clamp studies at each insulin dose. The glucose clamps with and without elevated plasma NEFA levels were performed in random order approximately 4 to 10 days apart.

At 7 AM, a hand vein was cannulated in a retrograde fashion and the hand was heated for sampling of arterialized blood. After each blood sample was taken this cannula was flushed with 0.15 mol/L NaCl in water. Beginning at 8 AM four basal blood samples were obtained at 10-minute intervals for measurement of plasma glucose concentration and specific activity, insulin, NEFA, and triglyceride concentrations. An intravenous infusion of insulin (Humulin R, Eli Lilly, Indianapolis, IN) diluted in 0.15 mol/L saline containing 1% wt/vol human albumin was begun from a Harvard syringe pump at 80 mU/m^2/min. Blood glucose was measured at 5-minute intervals and the blood glucose concentration clamped at 5.0 mmol/L by adjustment of the rate of infusion of a solution of 20% (wt/vol) glucose in water.[16] The 20% glucose solution was labeled with [3-^{3}H]-glucose to maintain plasma glucose specific activities during the clamp close to basal levels.[17] Potassium was given intravenously to maintain normal plasma levels. Blood samples for measurement of plasma glucose specific activity, insulin and NEFA concentrations were taken every 20 to 30 minutes until the last 30 minutes of the glucose clamps and at 10-minute intervals during the last 30 minutes.

Analytical Procedures

Plasma glucose was measured by a glucose oxidase method using a Yellow Springs analyzer (YSI 2700, Yellow Springs, OH). For determination of [^{3}H]-glucose specific activity, 0.65 mL of plasma were deproteinized with $Ba(OH)_2/ZnSO_4$.[18] After centrifugation the neutral supernatant was evaporated and the residue dissolved in 1 mL water. After adding 10 mL of scintillation fluid (Ecoscint, Manville, NJ), ^{3}H disintegrations per minute were determined in an ICN 36014 liquid scintillation counter (Titertek Instruments, Huntsville, AL) using an external standard to correct for quenching. Aliquots of the labeled glucose infusate were added to nonradioactive plasma and processed in parallel with the plasma samples to allow calculation of the [3-^{3}H]-glucose infusion rate. Blood (1.0 mL) for determination of plasma NEFA levels was placed into EDTA-coated microfuge tubes, immediately centrifuged (10 seconds, 14,000 × *g*) in an Eppendorf microcentrifuge, and the plasma immediately frozen on solid CO_2. Plasma samples were then stored at −70° C until assayed using an acyl-CoA oxidase-based colorimetric kit (WAKO NEFA-C, Richmond, VA) with intra- and interassay coefficients of variation (CVs) of 2.4% and 3.3%, respectively.

Plasma insulin was measured by a double-antibody technique.[19] The intra- and interassay CVs were 3.7% and 9.2%, respectively. Serum triglyceride was measured using a GPO-PAP kit (Boehringer Mannheim, Germany), with intra- and interassay CVs of 1.4% and 1.7%, respectively.

Calculations

The rates of total glucose appearance (Ra) and disappearance (Rd) were calculated from the [3-^{3}H]-glucose data using the non–steady-state equations of Steele et al.[20] A distribution volume of 0.19 L/kg and a pool fraction of 0.5 were used in the calculations.[21] HGO was calculated as the difference between total glucose Ra and the rate of exogenous glucose infusion.

Statistical Analysis

Results are expressed as mean ± SEM unless otherwise indicated. Differences between groups were sought using either Student's unpaired *t* test or 2-group (lean and obese) repeated measures (control and Intralipid glucose clamps) analysis of variance (ANOVA) where appropriate. Initially, the data were tested for an interaction effect between subject group and clamp type at the 5% level of significance. When no interaction was found, the main effects of subject group and clamp type were tested. When an interaction was present, general linear model pairwise contrasts were performed between groups for the glucose clamps with Intralipid and between control and Intralipid clamps for the 2 subject groups. Correlations were sought by Pearson's least-squares method. A *P* value less than .05 was considered statistically significant.

RESULTS

Plasma Glucose and Insulin Concentrations

Fasting and end of clamp plasma glucose concentrations were not significantly different between lean and obese subjects on either study day and were not influenced by the lipid and heparin infusions (Table 2). Fasting plasma insulin levels were higher in the obese than lean subjects on the control day (Table 2). Fasting plasma insulin levels increased by 44% in both the lean and obese subjects with lipid infusion ($P < .001$ by ANOVA). No interaction between subject group and effect of lipid infusion was found so that fasting insulin levels remained higher in the obese than lean subjects on the lipid day (Table 2). During the glucose clamps steady-state insulin levels were approximately 150 mU/L: they were not significantly different between the lean and obese subjects and did not differ significantly between the control and lipid studies (Table 2).

Table 2. Plasma Glucose, Insulin, and Lipid Levels in the Basal State and During the Last 30 Minutes of the 80-mU/m^2/min Hyperinsulinemic Euglycemic Clamps in the Absence (control) and Presence of an Infusion of Intralipid and Heparin in 15 Lean and 15 Obese Normal Subjects

	Control		Intralipid	
	Lean	Obese	Lean	Obese
Basal state				
Plasma glucose (mmol/L)	5.0 ± 0.1	5.2 ± 0.1	5.0 ± 0.1	5.3 ± 0.1
Plasma insulin (mU/L)	4.8 ± 0.6	9.1 ± 1.1*	6.9 ± 0.8§	13.1 ± 1.6†§
Plasma NEFA (μmol/L)	445 ± 54	404 ± 44	1279 ± 127§	1118 ± 79§
Serum triglyceride (mg/dL)	84 ± 9	182 ± 23‡	246 ± 54§	314 ± 35§
Last 30 min of clamp				
Plasma glucose (mmol/L)	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	5.1 ± 0.1
Plasma insulin (mU/L)	144 ± 8	153 ± 12	148 ± 10	168 ± 13
Plasma NEFA (μmol/L)	28 ± 8	32 ± 7	895 ± 80§	958 ± 131§
Serum triglyceride (mg/dL)	59 ± 9	155 ± 22‡	277 ± 40§	402 ± 51§

NOTE. Values are mean ± SEM. By ANOVA no difference in the effect of lipid on plasma glucose, insulin, NEFA, or triglyceride levels was found between the lean and obese subjects.

*$P < .005$, †$P < .002$, ‡$P < .001$ compared to values in the lean subjects in the same state.

§$P < .001$ compared to the control study by ANOVA.

Plasma NEFA and Triglyceride Concentrations

Fasting plasma NEFA concentrations on the control day did not differ between the lean and obese subjects but fasting triglyceride levels were higher in the obese subjects (182 ± 23 *v* 84 ± 9 mg/dL, $P < .001$). During the control glucose clamp studies plasma NEFA levels were suppressed to similar low levels in the lean and obese subjects (Table 2). The Intralipid and heparin infusions raised fasting plasma NEFA to similar levels (~1 mmol/l) in both groups and levels remained comparably elevated in the 2 groups throughout the glucose clamps. Thus, during the last 30 minutes of the clamps with lipid infusion, plasma NEFA levels were 958 ± 131 μmol/L in the obese subjects and 895 ± 80 μmol/L in the lean subjects. Serum triglyceride concentrations (fasting and during the clamp) increased significantly in both groups in response to the lipid infusions ($P < .001$ by ANOVA). No interaction between group and clamp type on serum triglyceride levels was found. With lipid infusion serum triglyceride levels were higher in the obese than in the lean subjects (Table 2, $P = .018$ by ANOVA).

Hepatic Glucose Production

In the basal state HGO equals the glucose disposal rate (GDR). HGO in the basal state in the control study was lower in the obese subjects (1.89 ± 0.07) than in the lean subjects (2.14 ± 0.08 mg/kg/min, $P < .05$). Elevation of plasma NEFA levels for 3 hours prior to the glucose clamps had no effect on basal HGO (and hence basal GDR) in either group (Fig 1). The effect of lipid infusion on HGO differed in the basal and clamp states ($P < .001$ by ANOVA). HGO was completely suppressed in both lean and obese subjects in the control glucose clamps, but in both groups elevated plasma NEFA levels resulted in impaired suppression of HGO (Fig 1, $P < .001$ by ANOVA). The magnitude of this defect was comparable in the 2 groups and residual HGO did not differ between the groups at the end of the lipid clamps (lean, 0.46 ± 0.13 mg/kg/min; obese, 0.32 ± 0.10 mg/kg/min; difference not significant [NS]).

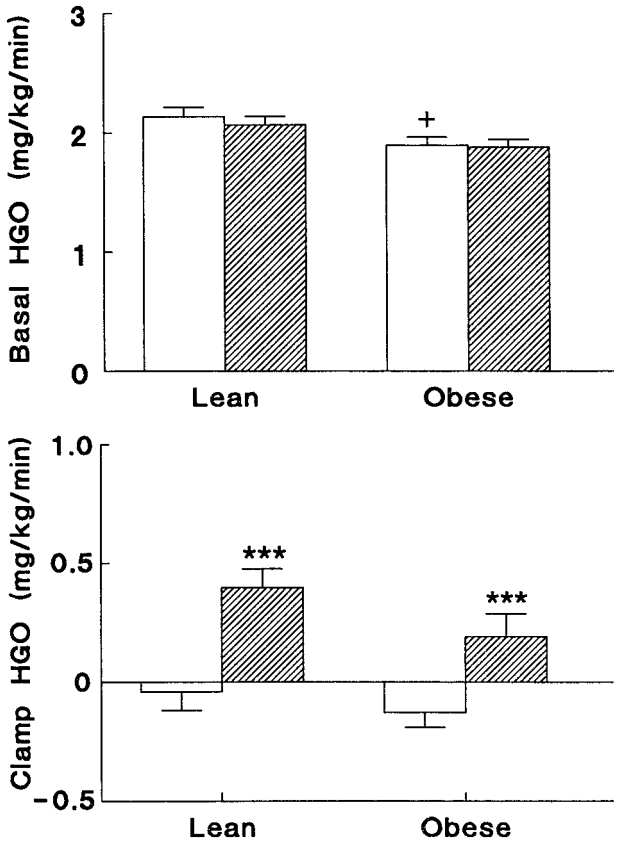

Fig 1. Rates of HGO in the 15 lean and 15 obese subjects in the basal state and during the last 40 minutes of the 5-hour 8-mU/m^2/min hyperinsulinemic euglycemic clamps in the absence (□) and presence of an Intralipid and heparin infusion (■) to raise plasma NEFA levels. Mean ± SEM. $^+P < .05$ *v* lean subjects. The effect of lipid infusion on HGO differed in the basal and clamp states ($P < .001$ by ANOVA) but not between lean and obese subjects. ***$P < .001$ compared to the saline control study at the end of the clamps.

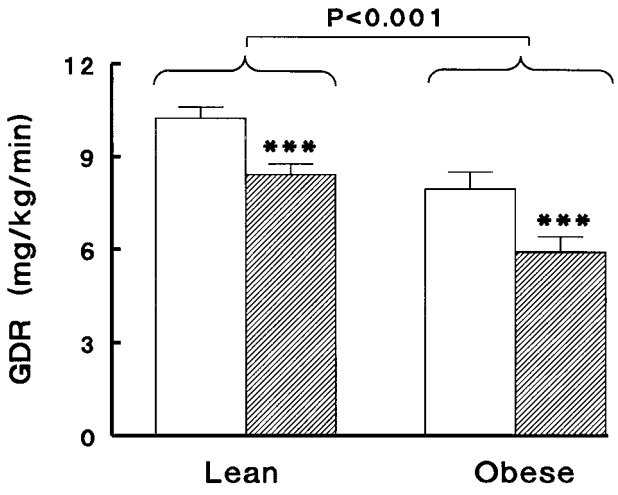

Fig 2. Whole body GDR in the 15 lean and 15 obese subjects during the last 40 minutes of the 5-hour 80-mU/m^2/min hyperinsulinemic euglycemic clamps in the absence (□) and presence of an Intralipid and heparin infusion (■) to raise plasma NEFA levels. Mean ± SEM. ***P < .001 compared to the saline control study by ANOVA. There was no significant interaction between subject group and clamp type. GDR during the clamps was lower in the obese than lean subjects (P < .001 by ANOVA).

Insulin-Stimulated Glucose Disposal Rates

In the lean subjects GDR increased approximately 5-fold during the control clamps to reach a steady-state level during the last 40 minutes of 10.24 ± 0.35 mg/kg/min. The increase was blunted in the obese subjects and their steady-state GDR was 22% lower than in the lean subjects (7.96 ± 0.55, $P <$ .002).

Elevated plasma NEFA levels led to a reduction in insulin-stimulated GDR in both the lean and obese subjects (Fig 2, $P <$ .001 by ANOVA). No interaction between subject group and clamp type was found. Thus, the effect of elevated NEFA was comparable in the 2 groups both in terms of the absolute reduction of GDR in the lean (1.82 ± 0.36 mg/kg/min decrement, $P <$ .001 for within-group difference) and obese subjects (2.03 ± 0.37 mg/kg/min decrement, $P <$ .001 for within-group difference) and the overall percentage reduction in GDR in the 2 groups (lean, 17.1% ± 3.1 % *v* obese, 24.5% ± 4.2 %; NS for lean *v* obese). The findings were similar for the 9 subjects in the obese group with a BMI of 30 kg/m^2 or more (reduction in GDR, 1.70 ± 0.46 mg/kg/min; % decrease in GDR, 22.4% ± 5.6 %). Similar results were also found when the 4 women in each of the 2 groups were excluded from the analyses (reduction in GDR: 11 lean men, 2.11 ± 0.43, *v* 11 obese men, 2.14 ± 0.40 mg/kg/min, NS; % decrease in GDR: 11 lean men, 19.6 ± 3.5 *v* 11 obese men, 25.4% ± 4.4%, NS). Combining the lean and obese groups, the percentage reduction in insulin stimulated GDR did not correlate with either BMI (Fig 3, $r = 0.08$, NS) or with a subject's insulin-stimulated GDR in the control study (Fig 3, $r = 0.11$, NS).

DISCUSSION

The possible role of NEFAs in contributing to peripheral and hepatic insulin resistance in obesity and type 2 diabetes has been the focus of many studies in recent years.[1-5,8] Most investigators have found that when plasma NEFA levels are elevated experimentally for several hours, defects of both insulin stimulated peripheral glucose disposal and suppression of HGO are induced. In general these studies have been conducted in healthy lean male subjects.[4,5,10,22-24] However, one study conducted in 7 obese women found no effect of elevated NEFA on insulin-stimulated GDR, although a defect in HGO suppression was induced.[13] Because a build up of fatty acyl CoAs in muscle has been implicated in the etiology of the defects of muscle insulin action induced by elevated NEFA,[6-9] and since these are already increased in conjunction with total intramyocellular lipid in insulin-resistant obese subjects,[7,8,11] we hypothesized that obese subjects may show little further impairment of insulin action when plasma NEFA are elevated. Our findings do not support this hypothesis.

As expected the obese subjects had fasting insulin levels that were nearly 2-fold higher than in the lean subjects, they had higher serum triglyceride levels (Table 2), and as a group were insulin-resistant as indicated by the 22% lower GDR at the end of the control glucose clamp study by comparison with the lean subjects (Fig 2). Contrary to our hypothesis, and in contrast to the findings of Bevilacqua et al[13] in the 7 obese women, elevation of plasma NEFA to approximately 1 mmol/L for 3

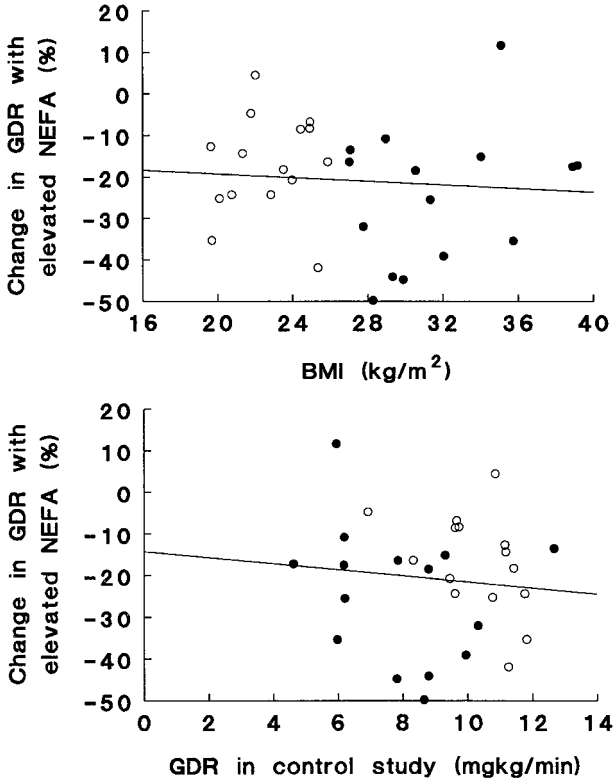

Fig 3. (Top) Relationship between the percentage change in GDR induced by elevated NEFA and BMI in the 30 subjects (○, lean; ●, obese), $r = 0.08$, NS. (Bottom) Relationship between the percentage change in GDR induced by elevated NEFA and insulin-stimulated GDR on the control study day in the 30 subjects (○, lean; ●, obese), $r = 0.11$, NS.

hours before and throughout the 5-hour glucose clamp had a similar effect on insulin-stimulated GDR in the lean and obese subjects, both in absolute terms and in terms of the percentage reduction in GDR (Fig 2). This was also the case in the nine obese subjects who had a BMI of 30 kg/m^2 or more. In this subset of subjects elevated NEFA levels decreased insulin-stimulated GDR on average by 1.7 mg/kg/m^2 ($P < .01$), corresponding to a 22.4% reduction in GDR compared to the control study day.

Although in a previous study we suggested a gender difference in susceptibility to fatty acid–induced insulin resistance,[14] in the current study we found no statistically significant difference by ANOVA between the responses of the 8 postmenopausal women and the 22 men. Hormonal status might explain the different findings as none of the women in this study were on estrogen replacement. Thus, insulin-stimulated GDR was decreased by 16.2% ± 6.4% in the 8 postmenopausal women when plasma NEFA were elevated compared to the control day ($P < .05$) and exclusion of the women from our analyses had little impact on the results or our conclusions. Although there was considerable variability between subjects in the effect of elevated NEFA on insulin-stimulated GDR, we found no relationship with BMI or with fasting plasma NEFA concentrations on the control day, which would be indicative of tissue exposure to NEFAs over a longer time frame. We conclude, therefore, that obese subjects, even though they may be insulin-resistant at baseline, are just as likely as lean subjects to exhibit a deterioration in peripheral tissue insulin action in response to a short-term elevation of plasma NEFA levels, and that the magnitude of this effect is also comparable. Given our previous findings of a difference in susceptibility to NEFA-induced insulin resistance between men and women,[14] it seems possible that the negative findings of Bevilacqua et al were due to the fact that the subjects studied were women.[13]

In keeping with previous studies,[14,22,23,25] elevation of plasma NEFA levels did not affect fasting glucose levels or basal HGO but did increase fasting insulin levels in both the lean and obese subjects (Table 2). The increase in fasting insulin levels could be due to increased insulin secretion[25-27] and/or decreased insulin clearance.[27,28] We did not measure plasma C-peptide levels in this study, but in a previous study we found increased fasting plasma C-peptide levels when plasma NEFA concentrations were elevated, implying an increase in insulin secretion.[26] Boden et al. showed that if the increase in basal insulin secretion seen with elevated NEFA levels is prevented by coinfusion of somatostatin and a basal insulin infusion, fasting glucose levels increase.[25] During the clamps, even though we used a rather high insulin infusion rate resulting in plasma insulin levels of approximately 150 mU/L, elevated plasma NEFA led to an impairment of HGO suppression (Fig 1), an effect that was comparable in the lean and obese subjects (Fig 1).

The Intralipid and heparin were infused at fixed doses of 60 ml/h and 900 U/h, respectively. Since the obese subjects were on average 25 kg heavier, on a weight basis they were given 25% less Intralipid and heparin than the lean subjects. Nonetheless, their plasma NEFA levels during the clamp were similar (Table 2). Since the main source of plasma NEFA under these conditions is the infused lipid, the data suggest that NEFA clearance might be lower in the obese subjects. Since muscle is an important site of NEFA uptake under these conditions[29,30] and an increase in intramyocellular lipid content can be demonstrated within the time frame of our study,[31-33] it is possible that the obese subjects exhibited a similar impairment of insulin stimulated GDR at a lower muscle NEFA uptake. Previous studies have suggested a lower capacity for fatty acid uptake/oxidation by muscle in obese subjects.[34]

In summary, the effect of a short-term elevation of plasma NEFA levels on insulin-stimulated GDR and supression of HGO is comparable in lean and moderately obese subjects.

ACKNOWLEDGMENT

We thank the CRC nursing, dietetic, and biostatistical staff for their excellent assistance.

REFERENCES

1. Randle PJ: Regulatory interactions between lipids and carbohydrates: The glucose fatty acid cycle after 35 years. Diabetes Metab Rev 14:263-283, 1998

2. Kruszynska YT: The role of fatty acid metabolism in the hypertriglyceridaemia and insulin resistance of type 2 (non-insulin dependent) diabetes. Diabetes Ann 9:107-139, 1995

3. Shulman GI: Cellular mechanisms of insulin resistance in humans. Am J Cardiol 84:3J-10J, 1999

4. Roden M, Price TB, Perseghin G, et al: Mechanism of free fatty-acid induced insulin resistance in humans. J Clin Invest 97:2859-2865, 1996

5. Boden G, Chen X, Ruiz J, et al: Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest 93:2438-2446, 1994

6. Thompson AL, Cooney GJ: Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance. Diabetes 49:1761-1765, 2000

7. Ellis BA, Poynten A, Lowy AJ, et al: Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. Am J Physiol 279:E554-E560, 2000

8. Kraegen EW, Cooney GJ, Ye JM, et al: The role of lipids in the pathogenesis of muscle insulin resistance and beta cell failure in type II diabetes and obesity. Exp Clin Endocrinol Diabetes 109:S189-S201, 2001 (suppl 2)

9. Yu C, Chen Y, Zong H, et al: Mechanism by which fatty acids inhibit insulin activation of IRS-1 associated phosphatidylinositol 3-kinase activity in muscle. J Biol Chem (in press)

10. Dresner A, Laurent D, Marcucci M, et al: Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest 103:253-259, 1999

11. Goodpaster BH, Theriault R, Watkins SC, et al: Intramuscular lipid content is increased in obesity and decreased by weight loss. Metabolism 49:467-472, 2000

12. Krssak M, Falk Petersen K, Dresner A, et al: Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: A ^{1}H NMR spectroscopy study. Diabetologia 42:113-116, 1999

13. Bevilacqua S, Bonadonna R, Buzzigoli G, et al: Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. Metabolism 36:502-506, 1987

14. Frias JP, Macaraeg GB, Ofrecio J, et al: Decreased susceptibility to fatty acid induced insulin resistance in women. Diabetes 50:1344-1350, 2001

15. World Health Organization: Diabetes Mellitus: Report of a

WHO Study Group. Tech. Rep. Ser. No. 727. Geneva, Switzerland, WHO, 1985, pp 80-82

16. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-E223, 1979

17. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic euglycemic glucose clamps: Comparison of unlabelled and labelled glucose infusates. Diabetes 36:914-924, 1987

18. Somogyi MJ: Determination of blood sugar. J Biol Chem 160: 69-73, 1945

19. Desbuquois B, Aurbach GD: Use of polyethylene glycol to separate free and antibody bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab 33:732-738, 1971

20. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

21. Wolfe RR: Tracers in Metabolic Research: Radioisotope/Mass Spectrometry Methods. New York, NY, Liss, 1984, pp 81-101

22. Kruszynska YT, Sears Worrall DS, et al: Fatty acid induced insulin resistance: Decreased muscle PI-3-kinase activation but unchanged Akt phosphorylation. J Clin Endocrinol Metab 87:226-234, 2002

23. 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

24. Yki-Jarvinen H, Puhakainen I, Koivisto V: Effect of free fatty acids on glucose uptake and nonoxidative glycolysis across human forearm tissues in the basal state and during insulin stimulation. J Clin Endocrinol Metab 72:1268-1277, 1991

25. Boden G, Jadali F: Effects of lipid on basal carbohydrate metabolism in normal men. Diabetes 40:686-692, 1991

26. Frias JP, Basabe L, Macaraeg G, et al: Lack of effect of a physiological elevation of plasma non-esterified fatty acid levels on insulin secretion. Diabetes Metab 26:133-139, 2000

27. Carpentier A, Mittelman S, Lamarcher B, et al: Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. Am J Physiol 39:E1055-E1066, 1999

28. Wiesenthal SR, Sandhu H, McCall RH, et al: Free fatty acids impair hepatic insulin extraction in vivo. Diabetes 48:766-774, 1999

29. Sidossis LS, Mittendorfer B, Chinkes D, et al: Effect of hyperglycemia-hyperinsulinemia on whole body and regional fatty acid metabolism. Am J Physiol 276:E427-E43, 1999

30. Furler SM, Cooney GJ, Hegarty BD, et al: Local factors modulate tissue-specific NEFA utilization: Assessment in rats using ^{3}H-(R)-2-bromopalmitate. Diabetes 49:1427-1433, 2000

31. Brechtel K, Dahl DB, Machann J, et al: Fast elevation of the intramyocellular lipid content in the presence of circulating free fatty acids and hyperinsulinemia: A dynamic (1) H-MRS study. Magn Reson Med 45:179-183, 2001

32. Chalkley SM, Hettiarachchi M, Chisholm DJ, et al: Five-hour fatty acid elevation increases muscle lipids and impairs glycogen synthesis in the rat. Metabolism 47:1121-1126, 1998

33. Boden G, Lebed B, Schatz M, et al; Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. Diabetes 50:1612-1617, 2001

34. Mandarino LJ, Consoli A, Jain A, et al: Interaction of carbohydrate and fat fuels in human skeletal muscle: Impact of obesity and NIDDM. Am J Physiol 270:E463-E470, 1996