

Decreased Susceptibility to Fatty Acid–Induced Peripheral Tissue Insulin Resistance in Women

Juan P. Frias, Gina B. Macaraeg, Jachelle Ofrecio, Joseph G. Yu, Jerrold M. Olefsky, and Yolanta T. Kruszynska

Elevation of plasma nonesterified fatty acid (NEFA) levels has been shown in various studies to induce peripheral tissue insulin resistance and impair the suppression of endogenous glucose production (EGP). These studies have been conducted predominantly in men. We compared the effects of elevated plasma NEFA levels on basal and insulin-stimulated glucose metabolism in 8 normal women (age 42 ± 8 years [mean \pm SD], BMI 25 ± 3 kg/m²) and 10 normal men (35 ± 6 years, 24 ± 3 kg/m²). Each subject underwent two 5-h 80 mU \cdot m⁻² \cdot min⁻¹ hyperinsulinemic-euglycemic clamps with measurement of glucose kinetics (intravenous [³-³H]glucose) and substrate oxidation. Plasma NEFA levels were elevated in one study for 3 h before and during the clamp (~ 1 mmol/l in both groups) by infusion of 20% Intralipid (60 ml/h) and heparin (900 U/h). In the control studies, the men and women had similar insulin-stimulated glucose disposal rates (R_d) and substrate oxidation rates. In the men, elevated NEFA levels decreased insulin-stimulated glucose R_d during the final 40 min of the clamp by 23% ($P < 0.001$). By contrast, no significant change in glucose R_d was found in the women (control 10.4 ± 1.1 , lipid study 9.9 ± 1.3 mg \cdot kg⁻¹ \cdot min⁻¹). Glucose R_d was also unchanged in six women studied at a lower insulin dose (40 mU \cdot m⁻² \cdot min⁻¹). During the last 40 min of the high-insulin dose clamps with elevated NEFA, glucose oxidation was decreased by 33% in the men ($P < 0.001$) and by 23% in the women ($P < 0.02$). Nonoxidative glucose R_d at this time was decreased by 15% in the men ($P = 0.02$) but was not significantly affected in women. Basal EGP was unaffected by elevation of plasma NEFA levels in both groups. Suppression of EGP during the glucose clamps, however, was impaired. At the insulin infusion rate used, the magnitude of this defect was comparable in men and women. In summary, our findings suggest that although the effects on EGP appear comparable, the inhibitory effects of NEFA on peripheral tissue insulin sensitivity are observed in men but cannot be demonstrated in women. *Diabetes* 50:1344–1350, 2001

From the Department of Endocrinology and Metabolism, University of California–San Diego, Veterans Affairs Medical Center, La Jolla, California.

Address correspondence and reprint requests to Yolanta T. Kruszynska, MD, Department of Endocrinology and Metabolism (9111G), Veterans Affairs Medical Center, 3350 La Jolla Village Dr., La Jolla, CA 92093.

Received for publication 19 September 2000 and accepted in revised form 21 February 2001.

CV, coefficient of variation; EGP, endogenous glucose production; NEFA, nonesterified fatty acid; R_d , disposal rate.

Increased plasma nonesterified fatty acid (NEFA) levels have been implicated in the pathogenesis of insulin resistance (1–3). Whole-body insulin sensitivity has been found to be inversely related to fasting plasma NEFA levels in healthy nondiabetic subjects (4) and also in the lean normoglycose-tolerant offspring of type 2 diabetic parents (5). In nondiabetic Pima Indians, fasting plasma NEFA levels were found to predict the subsequent development of type 2 diabetes (6).

Fatty acids affect both hepatic and peripheral tissue glucose metabolism (3). In the liver, they facilitate gluconeogenesis. Indeed, the inhibitory effect of insulin on hepatic glucose output may be in part indirect and mediated by suppression of adipose tissue lipolysis and plasma NEFA levels (7–11). In cardiac muscle and skeletal muscles with a predominance of red oxidative fibers, fatty acids compete with glucose for oxidative metabolism (1). During fasting, these effects of fatty acids on hepatic and peripheral tissue glucose metabolism play an important role in sparing glucose for tissues that cannot use fatty acids to meet their energy needs. However, in obesity and diabetes, impaired suppression of fatty acids after meals may result in impaired suppression of endogenous glucose production (EGP) and contribute to glucose intolerance (3, 12–14). Prolonged elevation of fatty acid levels may also impair insulin-mediated glucose uptake in muscle (2,3,15–19).

The hyperinsulinemic-euglycemic clamp has been used by many investigators to study the effects of elevated NEFA on peripheral and hepatic insulin sensitivity (2,3,15–21). These studies have shown that elevation of plasma NEFA concentrations by infusion of a triglyceride emulsion and heparin results in a time-dependent 15–50% reduction in insulin-stimulated whole-body glucose disposal rate (R_d) (2,3,15–21). The ability of insulin to suppress EGP is also impaired (16,20). These studies have been conducted almost exclusively in men; only two evaluated the effects of elevated plasma NEFA levels on insulin sensitivity specifically in women (22,23). Sivan et al. (23) found that a 4-h elevation of NEFA levels in pregnant women decreased insulin-stimulated glucose R_d by 28% but had no effect on EGP. Bevilacqua et al. (22) found that elevation of plasma NEFA levels by a 2-h infusion of Intralipid and heparin in seven obese women led to an impairment of suppression of EGP but did not affect peripheral glucose R_d . The lack of effect of elevated NEFA concentrations on insulin-stimulated glucose R_d in the latter study may have been due to the relatively short

duration of the lipid infusion, to the fact that these women were obese, and/or to differences in susceptibility to lipid-induced insulin resistance between male and female subjects.

Sex differences in lipoprotein metabolism have been reported. Women compared with men matched for age and total body fat mass generally have lower fasting plasma triglyceride and cholesterol levels (24) and a lower triglyceride response to a fat meal (25–27). These differences are largely explained by a more visceral distribution of body fat in men compared with women (26,27). The more visceral distribution of body fat and the consequent lipid abnormalities and insulin resistance are thought to be a major determinant of the increased risk of atherosclerotic cardiovascular disease in men (28,29). If men also showed a heightened susceptibility to NEFA-induced insulin resistance compared with women, this might be expected to compound the cardiovascular disease risk associated with visceral obesity, given the association between hyperinsulinemia and atherosclerotic risk in men (30). A difference in susceptibility of men and women to NEFA-induced insulin resistance would also have important implications for a potential role of NEFA in the pathogenesis of type 2 diabetes. Therefore, the aim of our study was to test the hypothesis that susceptibility to NEFA-induced insulin resistance differs between men and women.

RESEARCH DESIGN AND METHODS

Subjects. Eight normal women (age 42 ± 8 [SD] years, BMI 25 ± 3 kg/m²) and 10 normal men (35 ± 6 years, 24 ± 3 kg/m²) underwent two 5-h $80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic-euglycemic clamps: a control study and a study with elevated plasma NEFA levels. An additional six women (47 ± 3 years, 25 ± 3 kg/m²) underwent two 3-h hyperinsulinemic-euglycemic clamps with and without elevated plasma NEFA levels using a lower insulin infusion rate ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$). Each subject had normal glucose tolerance as determined by a 75-g oral glucose tolerance test. Two of the women undergoing the high insulin dose clamps were postmenopausal; one of them was on hormone-replacement therapy. All other women were premenopausal; they had regular menses and were studied during the follicular phase of their menstrual cycle. None of the men were on any medications. 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-h overnight fast. At 0300 h, an 18-gauge cannula was inserted in an antecubital vein, and a constant infusion of [³H]glucose ($0.35 \mu\text{Ci}/\text{min}$) (New England Nuclear, Boston, MA) was started. To elevate plasma NEFA levels, an infusion of 20% Intralipid and heparin ($900 \text{ U}/\text{h}$) was started 3 h before beginning one of the 5-h glucose clamps and 5 h before beginning one of the low-insulin dose 3-h glucose clamps. The Intralipid was infused at $60 \text{ ml}/\text{h}$ in the high insulin dose study and at $50 \text{ ml}/\text{h}$ in the six women who underwent the low-insulin dose study. The Intralipid and heparin infusions were continued throughout the glucose clamps. 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 ~ 5 –10 days apart.

At 0700 h, 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 $0.15 \text{ mol}/\text{l}$ NaCl in water. Beginning at 0800 h, four basal blood samples were obtained at 10-min 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 $0.15 \text{ mol}/\text{l}$ saline containing 1% wt/vol human albumin was begun from a Harvard syringe pump at either 80 or $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ during the 5- and 3-h glucose clamps, respectively. Blood glucose was measured at 5-min intervals and the blood glucose concentration clamped at $5.0 \text{ mmol}/\text{l}$ by adjustment of the rate of infusion of a solution of 20% (wt/vol) glucose in water (31). The 20% glucose solution was labeled with [³H]glucose to maintain plasma glucose-specific activities during the clamp close to basal levels (32). Potassium and phosphate were given intravenously to compensate for the intracellular movement of these

ions and to maintain normal serum plasma levels. Blood samples for measurement of plasma glucose-specific activity, insulin, and NEFA concentrations were taken every 20–30 min until the last 30 min of the glucose clamps and at 10-min intervals during the last 30 min.

Whole-body carbohydrate and lipid oxidation. Substrate oxidation rates in the basal state and during the $80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic glucose clamps were determined by indirect calorimetry using the Metascope II calorimeter (Cybermedic, Boulder, CO), as previously described (14). Oxygen consumption and CO₂ production were measured for 15 min during the second half of each 30-min period of the clamp, and the mean of the values during the last 10 min of the measurement interval was used for calculations. A timed (~ 5 h) basal urine sample and a postclamp urine sample were obtained for determination of basal and clamp urinary nitrogen excretion rates. The nonprotein respiratory quotient and carbohydrate and lipid oxidation rates were calculated using standard equations (14). Nonoxidative glucose R_d was calculated by subtracting the glucose oxidation rate from total glucose R_d .

Analytical procedures. Plasma glucose was measured by a glucose oxidase method using an analyzer (YSI 2,700; Yellow Springs Instruments, Yellow Springs, OH). For determination of [³H]glucose-specific activity, 0.65 ml plasma was deproteinized with $\text{Ba}(\text{OH})_2/\text{ZnSO}_4$ (33). After centrifugation, the neutral supernatant was evaporated and the residue was dissolved in 1 ml water. After adding 10 ml scintillation fluid (Ecoscint, Manville, NJ), ³H 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 [³H]glucose infusion rate. Blood (1.0 ml) for determination of plasma NEFA levels was placed in EDTA-coated microfuge tubes and immediately centrifuged (10 s , $14,000g$) in an Eppendorf microcentrifuge, and the plasma was immediately frozen on solid CO₂. Plasma samples were then stored at -70°C until assayed using an acyl-CoA oxidase–based colorimetric kit (NEFA-C; Wako, 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 (34). The intra- and interassay CVs were 3.7 and 9.2%, respectively. Serum triglyceride was measured using a GPO-PAP kit (Boehringer Mannheim, Mannheim, Germany), with intra- and interassay CVs of 1.4 and 1.7%, respectively. Urinary nitrogen excretion was calculated from the urine concentrations of creatinine, uric acid, and urea (35).

Calculations. The rates of total glucose appearance and disposal were calculated from the [³H]glucose data using the non-steady-state equations of Steele (36). A distribution volume of $0.19 \text{ l}/\text{kg}$ and a pool fraction of 0.5 were used in the calculations (37). EGP was calculated as the difference between total glucose R_a and the rate of exogenous glucose infusion.

Statistical analysis. Results are expressed as means \pm SE unless otherwise indicated. Separate two-group (men and women) repeated measures (control and Intralipid glucose clamps) analyses of variance were performed on the glucose kinetic and calorimetry data derived from the high-insulin dose clamps after verifying assumptions. Initially, the data were tested for a sex \times clamp-type interaction effect at the 5% level of significance. When no interaction was found, the main effects of sex and clamp type were tested. When interaction was present, general linear model pairwise contrasts were performed between sexes for the glucose clamps with Intralipid and between control and Intralipid clamps for the two subject groups. Although no formal adjustments were made for these three multiple pairwise comparisons for each outcome, *P* values are reported and commented on with respect to multiple testing. For the other data, statistical differences within groups were sought using Student's paired *t* test and between groups by Student's unpaired *t* test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Plasma glucose, NEFA, triglyceride, and insulin concentrations. Fasting and end-of-clamp plasma glucose, insulin, NEFA, and triglyceride concentrations for the women and men who underwent the 5-h $80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ glucose clamps are shown in Table 1. Plasma glucose and insulin concentrations were similar in the two studies, both in the fasting state and at the end of the glucose clamps, and were not different between the two groups. During the control glucose clamp studies, plasma NEFA levels were suppressed to very low levels in both men and women (Fig. 1). The Intralipid and heparin

TABLE 1

Plasma glucose, insulin, NEFA, and triglyceride concentrations after an overnight fast and at the end of the 80 mU · m⁻² · min⁻¹ hyperinsulinemic-euglycemic clamps in 8 normal women and 10 normal men in the absence and presence of an infusion of Intralipid and heparin

	Women (n = 8)		Men (n = 10)	
	Control	Lipid	Control	Lipid
Plasma glucose (mmol/l)				
Fasting	4.9 ± 0.2	4.8 ± 0.1	4.9 ± 0.1	5.0 ± 0.1
End of clamp	4.8 ± 0.1	4.9 ± 0.1	5.0 ± 0.0	5.1 ± 0.0
Plasma insulin (mU/l)				
Fasting	8.9 ± 1.5	9.6 ± 2.0	7.0 ± 1.7	7.8 ± 1.6
End of clamp	123 ± 9	128 ± 18	118 ± 7	117 ± 8
Plasma NEFA (μmol/l)				
Fasting	535 ± 81	1,148 ± 75*	351 ± 51	1,018 ± 60*
End of clamp	24 ± 5	809 ± 105*	15 ± 4	780 ± 80*
Plasma triglyceride (mg/dl)				
Fasting	145 ± 41	321 ± 70†	104 ± 21	223 ± 36*
End of clamp	97 ± 36	317 ± 80‡	71 ± 20	277 ± 51*

Data are means ± SE. **P* < 0.001, †*P* < 0.01, and ‡*P* < 0.005 compared with the control study.

infusions raised plasma NEFA to similar levels in both groups, and elevated levels were maintained during the clamps (Table 1, Fig. 1).

Plasma glucose, lipid, and insulin data for the six women who underwent the 3-h 40 mU · m⁻² · min⁻¹ hyperinsulinemic-euglycemic clamps are given in Table 2. The Intralipid/heparin infusions raised fasting plasma NEFA levels from 319 ± 35 to 883 ± 86 μmol/l (*P* < 0.001); NEFA levels during the last 30 min of the clamp were 682 ± 91 μmol/l (Table 2, Fig. 2). The slightly lower fasting and end-of-clamp plasma NEFA levels attained in these six women by comparison with the first study are consistent with the slightly lower Intralipid infusion rate used (50 ml/h). The fasting plasma glucose concentration was significantly higher after 5 h of Intralipid/heparin infusion than after saline administration (Table 2, *P* < 0.05). Fasting plasma insulin concentrations also tended to be higher, but did not reach statistical significance. During both glucose clamps, plasma insulin levels increased to ~80 mU/l within the first 30 min and did not change significantly thereafter.

Basal and insulin-stimulated glucose disposal rates. Basal glucose R_d in the control study was the same in men and women (2.03 ± 0.10 and 2.02 ± 0.08 mg · kg⁻¹ · min⁻¹, respectively) and rose approximately fivefold during the control 80 mU · m⁻² · min⁻¹ hyperinsulinemic clamps to reach a similar steady-state level during the last 40 min in men and women (10.78 ± 0.52 and 10.37 ± 1.11 mg · kg⁻¹ · min⁻¹, respectively) (Fig. 1). Elevation of plasma NEFA levels for 3 h before the glucose clamps had no effect on basal glucose R_d in either group (Fig. 1). Elevated plasma NEFA levels had a differential effect on insulin-stimulated glucose R_d in men and women (interaction *P* < 0.02, Fig. 1). Thus, in the men, glucose R_d during the last 40 min of the clamp was reduced by 23%, from 10.78 ± 0.52 to 8.37 ± 0.57 mg · kg⁻¹ · min⁻¹ (*P* = 0.0001), but was unchanged in the women.

In the six women who underwent the lower-dose insulin infusion clamps, elevation of plasma NEFA for 5 h before and throughout the 3-h clamp also had no effect on either basal or insulin-stimulated glucose R_d (Fig. 2). For example, during the final 40 min of the glucose clamp, glucose R_d was 7.43 ± 1.03 mg · kg⁻¹ · min⁻¹ in the Intralipid/

heparin study compared with 7.60 ± 0.84 mg · kg⁻¹ · min⁻¹ in the control study (*P* = NS).

Endogenous glucose production. In the basal state, EGP equals glucose R_d and was not affected by the 3-h Intralipid/heparin infusion in either men or women (Fig. 1). In the control glucose clamps, at the higher insulin dose, EGP was completely suppressed after 60 min in both men and women. In both groups, elevated plasma NEFA levels resulted in impaired suppression of EGP (Fig. 1, Table 3). No significant effects were found for the sex × clamp-type interaction or for sex, but there was a statistically significant difference between control and Intralipid glucose clamps (*P* < 0.001). In the six women studied at the lower insulin infusion rate, impaired EGP suppression was also evident (Fig. 2). During the final 40 min of the glucose clamps, EGP was higher in the Intralipid/heparin study compared with the control study (0.83 ± 0.24 vs. 0.03 ± 0.16 mg · kg⁻¹ · min⁻¹, *P* < 0.02).

Substrate oxidation. Glucose oxidation was increased during the glucose clamp to a similar extent in men and women, reaching levels during the last 40 min of 4.12 ± 0.21 mg · kg⁻¹ · min⁻¹ in men and 3.97 ± 0.17 mg · kg⁻¹ · min⁻¹ in women. Lipid oxidation was completely suppressed in both groups (Fig. 3). During the Intralipid/heparin clamps, the insulin-induced increase in glucose oxidation was blunted in both groups. In the men, glucose oxidation during the last 40 min of the clamp was 33% lower than in the control study (Fig. 3, *P* < 0.001); in the women, it was 23% lower (Fig. 3, *P* < 0.02). A corresponding enhancement of lipid oxidation was seen when plasma NEFA levels were elevated (Fig. 3).

Insulin led to a marked increase in nonoxidative glucose R_d in the control clamps to similar levels in men and women (Fig. 3). A significant interaction between sex and clamp type was found for insulin-stimulated nonoxidative glucose R_d (*P* = 0.03). Thus, nonoxidative glucose R_d during the clamps was unaffected by elevated NEFA levels in women, but was reduced by 15% in the men (Fig. 3, *P* = 0.02), which almost reaches statistical significance after a Bonferroni adjustment for three comparisons (significance level of 0.05/3 = 0.0167). Nonoxidative glucose R_d during

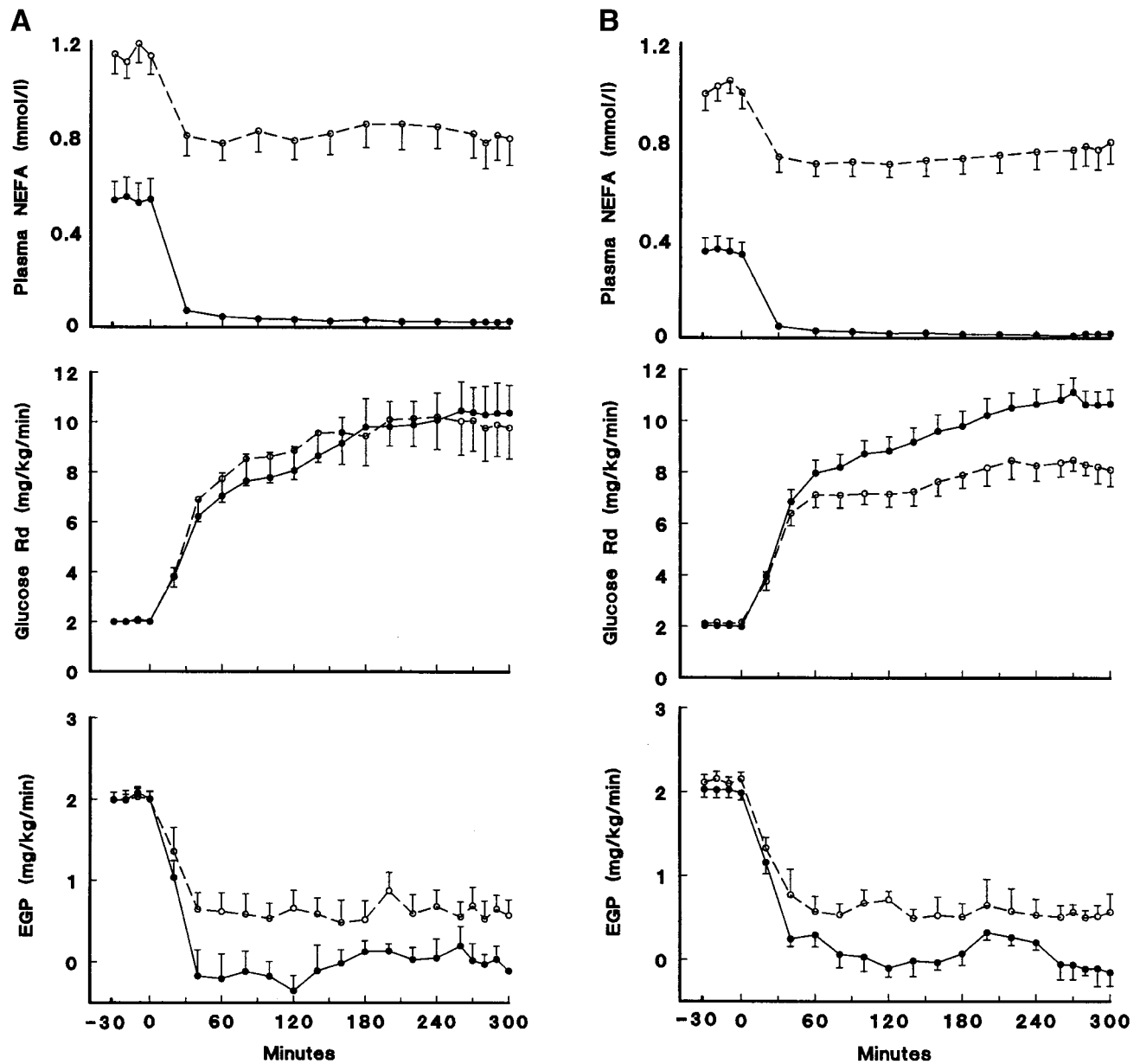


FIG. 1. Plasma NEFA concentrations, whole-body glucose R_d , and EGP in the 8 normal women (A) and 10 normal men (B) in the basal state after an overnight fast and during a 5-h $80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic-euglycemic clamp performed in the absence (●) and presence (○) of an infusion of Intralipid and heparin to raise plasma NEFA levels. Values are means \pm SE.

the last 40 min of the Intralipid clamps was significantly lower in men than in women ($P = 0.014$).

DISCUSSION

Peripheral tissue insulin resistance and hepatic overproduction of glucose are two key abnormalities underlying the pathogenesis of hyperglycemia in type 2 diabetes (38). There has been much interest in the possible role of NEFAs in contributing to both defects (1–3). Many studies have investigated the effects of short-term elevation of plasma NEFA levels achieved by infusion of a triglyceride emulsion and heparin on basal and insulin-stimulated glucose R_d and EGP (2,3,15–21). In general, these studies have shown that increased NEFA availability has little effect on basal EGP and glucose R_d but impairs insulin-

induced suppression of EGP and stimulation of glucose R_d . In reviewing these studies, we observed that they had been conducted almost exclusively in men. Only one study specifically evaluated the effects of elevated plasma NEFA in healthy nonpregnant women (22). In that study, a supra-physiological plasma NEFA elevation led to an impairment of insulin's ability to suppress EGP but had no significant effect on insulin-stimulated glucose R_d (22). The lack of effect of elevated NEFA levels on peripheral insulin sensitivity may have been due to the relatively short duration of the Intralipid/heparin infusion, to the fact that the women were obese, or to the female sex of the subjects. Because a difference in susceptibility to NEFA-induced insulin resistance between men and women would have important implications concerning the role of NEFA in the

TABLE 2

Plasma glucose, insulin, NEFA, and triglyceride concentrations in six women in the basal state after an overnight fast and during the last 30 min of the 3-h $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic-euglycemic clamps in the absence (control) and presence of an infusion of Intralipid and heparin (lipid)

	Control		Lipid	
	Basal	Clamp	Basal	Clamp
Plasma glucose (mmol/l)	5.2 ± 0.2	5.0 ± 0.0	$5.5 \pm 0.2^*$	5.0 ± 0.0
Plasma insulin (mU/l)	10.3 ± 1.6	76 ± 6	15.4 ± 3.6	84 ± 5
Plasma NEFA ($\mu\text{mol/l}$)	319 ± 35	4 ± 3	$883 \pm 86^\dagger$	$682 \pm 91^\dagger$
Plasma triglyceride (mg/dl)	163 ± 58	—	246 ± 46	—

Data are means \pm SE. * $P < 0.05$ and $^\dagger P < 0.001$ compared with the control study.

pathogenesis of type 2 diabetes, we compared the effect of experimental elevation of plasma NEFA on insulin-stimulated glucose R_d and EGP suppression in men and women.

The major findings of this study are that increased plasma NEFA levels produced insulin resistance in men, as reflected by the 23% decrease in insulin-stimulated glucose R_d (Fig. 1). In marked contrast, no inhibition of glucose R_d was observed in women, despite similar elevation of plasma NEFA levels (Fig. 1). During the Intralipid clamps, suppression of EGP was modestly blunted in both groups. The degree of insulin resistance induced by elevated plasma NEFA in male subjects is largely consistent with that found by others (3,16,18,20,21,39,40). Ferrannini et al. (39) found a strong correlation between insulin-stimulated glucose R_d and the percentage inhibition of glucose uptake caused by fatty acids during a clamp. Thus, in the obese women studied by Bevilacqua et al. (22), reduced insulin sensitivity could have been a factor in the lack of inhibition of glucose R_d by elevated NEFA levels. However, this cannot be a factor in our study because insulin sensitivity, as reflected by whole-body glucose R_d during the control clamps, was very similar in the male and female subjects (Fig. 1). Thiebaud et al. (40) found that elevated NEFA levels in normal subjects induced a more profound reduction in insulin-stimulated glucose R_d at lower steady-state plasma insulin concentrations: at a plasma insulin concentration of $\sim 62 \text{ mU/l}$, whole-body glucose uptake was reduced by 41%, whereas at $\sim 170 \text{ mU/l}$ it was reduced by only 28% (40). Because we found that glucose R_d was also unaffected in the six women studied at the lower insulin dose (Fig. 2), insulin concentration does not appear to have played a role in the lack of effect of elevated NEFA levels in the women.

Muscle glycogen deposition is the main determinant of nonoxidative glucose R_d at high physiological insulin concentrations under glucose clamp conditions. Using ^{31}P - and ^{13}C -nuclear magnetic resonance spectroscopy to measure gastrocnemius muscle glycogen levels in normal male subjects, Roden et al. (15) found that the decline in insulin-stimulated whole-body glucose uptake induced by elevated NEFA levels coincided with a reduction in the rate of glycogen deposition and that this was preceded by a defect in glucose transport/phosphorylation. Nonoxidative glucose metabolism in their study followed a time course similar to that of muscle glycogen synthesis and was reduced to a similar extent. Our data support the view that a defect of nonoxidative glucose R_d is an important determinant of the NEFA-induced defect in insulin-stimulated glucose R_d . The 15% reduction of nonoxidative glucose R_d

in the male subjects accounted for about one-third of the total defect in glucose utilization because nonoxidative glucose R_d accounted for approximately two-thirds of total

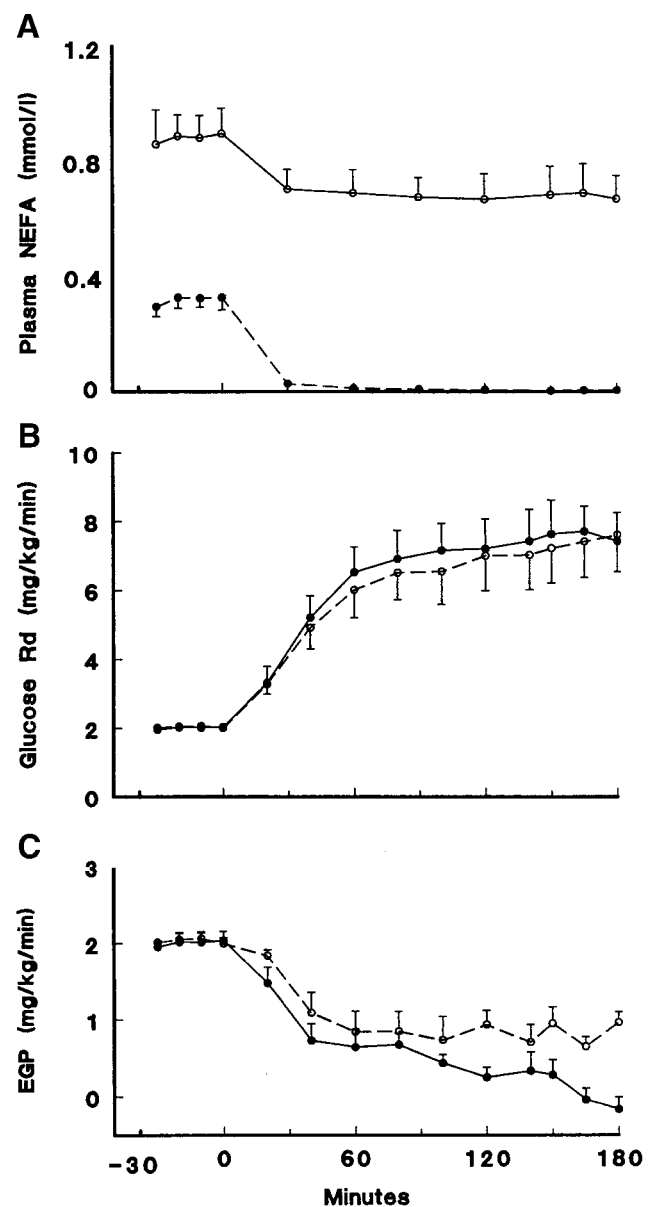


FIG. 2. Plasma NEFA (A), whole-body glucose R_d (B), and EGP (C) in six normal women in the basal state and during a 3-h $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic-euglycemic clamp in the absence (●) and presence (○) of an infusion of Intralipid and heparin. Values are means \pm SE.

TABLE 3

EGP and R_d in the basal state and during the last 40 min of the $80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic-euglycemic clamps in the 10 men and 8 women in the absence (control) and presence of an infusion of Intralipid and heparin (lipid) to raise plasma NEFA levels

	Women		Men	
	Control	Lipid	Control	Lipid
Basal				
EGP ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	2.02 ± 0.08	2.01 ± 0.10	2.03 ± 0.10	2.13 ± 0.08
End of clamp				
EGP ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	-0.02 ± 0.09	$0.61 \pm 0.16^*$	-0.11 ± 0.09	$0.58 \pm 0.11^\ddagger$
Glucose R_d ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	10.37 ± 1.11	9.89 ± 1.26	10.78 ± 0.52	$8.37 \pm 0.57^\ddagger$

Data are means \pm SE. In the basal steady-state situation, glucose R_d is numerically equivalent to EGP. $^*P < 0.01$, $^\ddagger P < 0.002$, and $^\ddagger P < 0.001$ compared with the control study.

glucose R_d . The absence of any decrease in nonoxidative glucose R_d in the women in conjunction with the rather small absolute decrease in oxidative glucose metabolism explains their unchanged whole-body glucose R_d during the clamps with elevated NEFA.

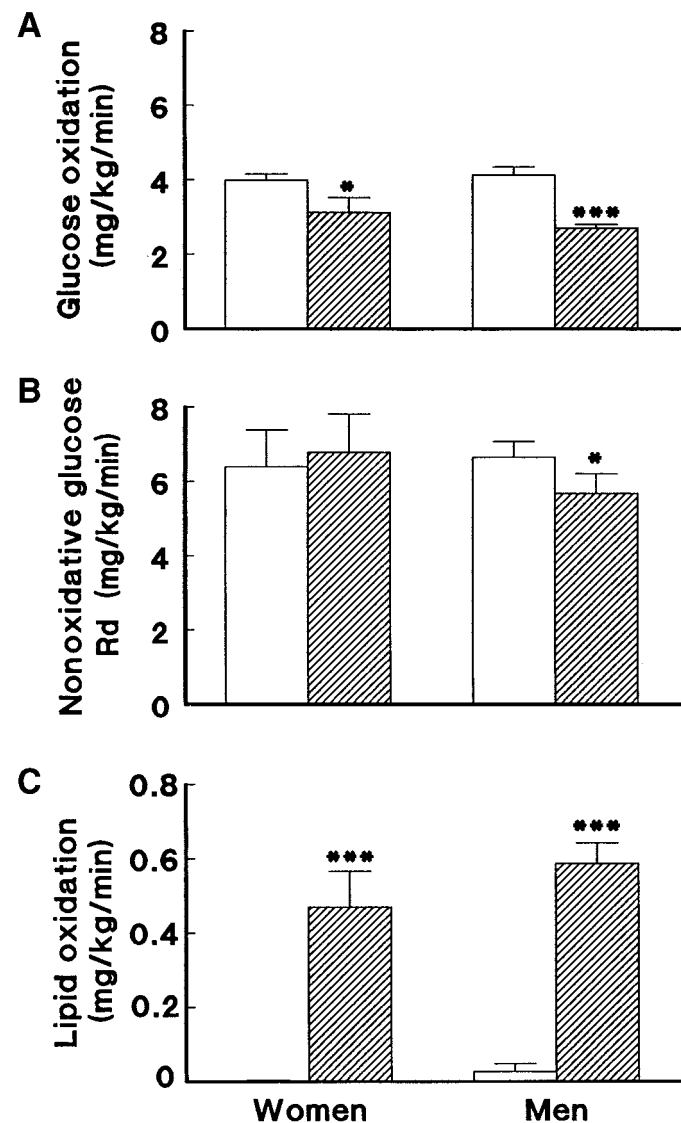


FIG. 3. Whole-body glucose oxidation (A), nonoxidative glucose R_d (B), and lipid oxidation rates (C) in 8 normal women and 10 normal men calculated for the last 40 min of the $80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ hyperinsulinemic-euglycemic clamps in the absence (\square) and presence (▨) of an infusion of Intralipid and heparin. Values are means \pm SE. $^*P < 0.02$ and $***P < 0.001$ compared with the control study.

Intramyocyte triglyceride depots are another source of fatty acids available to the myocyte for metabolism. The intramyocyte triglyceride content has been found to be a much stronger predictor of insulin sensitivity than BMI or percentage body fat content (41,42). Our findings that women are less susceptible to NEFA-induced peripheral tissue insulin resistance fit with recent data suggesting that women may have insulin sensitivity comparable to that of men, despite a higher intramyocyte fat content (43). The mechanisms underlying these sex differences are unclear; sex hormone status could clearly play a role. The one postmenopausal woman in our study who was not on hormone-replacement therapy had a 14.7% lower glucose R_d during the lipid study than during the control study, whereas the postmenopausal woman on hormone-replacement therapy showed no decrease in glucose R_d . Sex-hormone status could also explain why elevated NEFA levels induced a decrease in glucose utilization in the pregnant women studied by Sivan et al. (23). A difference in muscle fiber composition between the men and women in our study could also be a factor.

Even though we used a rather high insulin infusion rate, resulting in plasma insulin levels of $\sim 120 \text{ mU/l}$, elevated plasma NEFA led to an impairment of EGP suppression (Fig. 1). By contrast with the effects of elevated plasma NEFA on peripheral tissue glucose R_d , their effects on EGP at these insulin levels were comparable in the men and women (Fig. 1). An impairment of EGP suppression was also seen in the six women studied at the lower rate of insulin infusion (Fig. 2). Our findings in the women concur with those of Bevilacqua et al. (22), who also found that elevated NEFA levels impaired EGP suppression in the absence of any effect on peripheral glucose R_d .

To our knowledge, this is the first study evaluating the effects of elevated plasma NEFA levels on insulin sensitivity specifically in healthy nonobese women. Our findings show that women are less susceptible than men to the inhibitory effects of NEFA on peripheral tissue insulin sensitivity, whereas the effects on EGP appear to be comparable. Further studies are needed to determine whether premenopausal and postmenopausal women behave similarly in this respect and to clarify the mechanisms underlying the observed sex difference in susceptibility to NEFA-induced peripheral tissue insulin resistance.

ACKNOWLEDGMENTS

This study was supported by the Whittier Institute, the National Institutes of Health Grant DK-33649, and General Clinical Research Center Grant MO1-RR-00827.

We thank Dr. Reena Deutsch (Director, University of California–San Diego Clinical Research Center Biostatistics Unit) for assistance with the statistical analyses; Eva Brzezinski for helping with the indirect calorimetry measurements; and the Clinical Research Center nursing staff for their excellent assistance.

REFERENCES

- Randle PJ: Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years (Review). *Diabetes Metab Rev* 14:263–283, 1998
- Shulman GI: Cellular mechanisms of insulin resistance in humans. *Am J Cardiol* 84:3J–10J, 1999
- Kruszynska YT: The role of fatty acid metabolism in the hypertriglyceridaemia and insulin resistance of type 2 (non-insulin dependent) diabetes. In *The Diabetes Annual*. Marshall, SM, Home PD, Rizza RA, Eds. Amsterdam, Elsevier, 1995, p. 107–139
- Baldeweg SE, Golay A, Natali A, Balkau B, Del Prato S, Coppack SW: Insulin resistance, lipid and fatty acid concentrations in 867 healthy Europeans. *Eur J Clin Invest* 30:45–52, 2000
- Perseghin G, Ghosh S, Gerow K, Shulman GI: Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. *Diabetes* 46:1001–1009, 1997
- Paolisso G, Tataranni PA, Foley JE, Bogardus C, Howard BV, Ravussin E: A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM. *Diabetologia* 38:1213–1217, 1995
- Rebrin K, Steil GM, Getty L, Bergman RN: Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* 44:1038–1045, 1995
- Rebrin K, Steil GM, Mittelman S, Bergman RN: Causal linkage between insulin regulation of lipolysis and liver glucose output. *J Clin Invest* 98:741–749, 1996
- Sindelar DK, Chu CA, Rohlie M, Neal DW, Swift LL, Cherrington AD: The role of fatty acids in mediating the effects of peripheral insulin on hepatic glucose production in the conscious dog. *Diabetes* 46:187–196, 1997
- Lewis GF, Zimman B, Groenewoud Y, Vranic M, Giacca A: Hepatic glucose production is regulated by direct and extrahepatic effects of insulin in humans. *Diabetes* 45:454–462, 1996
- Prager R, Wallace P, Olefsky JM: Direct and indirect effects of insulin to inhibit hepatic glucose output in obese subjects. *Diabetes* 36:607–611, 1987
- Meylan M, Henny C, Temler E, Jequier E, Felber JP: Metabolic factors in the insulin resistance in human obesity. *Metabolism* 36:256–261, 1987
- Gomez F, Jequier E, Chabot V, Buber V, Felber JP: Carbohydrate and lipid oxidation in normal human subjects: its influence on glucose tolerance and insulin response to glucose. *Metabolism* 21:381–391, 1972
- Kruszynska YT, Mulford MI, Yu JG, Armstrong DA, Olefsky JM: Effects of nonesterified fatty acids on glucose metabolism after glucose ingestion. *Diabetes* 46:1586–1593, 1997
- Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI: Mechanism of free fatty-acid induced insulin resistance in humans. *J Clin Invest* 97:2859–2865, 1996
- Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994
- Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI: Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103:253–259, 1999
- Yki-Jarvinen H, Puhakainen I, Koivisto VA: 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
- Roden M, Krssak M, Stingl H, Grber S, Hofer A, Fornsinn C, Moser E, Waldhausl W: Rapid impairment of skeletal muscle glucose transport/phosphorylation by free fatty acids in humans. *Diabetes* 48:358–364, 1999
- Lee KU, Lee HK, Koh CS, Min HK: Artificial induction of intravascular lipolysis by lipid-heparin infusion leads to insulin resistance in man. *Diabetologia* 31:285–290, 1988
- Bonadonna RC, Zych K, Boni C, Ferrannini E, DeFronzo RA: Time dependence of the interaction between lipid and glucose in humans. *Am J Physiol* 257: E49–E56, 1989
- Bevilacqua S, Bonadonna R, Buzzigoli G, Boni C, Ciociaro D, Maccari F, Giorico MA, Ferrannini E: Acute elevation of free fatty acid levels leads to hepatic insulin resistance in obese subjects. *Metabolism* 36:502–506, 1987
- Sivan E, Homko CJ, Whittaker PG, Reece EA, Chen X, Boden G: Free fatty acids and insulin resistance during pregnancy. *J Clin Endocrinol Metab* 83:2338–2342, 1998
- Krotkiewski M, Bjorntorp P, Sjostrom L, Smith U: Impact of obesity on metabolism in men and women. *J Clin Invest* 72:1150–1162, 1983
- Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ: Postprandial plasma lipoprotein changes in human subjects of different ages. *J Lipid Res* 29:469–479, 1988
- Couillard C, Bergeron N, Prud'homme D, Bergeron J, Tremblay A, Bouchard C, Mauriege P, Despres J-P: Gender difference in postprandial lipemia: importance of visceral adipose tissue accumulation. *Arterioscler Thromb Vasc Biol* 19:2448–2466, 1999
- Laws A, Hoen HM, Selby JV, Saad MF, Haffner SM, Howard BV: Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status. *Arterioscler Thromb Vasc Biol* 17:64–71, 1997
- Nilsson PM, Lind L, Pollare T, Berne C, Lithell H: Differences in insulin sensitivity and risk markers due to gender and age in hypertensives. *J Hum Hypertens* 14:51–56, 2000
- Wingard DL, Suarez L, Barrett-Connor E: The sex differential in mortality from all causes and ischemic heart disease. *Am J Epidemiol* 117:165–172, 1983
- Modan M, Or J, Karasik A, Drory Y, Fuchs Z, Lusky A, Chetrit A, Halkin H: Hyperinsulinemia, sex, and risk of atherosclerotic cardiovascular disease. *Circulation* 84:1165–1175, 1991
- DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
- Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps: comparison of unlabeled and labeled glucose infusates. *Diabetes* 36:914–924, 1987
- Somogyi MJ: Determination of blood sugar. *J Biol Chem* 160:69–73, 1945
- 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
- Thorburn AW, Gumbiner B, Flynn T, Henry RR: Substrate oxidation errors during combined indirect calorimetry-hyperinsulinemic glucose clamp studies. *Metabolism* 40:391–398, 1991
- Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 82:420–430, 1959
- Wolfe RR: *Tracers in Metabolic Research: Radioisotope/Mass Spectrometry Methods*. New York, Alan R. Liss, 1984, p. 81–101
- Kruszynska YT, Olefsky JM: Cellular and molecular mechanisms of non-insulin dependent diabetes mellitus. *J Invest Med* 44:413–428, 1996
- Ferrannini E, Barrett EJ, Bevilacqua S: Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* 72:1737–1747, 1983
- Thiebaud D, DeFronzo RA, Jacot E, Golay A, Acheson K, Maeder E, Jequier E, Felber J-P: Effect of long chain triglyceride infusion on glucose metabolism in man. *Metabolism* 31:1128–1136, 1982
- Krssak M, Falk-Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI: Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ¹H NMR spectroscopy study. *Diabetologia* 42:113–116, 1999
- Perseghin G, Scifo P, DeCobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ¹H-¹³C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48:1600–1606, 1999
- Perseghin G, Scifo P, Pagliato E, Caloni M, Bertoli S, Del Maschio A, Luzzi L: Leptin, fatty acid metabolism, and insulin action differently interact in lean males and females (Abstract). *Diabetes* 49 (Suppl. 1):A301, 2000