Differential Effect of Insulin on Saturated and Unsaturated Fatty Acids

Raymond R. Townsend and Huawei Zhao

Fatty acids fulfill important roles in physiology. The plasma concentrations of fatty acids are principally regulated by insulin, which suppresses the release of fatty acids from lipid stores, and catecholamines, which increase their release from lipid stores. Although insulin regulates the concentration of plasma free fatty acids (FFAs), little is known about the relative effects of insulin on the saturated compared with the unsaturated plasma fatty acids. In the current study, we specifically measured the plasma concentration of 3 saturated and 4 unsaturated fatty acids along with an estimate of lipolytic activity using a stable isotope of glycerol during a 3-hour, 1-step, euglycemic clamp study in humans. The data showed the expected decline in plasma fatty acids from 0.26 ± 0.02 to 0.06 ± 0.01 μmol/mL. Saturated fatty acids were reduced from 0.12 ± 0.01 to 0.05 ± 0.005 μmol/mL and unsaturated fatty acids were reduced from 0.11 ± 0.01 to 0.01 ± 0.001 μmol/mL after 3 hours of insulin infusion. At baseline, the ratio of saturated to unsaturated fatty acid was 55:45, which increased to 82:18 by the end of study. The changes in fatty acids were evident within 1 hour. Whole body lipolytic rates were measured with deuterated glycerol and decreased from 1.48 ± 0.56 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to 0.75 ± 0.34 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Baseline postabsorptive plasma fatty acid concentrations were significantly correlated to insulin sensitivity (M value) as measured during the euglycemic clamp. There were no significant differences between the more insulin-sensitive subjects when compared with the more insulin-resistant subjects with respect to the relative decreases in saturated and unsaturated fatty acids during insulin infusion. These data demonstrate a sustained differential effect of insulin on the plasma fatty acid profile.

FATTY ACIDS SERVE important roles in physiology, including fuel sources, participants in membrane structure, mediators of metabolic processes, such as prostaglandin and leukotriene pathways, and the exertion of hemodynamic effects.[1,2] Most fatty acid transport is via esterification to glycerol and incorporation of these triglycerides into very–low-density lipoprotein (VLDL) particles, which are then released into the circulation. A smaller portion of nonesterified fatty acids are transported directly bound to serum proteins. The concentrations of these free fatty acids (FFA) are closely regulated by several systems, principally insulin, which suppresses the release of fatty acids from lipid stores, and catecholamines, which increase the release of fatty acids from lipid stores, in addition to other regulators.[3-5]

The plasma FFA profile is composed of both saturated and unsaturated fatty acids in roughly equal amounts. Intake of saturated fatty acids are thought to contribute to atherosclerosis, while unsaturated fatty acids may have a protective role in atherosclerosis.

Although insulin regulates the concentration of plasma FFA through its inhibitory effect on FFA release by lipolysis,[6] little is known about the relative effects of insulin on saturated compared with unsaturated plasma fatty acids. Recent data suggests that serum concentrations of specific fatty acids may be important in the pathogenesis of cardiovascular endpoints, such as myocardial infarction,[7] and clinical interest in fatty acids remains engaged because of their role in the pathogenesis of insulin resistance and type 2 diabetes.[8] We hypothesized that the plasma concentration of unsaturated fatty acids was reduced to a greater degree during insulin and glucose infusion when compared with saturated fatty acids based on preliminary observations of subjects in whom we measured lipolytic rates and individual plasma FFA concentrations during euglycemic clamp studies.[9-11] In the current study, we specifically measured the plasma concentration of 3 saturated (C14:0,C16:0, and C18:0) and 4 unsaturated fatty acids (C16:1,C18:1,C18:2, and C18:3) along with an estimate of whole-body lipolytic activity using the rate of appearance (Ra) of a stable isotope of glycerol during euglycemic clamp studies in humans. In addition, changes in fat oxidation were measured by indirect calorimetry during euglycemic clamp testing. Finally, to determine whether insulin sensitivity influenced the relative reduction in the saturated and unsaturated plasma FFA profiles, we compared the changes in saturated and unsaturated FFA in subjects who were more insulin sensitive compared with those who were less insulin sensitive, as determined by the M value (glucose uptake during stable hyperinsulinemia) during the euglycemic clamp study.

MATERIALS AND METHODS

All procedures were approved by the Institutional Review Boards of The University of Texas Medical Branch at Galveston and the University of Pennsylvania, and each subject gave informed, written consent. Details of the insulin sensitivity of some of these subjects have been previously published.[9,11,12]

Euglycemic Clamp Procedure

Each subject underwent a standard history and physical exam and a complete biochemical profile prior to study participation. No subject was receiving routine medication, and 6 subjects (5 men, 1 woman) with a history of essential hypertension had not received antihypertensive medication for at least 4 weeks prior to participation. Twenty-six of the 32 subjects (the normotensive subjects) underwent an oral

From the Department of Medicine, University of Pennsylvania, Philadelphia, PA; and the Department of Medicine, Michigan State University, East Lansing, MI.

Submitted September 8, 2001; accepted October 21, 2001.

Supported by General Clinical Research Center (GCRC) Grants No. M01-RR00073 and M01-RR00044, Grant No. 92G-620 from the American Heart Association (AHA) (Texas Affiliate), and Grant No. K24-DK-02684 from the National Institutes of Health (NIH).

Address reprint requests to Raymond R. Townsend, MD, University of Pennsylvania, 210 White Bldg, 3400 Spruce St, Philadelphia, PA 19104.

0026-0495/02/5106-0029$35.00/0

doi:10.1053/meta.2002.31344

glucose tolerance test (75 g of glucose) to exclude subjects with glucose intolerance. The other 6 subjects had no clinical history of diabetes and had normal fasting blood sugar concentrations.

The euglycemic clamp and indirect calorimetry studies were performed in the General Clinical Research Center (GCRC). Subjects were admitted to the GCRC at 5:00 to 6:00 PM on the evening prior to the euglycemic clamp study. A 12-hour urine collection was obtained (6:00 PM to 6:00 AM) to estimate protein oxidation from urea nitrogen excretion.[13] At 6:00 AM, an intravenous (IV) catheter was inserted in each arm. One catheter was placed in the hand or wrist and warmed to 55°C to 60°C (to "arterialize" the blood[14]), from which all blood sampling was performed. The other catheter was placed in the opposite forearm and used for all infusions. Subjects rested for approximately 30 minutes before any studies were initiated.

Beginning at 7:00 AM, blood was sampled every 10 minutes to demonstrate stable whole blood glucose concentration by the glucose oxidase method on a glucose analyzer (YSI model 23A; YSI, Yellow Springs, OH). At 9:00 AM, a euglycemic clamp was initiated. A priming bolus of regular human insulin ($\approx$ 0.5 U; Humulin R, Eli Lilly, Indianapolis, IN) was followed by a constant infusion at a dose of 40 $mU \cdot m^{-2} \cdot min^{-1}$ for 3 hours (9:00 AM to noon). Whole blood glucose concentrations were determined every 5 minutes, and a 15% dextrose solution was administered at a rate sufficient to maintain whole blood glucose concentration between 80 and 85 $mg \cdot dL^{-1}$ (4.4 to 4.7 $mmol \cdot L^{-1}$). Commencing with dextrose infusion, potassium chloride was administered at 0.1 $mmol \cdot kg^{-1} \cdot hr^{-1}$ until insulin infusion was complete. Following 3 hours of insulin infusion, all infusion solutions except dextrose were discontinued. The subjects were fed, and the dextrose infusion tapered and discontinued between noon and 1:30 PM.

Expired air was collected by a face mask system connected to a SensorMedic calorimeter (Sensormedic, Yorba Linda, CA) for determination of carbon dioxide production and oxygen usage just before and during the last 30 minutes of the euglycemic clamp study. Expired air was analyzed at 30-second intervals for a total of 9 minutes on each subject. Calorimetry was performed between 8:30 and 9:00 AM (basal) and again between 11:30 AM and noon during the last 30 minutes of insulin infusion.

Analyses

Plasma insulin levels were determined by radioimmunoassay (INC-STAR, Stillwater, MI). Plasma glycerol concentrations were measured with an AutoAnalyzer (Bayer-Technicon, Tarrytown, NY). Enrichment of plasma with $^2H_5$-glycerol was determined by gas chromatography/mass spectroscopy using an MSD 5971 system (Hewlett Packard, Palo Alto, CA) with an HP-1 12 $\times$ 0.2 mm fused silica capillary column after formation of the trimethyl-silyl derivative.[10,15] The rate of appearance of glycerol (glycerol Ra) was determined using the Steele equation for steady state isotope kinetics[16] and the nonsteady state[17] induced by insulin infusion. Plasma fatty acids were determined using gas chromatography as described previously.[10,18] Indirect calorimetry data were analyzed for substrate oxidation according to published formulae.[13]

Data

Data are expressed as mean $\pm$ SEM. The data were analyzed using the statistical package SigmaStat v2.0 (Jandel Scientific, San Rafael, CA). Differences in plasma FFA concentrations at the beginning compared with the end of the euglycemic clamp were compared with a paired *t* test. The relationship between insulin sensitivity (M value) and plasma FFA concentrations was evaluated with Pearson's product moment correlation (*r*). A Mann-Whitney test was performed when the data failed an Equal Variance test. Two-tailed *P* values for the *t* tests of less than .05 were considered significant.

Table 1. Demographics

Subjects	(M/F) 31/1
Age	30 $\pm$ 2 yr
Body mass index	23.5 $\pm$ 0.5 kg/m^2
Glucose	95 $\pm$ 2 mg/dL
Total cholesterol	174 $\pm$ 6 mg/dL
High-density lipoprotein	44 $\pm$ 2 mg/dL
Triglycerides	116 $\pm$ 11 mg/dL
Low-density lipoprotein-cholesterol	110 $\pm$ 6 mg/dL
FFA	0.26 $\pm$ 0.02 μmol/mL

RESULTS

Subject characteristics are presented in Table 1. The majority of the subjects were males ranging in age from 18 to 56 years.

The plasma concentrations of 7 fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3) were measured individually, and total fatty acid concentration represents the sum of these 7; saturated fatty acid is the sum of (C14:0 + C16:0 + C18:0), whereas unsaturated fatty acid is the sum of (C16:1 + C18:1 + C18:2 + C18:3) in this study. In general, these 7 fatty acids account for more than 90% of the plasma FFA in the postabsorptive state.

Figure 1A depicts the decline in total fatty acid concentration (thick solid line) during insulin infusion. Total FFA decreased from 0.26 $\pm$ 0.02 to 0.06 $\pm$ 0.01 μmol/mL. Also, the decline in saturated FFA (thin solid line) and unsaturated FFA (thin dashed line) is shown during the 180 minutes of insulin (and dextrose) infusion during euglycemic clamp conditions in Fig 1A. In Fig 1B, the relative concentrations of saturated FFA and unsaturated FFA at baseline and hourly intervals are shown in the pie charts. At 0 minutes (baseline) saturated FFA constituted 55% $\pm$ 2% of the total FFA, and at 180 minutes, saturated FFA constituted 82% $\pm$ 2% of the total FFA. As is evident following 60 minutes of insulin infusion and continuing throughout the entire 3 hours of insulin infusion, the percentage decline in unsaturated fatty acid was significantly greater than the decline in saturated fatty acid ($P < .001$ comparing proportions of saturated with unsaturated FFA at the end of study).

The changes in the glycerol Ra (not shown), a measure of whole body lipolysis, during the euglycemic clamp roughly paralleled the changes in total plasma FFA. The baseline glycerol Ra was 1.48 $\pm$ 0.56 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which declined to 0.75 $\pm$ 0.34 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at the end of insulin infusion. The absolute reduction in glycerol Ra (50%) was not as great as the reduction in total FFA (70%).

Fat oxidation roughly paralleled the changes in total plasma FFA. Indirect calorimetry estimates of fat and carbohydrate oxidation are shown in Fig 1C. Lipid oxidation declined from 0.8 $\pm$ 0.06 to 0.2 $\pm$ 0.06 $mg \cdot kg^{-1} \cdot min^{-1}$, while carbohydrate oxidation increased from 1.6 $\pm$ 0.1 to 3.4 $\pm$ 0.2 $mg \cdot kg^{-1} \cdot min^{-1}$.

For the whole group, the total glucose uptake (M value) during the euglycemic clamp demonstrated a significant correlation with the total FFA at baseline ($r = -.46$; $P < .01$) as shown in Fig 2. The subjects were subsequently divided into the more (n = 16; mean glucose uptake 10.3 $\pm$ 0.3 $mg \cdot kg^{-1} \cdot min^{-1}$) and less (n = 16; mean glucose uptake 6.3 $\pm$ 0.5 mg $\cdot$

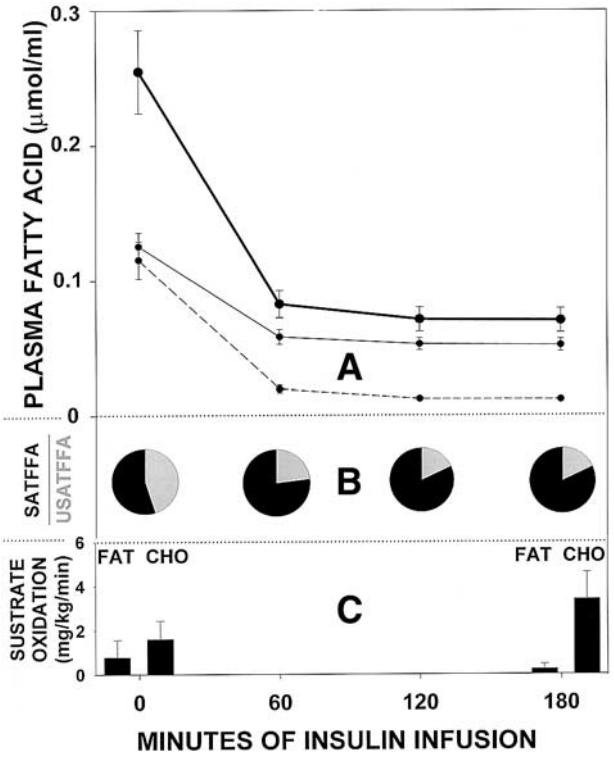

Fig 1. (A) Total (thick black line), saturated (thin sold black line), and unsaturated (thin dashed black line) FFA concentrations at baseline (0 minutes) and hourly throughout 3 hours (until 180 minutes) of insulin infusion during euglycemic clamp study (data are mean ± SEM). (B) Proportions of saturated (black) and unsaturated (grey) FFA in pie chart during each hour of euglycemic clamp and corresponding to same time points as FFA in (A). (C) Fat (FAT) and carbohydrate (CHO) oxidation at baseline (before insulin infusion) and at end of euglycemic clamp study (during the third hour of insulin infusion).

$kg^{-1} \cdot min^{-1}$) insulin-sensitive subjects by separating the entire group into 2 subgroups based on the M value. There were no significant differences in insulin suppression of total plasma FFA in the more compared with the less insulin-sensitive subjects, nor were there significant differences in the decrease in saturated compared with unsaturated FFA concentrations in the more compared with the less insulin-sensitive subjects.

DISCUSSION

These data show the expected suppression of lipolysis, total plasma FFA, and changes in fat and carbohydrate oxidation, which typically occur during a euglycemic insulin infusion. In addition, they show that whereas total plasma fatty acids decreased about 70% from baseline, the reduction in unsaturated fatty acids (decrease of 90%) was larger than the reduction in saturated fatty acids (decrease of 60%). Although both saturated and unsaturated FFA declined during insulin infusion, the 55:45 ratio of saturated:unsaturated FFA increased to a 77:23 ratio of saturated:unsaturated following 1 hour of insulin and dextrose infusion, increasing further to 82:18 during the last 2 hours of insulin infusion.

There is a small literature on the changes in plasma FFA profile following glucose load, the glucose load in general being given orally,[19,20] or IV push.[21] In the study of Nakamura et al,[20] 3 normal volunteers who received an oral glucose load of 100 g had a 40% decline in total FFA 1 hour later, with a reduction in saturated FFA of 22% and decline in unsaturated FFA of 54%, roughly similar to the changes in the current study, but with a small number of subjects studied and an oral route of glucose administration. In another human investigation by Yue et al,[19] the plasma FFA in both diabetic and nondiabetic subjects declined after an oral glucose tolerance load, with the largest changes in palmitate (the main saturated FFA) and oleate (the main unsaturated FFA). Our study differs from these investigations in that the administration of an oral or IV glucose load is typically accompanied by transient increases in both glucose and insulin, while in our study, the glucose and insulin levels were kept in a stable range for a period of 3 hours.

FFAs diminish during an insulin/dextrose infusion for at least 4 reasons: (1) suppressed release from cells (ie, decreased lipolysis, as confirmed by the decrease in Ra glycerol in the current study [and previous studies[22]], (2) inhibition of carrier-mediated transport across cell membranes,[23] (3) increased re-esterification relative to lipolytic rate within cells,[3] and (4) FFA removal by tissues.[24] The methods used in the current study were not designed to differentiate how these various insulin-mediated mechanisms could have differentially affected saturated versus unsaturated fatty acids, although the relatively greater suppression of plasma FFA compared with total lipolytic rates supports the mechanisms of increased intracellular re-esterification and decreased lipolysis as noted by others.[3]

The importance of fatty acids stem, in part, from their role in insulin sensitivity,[25] which, in turn, is thought to participate in the pathogenesis of atherosclerosis.[26] In this study, the plasma FFAs were higher in those with reduced insulin sensitivity as measured by euglycemic clamp methodology (Fig 2). How-

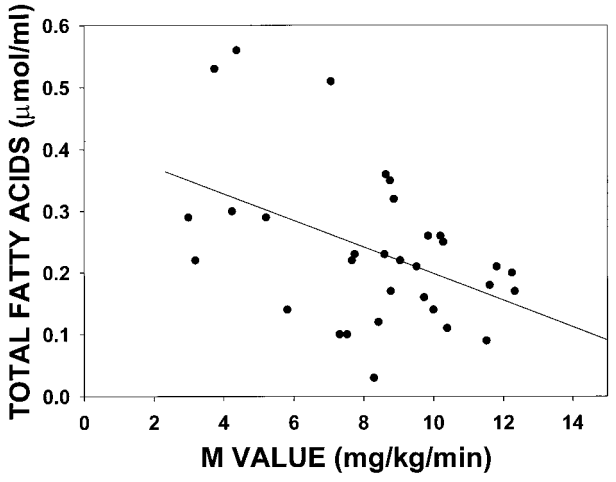

Fig 2. Plot of insulin sensitivity (M value each point representing the average glucose uptake during the last 30 minutes of the euglycemic clamp of each subject) *v* baseline total FFA concentration measured at the beginning (just prior to insulin infusion) of the euglycemic clamp. Solid line represents regression line, $r = -.46$; $P < .01$).

ever, the degree of suppression of both saturated and unsaturated FFA was the same when the insulin-resistant were compared with the insulin-sensitive subjects.

Although there were no significant differences in the degree of FFA suppression in the more insulin-resistant group compared with the more insulin-sensitive group, the remarkable findings in this study were the differential and sustained effects of the insulin infusion on the unsaturated FFA compared with the saturated FFA. This effect was apparent within an hour and maintained throughout the 3 hours of study. These findings, if extrapolated to increases in insulin concentration following ingestion of a meal, would suggest that subjects who have a more prolonged duration of insulin increase following food intake expose their tissues to relatively longer periods of saturated FFA excess. Thus, one possible mechanism whereby insulin resistance predisposes to atherogenesis could relate to a longer exposure of tissues to a relative excess in saturated compared with unsaturated FFA. Such a mechanism is supported by the finding that insulin-resistant subjects show a higher plasma insulin concentration and a more prolonged plasma insulin response after an oral glucose load.[27]

There are several limitations to this study. Individual FFA turnover was not directly measured, however, other studies have shown that the Ra of the main saturated FFA (palmitate) parallels that of total FFA.[3] Most of the study subjects were males (only 1 woman participated), thus these data do not readily generalize to women. The subjects were free of cardiovascular disease and were relatively young, thus the results may not be applicable to older subjects or those with clinical cardiovascular disease.

In summary, these data show a rapid and sustained reduction in plasma FFA during euglycemic clamp conditions that was paralleled by a similar (although smaller) decrease in lipolytic activity as measured by the glycerol Ra. The degree of saturated FFA reduction was less than the degree of unsaturated FFA reduction, and this was independent of the degree of insulin sensitivity present in subjects as measured by the M value on a euglycemic clamp study.

REFERENCES

1. Spector AA: Essentiality of fatty acids. Lipids 34:S1-S3, 1999 (suppl)
2. Haastrup AT, Stepniakowski KT, Goodfriend TL, et al: Intralipid enhances alpha1-adrenergic receptor mediated pressor sensitivity. Hypertension 32:693-698, 1998
3. Campbell PJ, Carlson MG, Hill JO, et al: Regulation of free fatty acid metabolism by insulin in humans: Role of lipolysis and reesterification. Am J Physiol 263:E1063-E1069, 1992
4. Arner P: Control of lipolysis and its relevance to development of obesity in man. Diabetes Metab Rev 4:507-515, 1988
5. Arner P, Kriegholm E, Engfeldt P, et al: Adrenergic regulation of lipolysis in situ at rest and during exercise. J Clin Invest 85:893-898, 1990
6. Nurjhan N, Campbell PJ, Kennedy FP, et al: Insulin dose-response characteristics for suppression of glycerol release and conversion to glucose in humans. Diabetes 35:1326-1331, 1986
7. Kooner JS, Baliga RR, Wilding J, et al: Abdominal obesity, impaired nonesterified fatty acid suppression, and insulin-mediated glucose disposal are early metabolic abnormalities in families with premature myocardial infarction. Arterioscler Thromb Vasc Biol 18: 1021-1026, 1998
8. Sniderman AD, Cianflone K, Arner P, et al: The adipocyte, fatty acid trapping, and atherogenesis. Arterioscler Thromb Vasc Biol 18: 147-151, 1998
9. Townsend RR, DiPette DJ: Pressor doses of angiotensin-II increase insulin-mediated glucose uptake in normotensive man. Am J Physiol 265:E362-E366, 1993
10. Townsend RR: The effects of angiotensin-II on lipolysis in humans. Metabolism 50:468-472, 2001
11. Townsend RR, DiPette DJ, Lieux TR, et al: The role of the renin-angiotensin system in insulin sensitivity in normotensive subjects. Am J Med Sci 305:67-71, 1993
12. Townsend RR, Zhao H: Plasma renin activity and insulin sensitivity in normotensive subjects. Am J Hypertens 7:894-898, 1994
13. Jéquier E, Felber J-P: Indirect calorimetry. Baillieres Clin Endocrinol Metab 1:911-935, 1987
14. McGuire EAH, Helderman JH, Tobin JD, et al: Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 41:565-573, 1976
15. Wolfe RR: Determination of isotopic enrichment by gas chromatography-mass spectroscopy, in Wolfe RR (ed): Radioactive and Stable Isotope Tracers in Biomedicine, vol 1. New York, NY, Wiley-Liss, 1992, pp 49-86
16. Steele R, Wall J, DeBodo R, et al: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. Am J Physiol 187:15-24, 1956
17. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 82:420-430, 1959
18. McDonald-Gibson RG, Young M: The use of an automatic solids injection system for quantitative determination of plasma long chain non-esterified fatty acids by gas-liquid chromatography. Clin Chim Acta 53:117-126, 1974
19. Yue KT, Davis JW, Phillips PE, et al: Effect of glucose on plasma concentrations of individual non-esterified fatty acids of non-diabetic and insulin-independent diabetic men. Postgrad Med J 57:622-626, 1981
20. Nakamura H, Faludi G, Spitzer JJ: Changes of individual free fatty acids during glucose tolerance test. Diabetes 16:175-180, 1967
21. Cunnane SC, Jenkins DJ, Wolever TM: Response of individual serum free fatty acids to oral and intravenous glucose: Relation to adipose tissue composition. Nutrition 9:423-429, 1993
22. Hales CN, Luzio JP, Siddle K: Hormonal control of adipose-tissue lipolysis. Biochem Soc Symp 43:97-135, 1978
23. Abumrad NA, Perry PR, Whitesell RR: Insulin antagonizes epinephrine activation of the membrane transport of fatty acids. J Biol Chem 261:2999-3001, 1986
24. 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
25. Manco M, Mingrone G, Greco AV, et al: Insulin resistance directly correlates with increased saturated fatty acids in skeletal muscle triglycerides. Metabolism 49:220-224, 2000
26. Folsom AR, Rasmussen ML, Chambless LE, et al: Prospective associations of fasting insulin, body fat distribution, and diabetes with risk of ischemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Diabetes Care 22:1077-1083, 1999
27. Swislocki A, Hoffman B, Reaven G: Insulin resistance, glucose intolerance and hyperinsulinemia in patients with hypertension. Am J Hypertens 2:419-423, 1989