Effects of Fat on Insulin-stimulated Carbohydrate Metabolism in Normal Men

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Abstract

We have examined the onset and duration of the inhibitory effect of an intravenous infusion of lipid/heparin on total body carbohydrate and fat oxidation (by indirect calorimetry) and on glucose disappearance (with 6,6 D_2-glucose and gas chromatography-mass spectrometry) in healthy men during euglycemic hyperinsulinemia. Glycogen synthase activity and concentrations of acetyl-CoA, free CoA-SH, citrate, and glucose-6-phosphate were measured in muscle biopsies obtained before and after insulin/lipid and insulin/saline infusions. Lipid increased insulin-inhibited fat oxidation (+40%) and decreased insulin-stimulated carbohydrate oxidation (−63%) within 1 h. These changes were associated with an increase (+489%) in the muscle acetyl-CoA/free CoA-SH ratio. Glucose disappearance did not decrease until 2–4 h later (−55%). This decrease was associated with a decrease in muscle glycogen synthase fractional velocity (−82%). The muscle content of citrate and glucose-6-phosphate did not change. We concluded that, during hyperinsulinemia, lipid promptly replaced carbohydrate as fuel for oxidation in muscle and hours later inhibited glucose uptake, presumably by interfering with muscle glycogen formation. (*J. Clin. Invest.* 1991. 88:960–966.) Key words: acetyl-coenzyme • carbohydrate oxidation • carbohydrate storage • citrate • muscle glycogen synthase

Introduction

More than 25 years ago Randle et al. (1) demonstrated that increased availability of fatty acids enhanced fat oxidation (FAT OX)[1] and decreased carbohydrate oxidation (CHO OX) and glucose uptake in perfused rat heart and to a lesser extent in rat diaphragm. Based on these findings, they proposed a glucose–fatty acid cycle presumed to be of fundamental importance for the control of blood glucose and free fatty acid (FFA) concentrations and insulin sensitivity (1). Inasmuch as FFA were known to be frequently elevated in obesity and non–insulin-dependent diabetes mellitus (NIDDM), they also postulated that increased FAT OX contributed to the impaired glucose tolerance commonly associated with these conditions (2, 3). Subsequently, many (4–7), but not all (8), investigators were unable to reproduce the fatty acid–mediated inhibition of glucose uptake in rat diaphragm and striated muscle that Randle et al. had observed in perfused rat hearts. (Effects of fatty acids on CHO OX were not examined in these studies.) More recently, several groups (9–17) have examined glucose–fatty acid interactions in vivo during hyperinsulinemia, when most of the glucose uptake occurs in muscle (18). Practically all found that raising FFA increased FAT OX and decreased CHO OX. Some also found inhibitory effects of lipid on glucose uptake (11, 16) but most did not (12, 14, 15, 17). Thus, although there seems to be growing acceptance of the idea that increasing FAT OX will reduce CHO OX, at least in vivo and under hyperinsulinemic conditions, there remains much controversy as to whether fatty acids also inhibit glucose uptake. Contributing to this uncertainty is the fact that none of the critical enzyme and substrate changes predicted by the glucose–fatty acid cycle to occur in response to increased FAT OX have been demonstrated in human muscle. Furthermore, it is not known whether the time of onset and the duration of the fatty acid–induced alterations are the same or are different for CHO OX and glucose uptake. Noteworthy in this respect are recent findings by Bonnadonna et al. (15), who suggested that the fatty acid effects depended on the time of the fat infusion.

In the present study we have therefore investigated the onset and duration of the inhibitory effect of intravascular lipolysis, produced by intravenous infusion of triglycerides and heparin, on total body CHO OX and glucose disappearance rates (G_{Rd}) in normal men during euglycemic hyperinsulinemia. In addition, we have obtained muscle biopsies from these individuals before and after lipid and saline infusions for measurement of glycogen synthase (GS) activity and of several important intermediates of fat and CHO metabolism.

Methods

Subjects

18 healthy, normal weight men were studied. Their ages, weights, and heights are shown in Table I. None of the subjects had a family history of diabetes or other endocrine disorders and none were taking any medications. Their weights were stable for at least 2 mo and their diets contained a minimum of 250 g/day of carbohydrates for at least 2 d before the men were studied. Informed written consent was obtained from all after explanation of the nature, purpose, and potential risks of each study. The study protocol was approved by the Institutional Review Board for Human Research of Temple University. Studies were performed in the General Clinical Research Center of Temple University Hospital and began between 8 and 9 a.m. after an overnight fast. The subjects were studied reclining in bed. A short polyethylene catheter was inserted into an antecubital vein for infusion of all test sub-

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Table I. Study Subjects

Study	Age	Weight	Height
	yr	kg	cm
1 (n = 6)	26.0±1.2	79.4±4.4	180.3±3.1
2 (n = 6)	25.2±1.5	82.6±5.0	181.8±2.7
3 (n = 6)	26.2±1.8	82.5±3.0	181.5±3.1

stances. Another catheter was placed into a forearm vein of the same arm to supply the Biostator (Ames Lifescience Instruments, Elkhart, IN) with a continuous flow (2 ml/h) of blood. A third catheter was inserted into a contralateral forearm vein for blood sampling. This arm was kept at 70°C with a heating blanket to arterialize venous blood.

Experimental design

Study 1. Six subjects were studied. 6,6 D_2-glucose (Tracer Technologies, Somerville, MA) was infused i.v. for 7 h (−60 to 360 min) starting with a bolus of 30 μmol/kg followed by a continuous infusion of 0.3 μmol/kg·min. At 0 min, LIPOSYN II (Abbott Laboratories, North Chicago, IL), a 20% triglyceride emulsion (10% safflower, 10% soy bean oil, and 2.14 g of glycerol per 100 ml) plus heparin (0.4 U/kg min) were infused at a rate of 1.5 ml/min for 6.5 h. Regular human insulin (Humulin R, Eli Lilly & Co., Indianapolis, IN) was infused i.v. at a rate of 1 mU/kg min for 6.5 h starting at 0 min. Glucose concentrations were clamped at ∼ 85 mg/dl by a feedback-controlled glucose infusion (Biostator). The first muscle biopsy was performed before the start of the infusions (between −90 and −60 min); a second biopsy was obtained at the end of the studies (between 360 and 390 min).

Study 2. Six subjects were studied. The protocol for study 2 was identical to that of study 1 except that saline (1.5 ml/min for 6.5 h) was infused instead of lipid/heparin.

Study 3. Six subjects were studied. Infusions of 6,6 D_2-glucose and insulin were as in study 1. Lipid/heparin was infused for 2 h starting at 0 min at a rate of 1.5 ml/min. At 120 min the lipid/heparin infusion was discontinued and saline was infused for another 4 h.

Glucose turnover

Glucose turnover was determined with 6,6 D_2-glucose. Plasma glucose was isolated from blood drawn at 30-min intervals for determination of isotope enrichment as described (19) with a gas chromatograph-mass spectrometer (model 4610-B, Finnigan-MAT, San Jose, CA). The penta-acetyl derivative of glucose was measured by the electron impact mode at 70 eV. Ions were measured at m/e 242 and 244, respectively. Rates of glucose appearance (G_{Ra}) and disappearance (G_{Rd}) were calculated from the isotope enrichment for 30-min intervals using Steele's equation for non–steady-state conditions (20). In all three studies, G_{Ra} during hyperinsulinemia were frequently lower than glucose infusion rates (GIR) resulting in negative values for hepatic glucose production. This problem has recently been attributed to errors in Steele's non-steady-state equation (21). We have assumed that endogenous glucose production was completely suppressed whenever the isotopically determined G_{Ra} was equal or smaller than GIR.

Rates of CHO storage (CHO STOR) were obtained by subtracting rates of CHO OX (see below) from G_{Rd}.

Indirect calorimetry

Respiratory gas exchange rates were determined as previously described before and at 30-min intervals during lipid/heparin or saline infusions with a metabolic measurement cart (Beckman Instruments, Inc., Palo Alto, CA) (22). Rates of protein oxidation were estimated from urinary nitrogen excretion after correction for changes in urea nitrogen pool size (23). Rates of protein oxidation were used to determine the non–protein respiratory quotient (npRQ). It was assumed that for each gram of N excreted in the urine, 6.02 liters of O_2 were

consumed and 4.75 liters of CO_2 were produced (RQ = 0.79). Rates of CHO OX and FAT OX were determined with the npRQ tables of Lusk, which are based on an npRQ of 0.707 for 100% FAT OX and 1.00 for 100% CHO OX.

Muscle biopsies and extractions

Biopsies were obtained from the lateral aspect of the vastus lateralis muscle ∼ 15 cm above the patella from six of six subjects in study 1 and from four of six subjects in study 2. The skin was cleaned with betadine and anesthetized with 1% lidocaine without epinephrine in a field block pattern (at 2 × 3 in.). (We have found that injection of lidocaine too close to the biopsy site interfered with the measurement of acetyl-CoA.) After an incision (∼ 1 in.) was made through the skin, subcutaneous tissue, and fascia, ∼ 150 mg of muscle was mobilized and excised. The muscle was dropped immediately into isopentane, kept at its freezing point (−160°C) by liquid nitrogen. The frozen muscle was stored at −70° until it was aliquoted into three separate portions. One portion was extracted with fluoride buffer according to Hagg et al. (24) for measurement of glycogen synthase. A second portion was extracted with perchloric acid according to Allred and Guy (25) for measurement of glucose-6 phosphate (G-6-P) and citrate. The third portion was extracted with methanol-TCA for measurement of acetyl-CoA and free CoA-SH.

GS assay

GS was assayed by a modification of the method of Thomas et al. (26). Reactions were started by addition of 30-μl aliquots of the muscle extract to 60 μl of a reaction mixture containing 20 mM EDTA, 25 mM sodium fluoride, 50 mM Tris-HCl, 1% glycogen, 0.7 μCi [U-^{14}C]UDP glucose, 0.3 mM UDP glucose, and 0–10 mM G-6-P. The reaction was terminated after 15 min by precipitating 75-μl aliquots of the reaction mixture on 2 × 2-cm squares of filter paper which were dropped into cold 66% ethanol, washed, dried, and counted. GS activity was calculated as micromoles of UDP glucose incorporated into glycogen per minute per milligram of protein. Results are expressed as the fractional velocity of GS activity, i.e., the activity of GS at 0.1 mM G-6-P divided by the activity at 10 mM G-6-P. This is an indicator of the active form of GS and believed to be a sensitive parameter of in vivo GS activity (27).

Metabolite assays

HPLC analysis of Acetyl-CoA and free CoA-SH. Frozen muscle samples were minced in 200–400 μl of methanol-TCA (10%) at −20°C. After 5 min at −20°C, 10 volumes of ice cold 10% TCA in H_2O was added to the sample, followed by sonication in ice for 1 min. The supernatant of the TCA extract was washed five times with ether, which raised the pH to ∼ 5.0 (28). The acid-free extract was then lyophilized and stored at −20°C until analysis. HPLC analysis was performed as described (29) using a Waters Associates (Milford, MA) Nova-Pak C18 5-μm reverse-phase column. The two mobile-phase solvents were 0.1 M KH_2PO_4 (A) and 0.1 M KH_2PO_4 containing 40% acetonitrile (B), both at pH 5.0. Acetyl-CoA and free CoA-SH were determined at 260 nm.

Citrate was assayed in a coupled end-point assay with citrate lyase and malate dehydrogenase (30). G-6-P was assayed in a coupled end-point assay with hexokinase and G-6-P dehydrogenase (31).

Analytical procedures

Plasma glucose was measured with a glucose analyzer (Beckman Instruments, Inc., Palo Alto, CA). Serum insulin (32) was determined by radioimmunoassay. Blood urea nitrogen (33), was measured colorimetrically. Urinary nitrogen was measured by the method of Kjeldahl (34). Lactate (35) and pyruvate (36) were measured enzymatically.

Statistical analysis and calculations

All data were expressed as the mean±SEM. Statistical significance was assessed using MANOVA and Student's two-tailed paired or unpaired t test.

Results

Insulin, glucose, and GIR (Fig. 1)

In all three studies, serum insulin was raised about eightfold from 7.5±1.0 to a mean of 58±5 μU/ml by infusion of insulin (1 mU/kg·min). Plasma glucose was clamped at 88±2 mg/dl (CV 11.2%). For the first 3 h of insulin infusion, GIR were comparable during insulin/saline or insulin/lipid infusions. The inhibitory effect of lipid became statistically significant at 210 min when GIR had risen to 6.3±0.4 mg/kg·min with saline, while remaining at 3.7±0.5 mg/kg·min with lipid ($P < 0.05$). When lipid was replaced by saline at 120 min in study 3, 3 h elapsed before the inhibitory effect of lipid disappeared and GIR increased significantly.
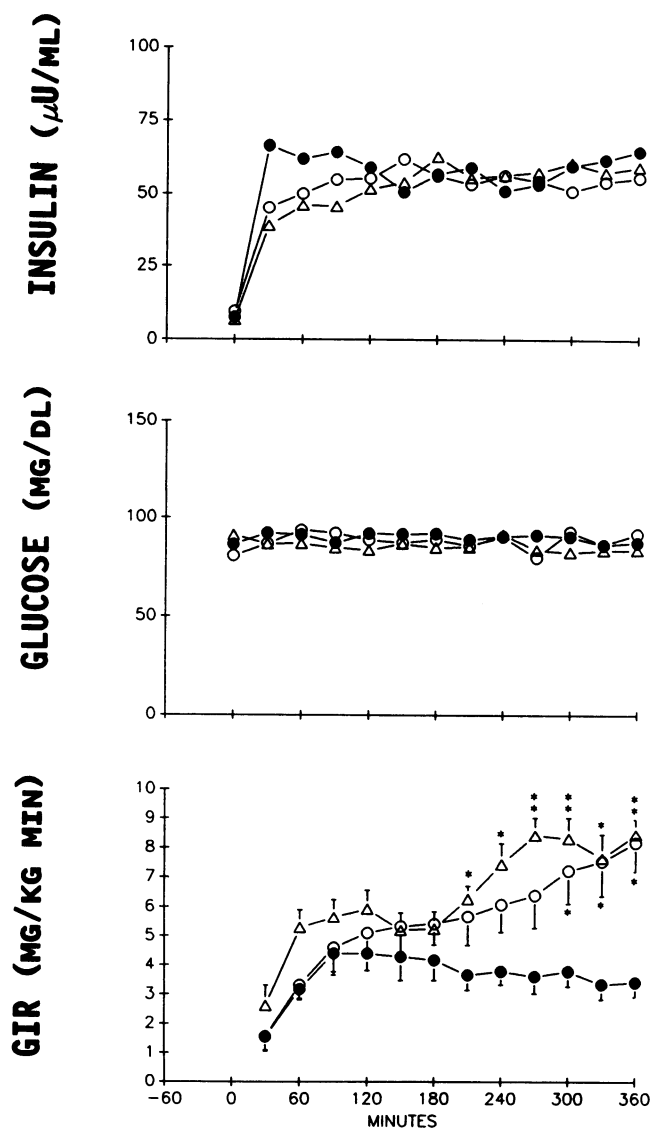

Figure 1. Peripheral venous insulin and glucose concentrations and (GIR) in healthy men during study 1 (lipid infusion during euglycemic hyperinsulinemia, •, $n = 6$), study 2 (saline infusion during euglycemic hyperinsulinemia, △, $n = 6$), and study 3 (euglycemic hyperinsulinemia plus lipid from 0 to 2 h and saline from 2 to 4 h, ○, $n = 6$). *$P < 0.05$, **$P < 0.01$ comparing studies 2 with 1 and 3 with 1.
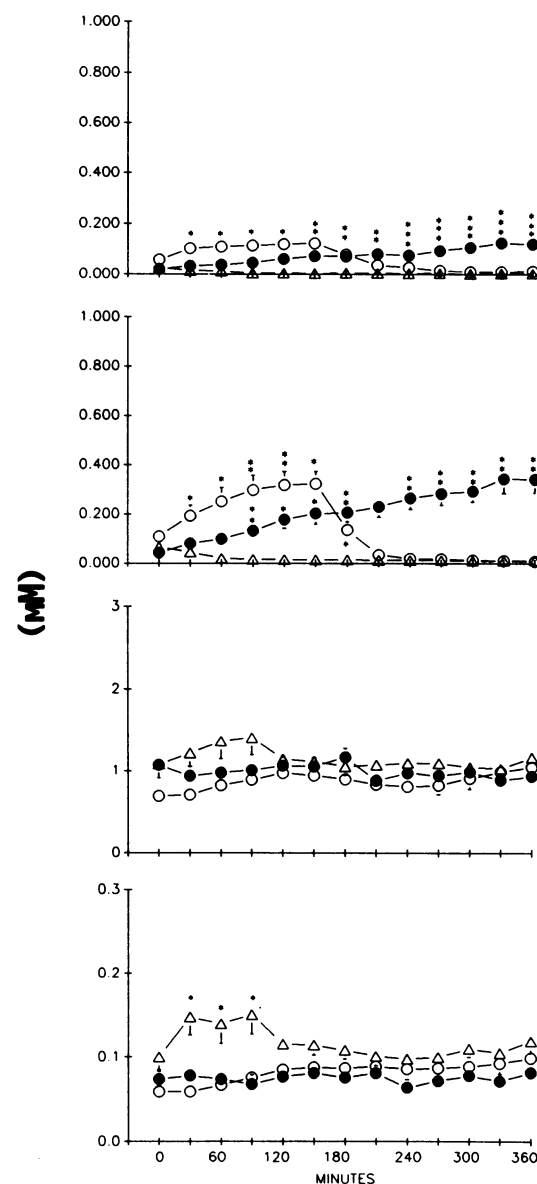

Figure 2. Acetoacetate, β-OH-butyrate, lactate, and pyruvate concentrations in studies 1–3. In study 1, lipid/heparin was infused from 0 to 360 min. In study 2, saline was infused from 0 to 360 min. In study 3, lipid/heparin was infused from 0 to 120 min and saline from 120 to 360 min. Symbols and numbers of experiments as in Fig. 1. All studies were performed under euglycemic hyperinsulinemic conditions. Mean±SE. *$P < 0.05$, **$P < 0.05$, ***$P < 0.005$ comparing studies 1 with 2 and 3 with 2.

Ketone bodies, lactate, and pyruvate (Fig. 2)

Insulin/lipid infusion in study 1 resulted in small increases in plasma concentrations of acetoacetate (from 0.02 to 0.12 mM, $P < 0.001$) and β-hydroxybutyrate (from 0.04 to 0.34 mM, $P < 0.001$). The accumulation of ketone bodies observed during studies 1 and 3 were, however, too small to affect the calculated rates of CHO OX and FAT OX. After lipid was replaced by saline at 120 min in study 3, both ketone bodies returned to basal levels within 1 h. Insulin/saline or insulin/lipid infusions had no effect on plasma lactate and lactate/pyruvate ratios (not

shown). Pyruvate concentrations were slightly higher during insulin/saline as compared to insulin/lipid infusions between 30 and 90 min ($P < 0.05$).

Onset and duration of effect of lipid on FAT OX, and CHO OX (Fig. 3)

Panel 1. During insulin/lipid infusion, FAT OX rose from 0.63 ± 0.13 mg/kg·min at 0 min to 0.91 ± 0.1 mg/kg·min at 60 min ($P < 0.05$) and continued to rise to 1.36 ± 0.12 mg/kg·min at 360 min. During insulin/saline infusion, FAT OX decreased from 0.70 ± 0.28 mg/kg·min at 0 min to 0.40 ± 0.23 mg/kg·min at 360 min. After lipid was replaced by saline in study 3, FAT OX fell significantly within 30 min from 1.3 ± 0.18 (at 120 min) to 0.93 ± 0.08 mg/kg·min (at 150 min) ($P < 0.05$) and then continued to fall to reach 0.39 ± 0.14 mg/kg·min at 360 min.

Panel 2. During insulin/saline CHO OX increased from 0.85 ± 0.5 (0 min) to 1.93 ± 0.14 mg/kg·min 30 min later and then continued to rise to reach 3.15 ± 0.33 mg/kg·min at 360 min. During insulin/lipid infusion CHO OX decreased slowly

from 1.6 ± 0.33 (0 min) to 0.71 ± 0.06 mg/kg·min (360 min). After lipid was replaced by saline in study 3, CHO OX increased within 60 min to values indistinguishable from those seen during insulin/saline infusions.

Panel 3. To better illustrate these directional changes, CHO OX was normalized with basal values (0 min) being set at 100%. As can be seen, the difference between insulin/lipid and insulin/saline infusions became statistically significant at 30 min.

Onset and duration of lipid on glucose uptake (G_{Rd}) (Fig. 4)

In contrast to its early effect on CHO OX, insulin/lipid infusion had no effect on G_{Rd} or G_{Ra} for at least 3 h. After 3 h, G_{Rd} and G_{Rd} plateaued at $\sim$ 4 mg/kg·min during insulin/lipid, whereas it continued to rise to between 7 and 8 mg/kg·min during insulin/saline infusion. The difference between insulin/lipid and insulin/saline infusions, however, did not become statistically significant until 330 min. Similarly, after lipid was replaced by saline in study 3, G_{Rd} and G_{Rd} remained inhibited for 3 h before rising.

Correlations between G_{Rd}, FAT OX, and CHO OX

G_{Rd} did not correlate with FAT OX ($r = -0.19$, $P < 0.04$) and correlated only weakly with CHO OX ($r = 41$, $P < 0.0001$).

GS, acetyl-CoA, free CoA-SH, G-6-P, and citrate in muscle biopsies (Fig. 5)

GS activity, expressed as fractional velocity, i.e., GS activity at a subsaturating (0.1 mM) G-6-P concentration divided by GS
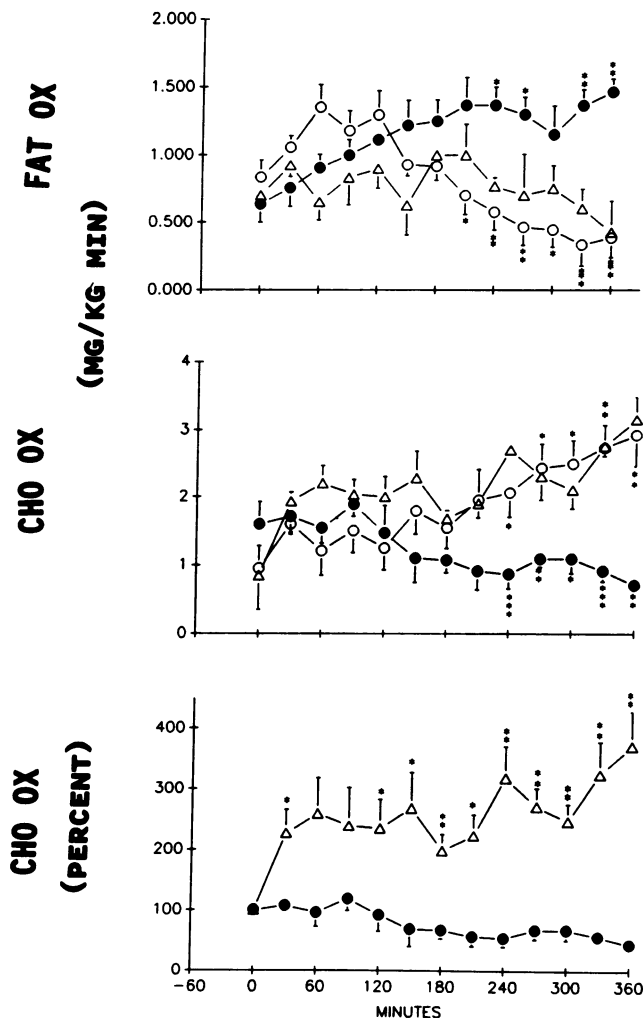

Figure 3. Rates of FAT OX and CHO OX in healthy men during lipid or saline infusions. Symbols and number of experiments were as in Fig. 1. In the bottom panel, CHO OX was normalized by setting 0 min values as 100%. Shown are mean±SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ comparing studies 1 with 2 and 3 with 1.
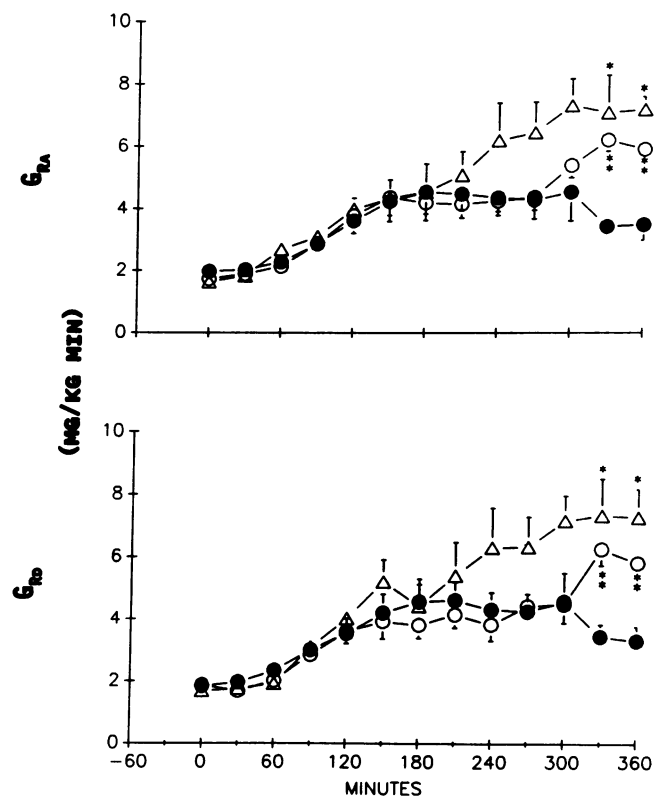

Figure 4. G_{Ra} and G_{Rd} in healthy men during lipid or saline infusions. Symbols and number of experiments were as in Fig. 1. Shown are mean±SE. *$P < 0.05$, **$P < 0.01$ comparing studies 2 with 1 and 3 with 1.
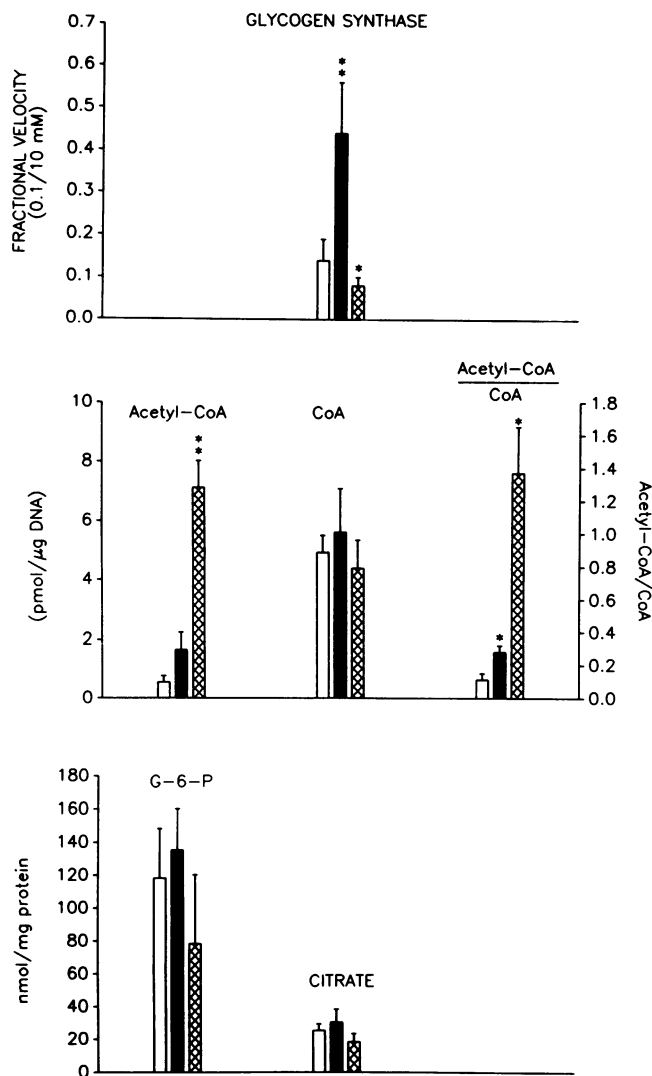
Figure 5. Upper panel: glycogen synthase activity in muscle biopsies expressed as fractional velocity (activity at 0.1 mM G-6-P divided by activity at 10 mM G-6-P) before infusions (*open bars, n* = 10), after 6 h of euglycemic hyperinsulinemia (*solid bars, n* = 4) and after 6 h of lipid plus euglycemic hyperinsulinemia (*cross-hatched bars, n* = 6). *Middle panel:* acetyl-CoA and free CoA-SH and the acetyl-CoA/free CoA-SH ratio in muscle biopsies. Symbols and number of experiments as in the upper panel. *Lower panel:* G-6-P and citrate concentrations in muscle biopsies. Mean±SE. *$P < 0.05$, **$P < 0.005$ comparison with the preceding column.

activity at a saturating (10 mM) concentration of G-6-P, increased from 0.14±0.05 before to 0.44±0.12 after insulin/saline infusion (study 2) ($P < 0.02$). After insulin/lipid infusion (study 1) GS fractional velocity was 0.08±0.02; i.e., it failed to rise in response to insulin. GS fractional velocity correlated positively with G_{Rd} ($r = 0.61$) (Fig. 6).

Acetyl-CoA concentration was 1.65±0.6 pmol/μg DNA after insulin/saline and 7.12±0.90 pmol/μg DNA after insulin/lipid infusion (+432%, $P < 0.03$) whereas the acetyl-CoA/free CoA-SH ratio was 0.28±0.04 during insulin/saline and 1.37±0.28 during insulin/lipid (+489%, $P < 0.03$).

Insulin/saline or insulin/lipid infusions had no significant effects on G-6-P or on citrate concentrations.
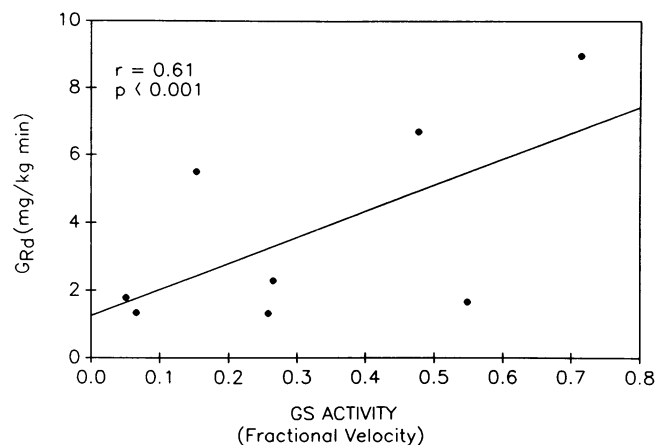

Figure 6. Correlation between GS activity (fractional velocity) and G_{Rd}. Data from studies 1 and 2.

Discussion

Effect of lipid on FAT OX and CHO OX. Lipid infusion was followed within 60 min by an increase in FAT OX and by a decrease in insulin-stimulated total body CHO OX (study 1). This effect was reversed, also within 1 h, after FAT OX had declined after replacement of insulin/lipid infusion by insulin/saline (study 3).

These observations confirm and expand reports by others which, while not specifically investigating time/effect relationships, did show that fat infusion increased total body FAT OX and decreased insulin-stimulated CHO OX within the usual 2-h studies (10–17). Thus, there is convincing evidence demonstrating that elevation FAT OX leads to a prompt decrease in insulin-stimulated CHO OX in vivo. These findings are compatible with that part of Randle's glucose–fatty acid cycle hypothesis which postulated that rising plasma FFA concentrations increased FAT OX and inhibited CHO OX in striated muscle via a rise in the mitochondrial acetyl-CoA/CoA ratio and inhibition of pyruvate dehydrogenase. In further support of this hypothesis, we have demonstrated in this study that lipid infusions produced large increments in acetyl-CoA (+432%) and in the acetyl-CoA/free CoA-SH ratio (+489%) in human skeletal muscle.

Effect of lipid on G_{Rd}. Randle's hypothesis, however, also postulated that increased FAT OX caused muscle citrate and G-6-P concentrations to rise (the latter by inhibition of phosphofructokinase 1) and glucose uptake to fall (by inhibition of hexokinase). In this study, we found that lipid induced increments in FAT OX did neither increase citrate nor G-6-P concentrations in muscle and did not reduce G_{Rd} (G_{Rd} is equivalent with glucose uptake) for at least 3 h. On the other hand, once established, the inhibition of G_{Rd} lasted for several hours even after FAT OX had fallen (in study 3). It must be pointed out, however, that the validity of our G_{Rd} values depended on the accuracy of the glucose turnover measurements. As mentioned (see Methods) the stable isotope method used underestimated G_{Ra} (and thus G_{Rd}) during hyperinsulinemia. The error, however, appeared to be relatively minor (negative values for hepatic glucose output ranged from 0.5 to 1.0 mg/kg·min). Moreover, the error was similar in all three studies and thus did not invalidate our observation that lipid inhibited CHO OX

first and G_{Rd} hours later. The long delay between the rise in FAT OX and the inhibition of G_{Rd}, the lack of correlation between FAT OX and G_{Rd} and the lack of increases in muscle citrate and G-6-P concentrations cannot easily be explained by the Randle hypothesis and suggest that the effects of fat on rat heart muscle are different from those on human striated muscle. Others have arrived at similar conclusions. Lillioja et al. (37), studying lipid turnover in nondiabetic Pima Indian women during hyperinsulinemic clamps, concluded that the Randle cycle was probably operative for the regulation of CHO OX but not for the regulation of the nonoxidative component of G_{Rd}. Yki-Yarvinnen et al. (38) found that the rate-limiting step for G_{Rd} during hyperinsulinemia appeared to be beyond the glucose transport step.

What caused the late decrease in G_{Rd}? We found that G_{Rd} correlated poorly with FAT OX and CHO OX but closely with GS activity. GS activity is generally considered to be the rate-limiting step in glycogen synthesis (39), which has recently been demonstrated to account for nearly all of CHO storage during euglycemic hyperinsulinemia (40). It appeared, therefore, that G_{Rd} declined eventually because of a problem with glycogen synthesis, which developed after several hours of insulin/lipid infusion.

The mechanism by which lipid inhibited GS activity was not explored. It is, however, known that as muscle glycogen stores fill up, GS activity decreases, at least partially owing to a decrease in the active form of GS (41–43). This may have occurred in our study where a marked fall in the active form of GS was seen after several hours of lipid infusion. Bjorntorp et al. (44) have estimated on the basis of forearm glucose uptake studies that at rest, when no utilization of glycogen has occurred, the uptake of glucose into human muscle was limited to ~ 20 g/d. We have calculated that after 5½ h of insulin/lipid infusion, i.e., at the time when G_{Rd} started to decrease sigificantly, ~ 23.4 g of glucose had been prevented from being oxidized and presumably had been shunted for the most part into muscle glycogen formation. We would, therefore, like to propose the following hypothesis: during the initial 3–4 h of insulin/lipid infusion, when G_{Rd} was normal but CHO OX was diminished, the glucose which entered the muscle, but could not be oxidized, was stored as glycogen. At least, we found no evidence that glucose was shunted in any substantial amount into other forms of nonoxidative glucose disposal such as lactate production or lipid synthesis (plasma lactate levels, presumably reflecting lactate production, did not change and npRQ values never exceeded 1.0). Blood lactate levels, however, may not accurately reflect lactate turnover and the possibility cannot be ruled out that there was a small accumulation of lactate in muscle or that some lactate was used for gluconeogenesis. After 4–5 h, muscle storage capacity was saturated and G_{Rd} had to decrease since all major pathways of glucose utilization were now blocked. There are, however, alternative pathways by which lipid could have affected G_{Rd}. For instance, long chain acyl-CoA was likely to increase in muscle and may have inhibited GS activity. Palmityl-CoA has been shown to dissociate active tetrameric liver glycogen synthase into monomers which were unable to bind to the primer glycogen (45). Furthermore, we cannot rule out the possibility that lipids may have inhibited glucose transport directly. Hissin et al. have shown that feeding of high fat/low CHO diets for 21 days to rats reduced the number of glucose transporters in adipocytes plasma membranes and decreased glucose transport (46). Clearly, fur-

ther studies are needed to determine which of these mechanisms was operative.

The delayed inhibitory effect of lipid infusion on G_{Rd} may explain why the in vitro studies (4–7), which were short, lasting only from 45 to 150 min, failed to show lipid effects on glucose uptake. In addition, it may help to reconcile several contradictory in vivo reports. For instance, several groups have failed to detect effects of lipid infusions on glucose uptake during hyperinsulinemic clamps (12, 14, 15, 17), while others have found only marginal (−15%) inhibition (10). All these studies lasted for only 2 h. According to our data, 2 h is not sufficient for the inhibitory effect of FAT OX on G_{Rd} to develop. By comparison, groups who infused lipid for 3–4 h did observe inhibition of glucose uptake (11, 16). Bonnadonna et al. (15), in the only other study on the time dependency of lipid and glucose interaction, reported that lipid infusion, when started simultaneously with the insulin clamp, resulted in inhibition of insulin-mediated glucose disposal after 3–4 h. In contrast, when the lipid infusion was started 2 h after commencement of the insulin clamp and continued for another 2 h, there was no inhibition of glucose disposal. They interpreted their findings as indicating that 2 h of hyperinsulinemia had rendered the body relatively refractory to the action of lipid on glucose disposal. Our findings suggested that lipids infused for 4 h allowed sufficient time for the inhibition of glucose disposal to develop whereas lipid infused for only 2 h did not.

Clinical relevance. Patients with NIDDM commonly have long histories of excessive caloric intake (frequently in the form of fat), lack of physical exercise, increased plasma FFA concentrations, and increased FAT OX. Impaired CHO STOR has been recognized as one of the earliest problems (47–49). As shown in this and other studies, many of these abnormalities including impaired glycogen synthesis can be produced by fat infusion in healthy individuals. It is, therefore, likely that an excess of FFA may contribute to the insulin resistance associated with NIDDM in susceptible individuals. Of particular interest, in this respect, is a recent report by Pascoe and Storlien (50), describing development of fasting hyperglycemia in rats with mildly compromised β-cell function after being fed a high fat diet for only 1 wk.

In summary, we have shown in normal men that insulin/lipid infusion caused prompt inhibition of CHO OX which was followed after several hours by a fall in G_{Rd}. This fall in G_{Rd} was associated with a drastic decrease in muscle GS activity. On the basis of these observations we concluded that lipid reduced G_{Rd} by interfering with glycogen synthesis. Putative mechanism included inhibition of GS by saturation of glycogen storage or by long chain acyl-CoA.

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