Effects of Nonesterified Fatty Acid Availability on Tissue-Specific Glucose Utilization in Rats In Vivo

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Abstract

The pathophysiological significance of the glucose-fatty acid cycle in skeletal muscle in vivo is uncertain. We have examined the short term effects of increased availability of nonesterified FFA on tissue-specific glucose uptake and storage in rat tissues in vivo basally and during a hyperinsulinemic (150 mU/liter) euglycemic clamp. Circulating FFA were elevated to 2 mmol/liter (FFA 1) or 4 mmol/liter (FFA 2). Elevated FFA produced a dose-dependent inhibition of myocardial glucose utilization in both basal (FFA1, 42%; FFA2, 68%; $P < 0.001$, by analysis of variance) and clamp groups (FFA1, 39%; FFA2, 49%; $P < 0.001$) and also suppressed brown adipose tissue glucose utilization during the clamp (−42%, $P < 0.001$). In contrast to heart, glucose utilization in skeletal muscle was suppressed by FFA only in the FFA1 basal group (−36%, $P < 0.001$); in other groups (e.g., FFA2 clamp) elevated FFA produced increased skeletal muscle glucose utilization (+68%, $P < 0.001$) that was directed toward glycogen (+175%, $P < 0.05$) and lipid deposition (+125%, $P < 0.005$). FFA stimulated basal glucose utilization in white (e.g., FFA2, +220%, $P < 0.005$) and brown adipose tissue (e.g., FFA2, +200%, $P < 0.005$). Thus elevated FFA can acutely inhibit glucose utilization in skeletal muscle in addition to cardiac muscle in vivo supporting a possible role for the glucose-fatty acid cycle in skeletal muscle in acute insulin resistance. However, at high levels or with elevated insulin, FFA stimulates glucose utilization and storage in skeletal muscle. By promoting accumulation of glucose storage products, chronic elevation of FFA may lead to skeletal muscle (and therefore whole body) insulin resistance.

Introduction

An inhibitory effect of fatty acid oxidation on glucose oxidation in heart and skeletal muscle was first proposed more than 20 yr ago as one component of the 'glucose–fatty acid cycle' of Randle et al. (1). In that report and others (2, 3), the potential importance of this cycle to the acute manifestation of insulin resistance in hyperlipidemic states such as obesity and diabetes has been emphasized.

The importance of the cycle to the physiology and pathology of fuel homeostasis is dependent on details of its operation in skeletal muscle since this tissue accounts for the largest part of glucose disposal by insulin-sensitive tissues in vivo (4, 5). In contrast to heart, where there is no doubt that the cycle operates in vitro (1, 6) and in vivo (7) under physiological conditions, the evidence in skeletal muscle is that the cycle operates only under a restricted range of conditions. For example, Zorzano et al. (8) found evidence of fatty acid-induced inhibition of glycolysis during recovery from exercise but not during prolonged fasting in rat skeletal muscle. Similarly, in vivo studies in man, while not directly measuring skeletal muscle glucose utilization, have been consistent with the operation of the cycle in muscle under some, but not all, conditions tested (9, 10). The factors that control the relationship between fatty acid and glucose utilization in muscle are not clear.

With the recent development of techniques based on systemic administration of radiolabeled 2-deoxyglucose and glucose to measure tissue-specific glucose utilization and storage in vivo (4, 5), it is now possible to assess directly the effect of increased fatty acid availability on skeletal muscle glucose utilization. We describe here the effects of acute elevations of circulating fatty acids on skeletal muscle and heart glucose metabolism in rats in vivo under basal conditions and in the presence of moderate physiologic hyperinsulinemia during a euglycemic clamp.

Methods

Animals. Adult male Wistar rats (body weight 350–400 g) with free access to food and water and subject to controlled lighting (lights on from 0600–1800 hours) were used for study. All animals were deprived of food for 5 h before study. This work was approved by the St. Vincent's Hospital Animal Ethics Committee and complies with the National Health and Medical Research Council of Australia guidelines for animal experimentation.

Protocol. A detailed description of the preparation of the animals for study has been given previously (11). Briefly, animals were prepared with chronic cannulae in the jugular vein and carotid artery, which were exteriorized via a head piece. Animals were housed individually in enclosed air-conditioned cages and were conscious and undisturbed before and during the study. All studies were conducted 48 h after surgery when postoperative weight loss is complete (11), corticosterone levels have returned to normal (11), and food consumption is 85–90% of preoperative intake (12). Animals were studied either in the basal state or during a hyperinsulinemic, euglycemic clamp (11). Hyperinsulinemia was achieved using a constant infusion of porcine insulin (0.25 U/kg-h, commenced at $t = 0$ min), which produces circulating insulin levels within the physiological range (~ 150 mU/liter, reference 11). Blood glucose concentration was maintained constant at basal levels by a variable rate glucose infusion adjusted according to five minutely estimations of blood glucose concentration. Circulating concentrations of nonesterified FFA were raised acutely by a priming bolus of heparin (10 U) at $t = 0$ min, followed by constant infusion of a mixture of heparin and a 20% wt/vol triglyceride emulsion (Intralipid, Travenol, Sydney, Australia). Two infusion rates were used: 0.6 or 1.2 ml of triglyceride emulsion/h (referred to as FFA1 and FFA2 groups, respectively) combined with heparin at 40 U/h. Studies were of 120–135-min duration with bolus tracer administration (see below) 45 min before killing with systemic

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nembutal (150 mg/kg). Tissues were rapidly removed, freeze clamped in aluminium tongs cooled in liquid N_2, and stored at −70°C for later analysis. Muscles removed were the soleus, plantaris, the red and white portions of the medial gastrocnemius, extensor digitorum longus, the red and white quadriceps, diaphragm, and heart. Samples of epididymal and inguinal white adipose tissue, and interscapular brown adipose tissue were also taken. Serum samples for FFA and insulin determinations were obtained at t = 75 min and at the end of the study.

Indices of tissue specific glucose metabolism. Estimates of total rates of glucose utilization, glucose incorporation into glycogen and lipid fractions, and glycogen content in individual tissues were obtained using methods described in detail elsewhere (4). Briefly, an index of total glucose utilization (glucose metabolic index, $R_{g'}$)[1] was calculated from the accumulation in tissues of 2-[^{3}H]deoxyglucose-6-phosphate after a systemic bolus of 2-[^{3}H]deoxyglucose. $R_{g'}$ may differ from the actual rate of tissue glucose utilization by a dimensionless constant (the "lumped constant" (13) that relates to and varies between limits imposed by the relative affinities of glucose and 2-deoxyglucose for glucose transport and phosphorylating activities, i.e., the lumped constant will tend towards the ratio describing the relative affinity of glucose and 2-deoxyglucose for the rate-limiting step for glucose uptake (14). In skeletal muscle, recent evidence suggests that both the lumped constant (5) and the relative affinity of 2-deoxyglucose for the glucose transporter (15) are approximately unity and hence the relative affinity for phosphorylation is also close to unity. Therefore $R_{g'}$ should accurately reflect true rates of glucose utilization in muscle independent of conditions. Consistent with this, during maximal insulin stimulation in rats when skeletal muscle dominates whole body glucose disposal and phosphorylation is likely to be the rate-limiting process, the plasma kinetics of glucose and deoxyglucose are indistinguishable (4); moreover changes in whole body insulin-induced glucose utilization created by exercise training (16) or diet (12) have correlated well with changes in $R_{g'}$ in muscle.

Tissue-specific rates of glucose incorporation into glycogen (4) and total lipids in selected muscles were calculated from the accumulation of ^{14}C in these products after a systemic bolus of [U-^{14}C]glucose. The calculation for lipid was identical to that for glycogen and represents the net rate of conversion of circulating glucose to lipid products. An approximate estimate of glycolytic flux (Gf) was obtained from the difference between $R_{g'}$ and the rate of glucose incorporation into glycogen (17). This calculation assumes that the "lumped constant" (13) for skeletal muscle is unity (5, 15). Total lipid extracts were saponified and separated into glycerol and fatty acid fractions. In all cases, > 90% of total lipid ^{14}C was recovered in the glycerol fraction, which suggests that minimal de novo lipogenesis occurs in muscle under the conditions of these studies. $R_{g'}$ was determined for all tissues but measurement of rates of glycogen synthesis and glycogen mass were restricted to the most rapidly excised muscles (soleus and the red and white gastrocnemius). Rates of glucose incorporation into lipids were determined in the red and white quadriceps, plantaris, and diaphragm.

Whole body glucose turnover. Estimates of the whole body rate of glucose utilization ($R_{d'}$)[2] and hepatic glucose production ($R_{a'}$) were obtained from the plasma kinetics of 2-[^{3}H]deoxyglucose using a procedure similar to that described elsewhere for [^{14}C]glucose (18). [^{14}C]-glucose was not suitable for this purpose in the present study due to the probable stimulation of recycling of ^{14}C by elevated lipids in the basal state, which leads to a variable underestimation of glucose turnover. Consistent with this, in the present study rates of plasma disappearance of [^{3}H]deoxyglucose and [^{14}C]glucose were highly correlated in the presence of hyperinsulinemia (r = 0.88, n = 16, $P < 0.001$) but not in the basal state (r = 0.43, n = 15, NS). We have shown elsewhere that at maximally stimulating serum insulin concentrations, where ^{14}C recycling and urinary loss of 2-[^{3}H]deoxyglucose are negligible, the plasma kinetics of 2-[^{3}H]deoxyglucose and [^{14}C]glucose are indistinguishable (4). In addition, we have since shown that the urinary loss of 2-deoxyglucose (which leads to a significant overestimation of R_d at physiological insulin levels using this methodology) is well described by a first order process with a rate constant of 0.036 min^{-1} (unpublished observation). Our estimates of $R_{a'}$ and $R_{d'}$ have therefore been corrected for this process using the relationship (adapted from reference 18): $R_{d'} = PG([dose/\int_0^\infty C^*dt] - kV)$ where: PG = plasma glucose concentration, $dose$ = the bolus dose of 2-[^{3}H]deoxyglucose in dpm, $\int_0^\infty C^*dt$ = the area to infinity under the plasma disappearance curve of 2-[^{3}H]-deoxyglucose (calculated from a double exponential curve fit), V = the apparent distribution volume of 2-[^{3}H]deoxyglucose (calculated from a double exponential curve fit), and k = a first order rate constant describing urinary loss of 2-[^{3}H]deoxyglucose (= 0.036 min^{-1}). In the basal state under steady-state conditions, $R_{a'} = R_{d'}$ by definition. In the euglycemic clamp studies, $R_{a'}$ was obtained by subtracting the glucose infusion rate required to maintain basal blood glucose level, from $R_{d'}$ determined from 2-[^{3}H]deoxyglucose kinetics.

Other analytical methods. Plasma glucose, serum insulin (rat or porcine), and plasma tracer concentrations were determined as described previously (4). The insulin assay used reacts identically with porcine and human insulin. Serum nonesterified fatty acids were determined using an acyl-CoA oxidase based colorimetric kit (WAKO NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan). Serum for fatty acid estimation was separated and frozen immediately, stored at −70°C, and thawed immediately before assay. Serum triglycerides were measured on Cobas FARA centrifugal analyzer (Roche Diagnostica, Basle, Switzerland) using a glycerol kinase-based kit method (MA-Kit 10).

Materials. 2-[2,6-^{3}H]deoxyglucose and [U-^{14}C]glucose were obtained from Amersham Australia, Sydney. Porcine insulin for infusion and human and rat insulin standards for RIA were obtained from NOVO Industri, Bagsvaerd, Denmark.

Statistical analyses and presentation of data. Statistical comparisons between groups were performed using one-way or two-way analysis of variance (ANOVA) as indicated. Our primary aim was to assess the effects of increased circulating FFA on muscle and adipose tissue and only secondarily to detect differences in behavior of different muscle types and fat depots. We have therefore calculated average muscle and adipose tissue parameters for the test groups by expressing the uptake per unit mass parameters from individual tissues from each animal as percentage of the mean value for that tissue in the appropriate control group. These normalized individual tissue parameters were then meaned to provide 'average' muscle and adipose tissue parameters for each rat. In the course of this analysis, it became clear that heart and brown adipose tissue were special cases and these two tissues are therefore analyzed separately. Data are presented in this way in the figures for clarity and as a visual analogy to the ANOVA test procedure. However, all tests of significance were performed on the untransformed data presented in tabular form. All numerical data are presented as mean±SEM.

Results

Circulating lipid and insulin levels. Circulating insulin levels were not affected by triglyceride plus heparin infusion in either the basal or clamp groups (Table I). Insulin levels during the euglycemic clamp were within the physiological range. In the control group, the FFA level was suppressed during the euglycemic clamp (Table I). Triglyceride plus heparin infusion produced dose-dependent elevations in circulating FFA (Table I) with no difference in levels achieved between the basal and clamp groups. Serum triglycerides were not affected by insulin

1. *Abbreviations used in this paper:* ANOVA, analysis of variance; Gf, glycolytic flux; $R_{a'}$, rate of disposal; $R_{d'}$, rate of production; $R_{g'}$, glucose metabolic index.

2. $R_{d'}$ and $R_{a'}$ designate approximate estimates of rates of whole body glucose disposal (R_d) and production (R_a) based on the plasma kinetics of 3H-2-deoxyglucose. This terminology is consistent with the use of $R_{g'}$ to designate 2-[^{3}H]deoxyglucose based estimates of rates of individual tissue glucose utilization.

Table I. Circulating FFA and Insulin

	Control		FFA1		FFA2	
	Basal	Clamp	Basal	Clamp	Basal	Clamp
FFA (*mmol/liter*)	1.0±0.1	0.2±0.1	2.2±0.2	2.2±0.2	4.7±0.7	4.4±0.7
Insulin (*mU/liter*)	16±5	151±14	28±6	125±16	34±7	170±23

Serum FFA and insulin under basal conditions and during a hyperinsulinemic euglycemic clamp in control, FFA1, and FFA2 groups. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter, respectively. Individual data are the mean of the 75 min and final samples. Mean±SEM (n = 5–6 in each group).

elevation and were 0.35±0.05, 0.92±0.11, and 1.68±0.39 mmol/liter in the control, FFA1, and FFA2 groups, respectively.

Systemic glucose flux. Plasma glucose levels were not affected by any treatment (Table II). Insulin-induced stimulation of R_d was not significantly affected by elevated FFA but insulin-induced suppression of R_a was abolished at the higher triglyceride infusion rate (Table II).

Tissue parameters

SKELETAL MUSCLE AND HEART

Total glucose utilization. The effects of elevated FFA on heart and skeletal muscle total glucose utilization (R_g) are detailed in Table III and summarized in Fig. 1. In the basal state, increases in circulating FFA produced dose-dependent decreases in R_g in heart, which were consistent with the predictions of the glucose–fatty acid cycle (1). In contrast, R_g in skeletal muscle was suppressed only in the FFA1 group and remained at control levels in the FFA2 group. With elevated insulin, elevated FFA produced a dose-related suppression of R_g in heart as in the basal state. The opposite effect was found in skeletal muscle where elevated FFA levels were associated with substantial dose-related elevations of R_g.

Table II. Glucose Turnover Parameters

		Plasma glucose	GIR	R_a	R_d
		mmol/liter		*mg/kg per min*	
Control	Basal	6.6±0.2	—	9.4±0.5	9.4±0.5
	Clamp	6.4±0.3	16.0±1.6	5.5±1.2*	21.5±2.3*
FFA1	Basal	6.5±0.3	—	9.1±0.5	9.1±0.5
	Clamp	6.4±0.2	19.4±1.3	5.8±1.1*	25.2±0.9*
FFA2	Basal	6.2±0.3	—	7.9±1.1	7.9±1.1
	Clamp	6.9±0.3	15.3±2.0	9.0±2.2	24.3±3.6*

Plasma glucose concentration and systemic glucose turnover parameters under basal conditions and during a hyperinsulinemic euglycemic clamp in control, FFA1, and FFA2 groups. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter, respectively. GIR is the glucose infusion rate required to maintain basal blood glucose level during insulin infusion. R_a and R_d are estimates of hepatic glucose output and systemic glucose utilization, respectively, obtained from the plasma kinetic behavior of 2-[^{3}H]deoxyglucose as described in Methods. Note that in the steady state in the absence of an exogenous glucose infusion (basal groups) $R_a = R_d$.

* Significantly different from the respective basal group $P < 0.05$ (One-way ANOVA).

Glycogen synthesis. The effects of elevated FFA on skeletal muscle glycogen mass and rates of glycogen synthesis are detailed in Table IV and summarized in Fig. 2. In the basal state, a similar pattern to that of R_g was seen in rates of glucose incorporation into glycogen and in glycogen mass in skeletal muscle except that the FFA2 group showed elevations above control levels. With elevated insulin, effects of FFA on glycogen synthesis and mass again paralleled effects on total glucose utilization. Increased rates of glycogen synthesis accounted quantitatively for the increased rates of total glucose utilization.

Lipid deposition. The effects of elevated FFA on glucose incorporation into total lipid in skeletal muscle are detailed in Table V and summarized in Fig. 2. In the basal state, rates of glucose incorporation into lipid tended to increase with elevated FFA but this effect was not significant. With elevated insulin, glucose incorporation into lipid was increased by elevated FFA. In all conditions lipid deposition was only a minor component of total glucose utilization in skeletal muscle and was therefore ignored in the calculation of Gf.

Gf. The effects of elevated FFA on estimated Gf are detailed in Table IV. In the basal state, Gf tended to be suppressed by elevated FFA but this effect was not significant. With elevated insulin, Gf was not affected by elevated FFA but was markedly increased compared with the basal state.

ADIPOSE TISSUE

The effects of elevated FFA on white and brown adipose tissue total glucose utilization (R_g) are detailed in Table III and summarized in Fig. 3. Basal R_g was increased in both white and brown adipose tissue depots with increasing FFA. With elevated insulin, R_g in white adipose tissue was not affected by elevated FFA but was suppressed in brown adipose tissue which showed a similar pattern to that of heart.

Discussion

Increasing the circulating FFA concentration inhibited glucose utilization in a dose-dependent manner in the heart, in the basal state, and at physiological elevated serum insulin. In skeletal muscle, two seemingly independent effects were seen; inhibition of glucose utilization was seen only at the lower FFA delivery rate in the basal state; at higher FFA levels in the basal state, glucose utilization was increased back to control levels, and with elevated insulin glucose utilization was increased by FFA in a dose-dependent manner. This increased glucose utilization was accounted for predominantly by increased glycogen synthesis with a much smaller component due to incorporation into the glycerol component of total lipids. While the increased rate of lipid deposition only accounts for a small percentage of glucose flux, the rate of fatty acid accumulation it represents is substantial compared with the rate of glycogen synthesis when expressed as energy equivalents (e.g., ~ 50% in the FFA2 clamp group).

The major findings in this study rely on the assumption that elevated FFA do not affect the relationship between rates of 2-deoxyglucose-6-phosphate accumulation and rates of glucose metabolism. Such an effect could result either from a change in the "lumped constant" (13) or from a change in the rate of loss of 2-deoxyglucose-6-phosphate from tissues. The former is unlikely in skeletal muscle for reasons given above (see discussion of the "lumped constant" in Methods), and the latter is also improbable since rates of loss of 2-deoxyglucose-6-phosphate from muscle are negligible (19) and a decrease in this rate could not account for the large increases seen in 2-deoxyglucose accumulation. Within this study, the similar patterns of 2-[^{3}H]deoxyglucose-based and [^{14}C]glucose-based measures of glucose metabolism in muscle are mutually supportive.

Table III. Glucose Metabolic Index (R_g)

Tissue	Basal			Clamp		
	Control	FFA1	FFA2	Control	FFA1	FFA2
	μmol/100 g per min					
Soleus	6.7±1.8	5.2±1.6	5.5±1.1	22±3	28±3	29±4
Red quadriceps	3.0±0.4	1.6±0.2	3.1±0.5	12±1	19±3	17±3
Red gastrocnemius	3.0±0.3	1.7±0.1	3.3±1.0	14±3	16±1	21±5
Extensor digitorum longus	6.0±1.6	1.9±0.2	3.4±1.2	7.9±1.0	6.9±0.5	11±3
Plantaris	2.4±0.3	1.7±0.1	2.7±0.3	4.1±1.2	8.9±1.4	11±3
White quadriceps	1.7±0.3	0.9±0.2	1.9±0.7	3.8±0.5	4.8±0.8	8.5±2.7
White gastrocnemius	1.2±0.1	1.2±0.1	2.0±0.6	2.4±0.4	3.0±0.5	5.1±1.6
Diaphragm	9.4±2.3	5.3±0.9	7.1±1.2	31±7	34±4	38±7
Heart	67±14	39±4	21±4	90±8	54±3	46±5
Epididymal (white) adipose tissue	0.5±0.1	0.4±0.1	0.9±0.2	2.0±0.4	2.3±0.6	2.2±0.4
Inguinal (white) adipose tissue	0.4±0.1	0.3±0.1	1.7±0.5	1.2±0.2	1.7±0.2	1.7±0.3
Interscapular (brown) adipose tissue	7.8±2.3	20±15	23±6	82±16	47±6	48±7

Glucose metabolic index (R_g) under basal conditions and during a hyperinsulinemic, euglycemic clamp in control, FFA1 and FFA2 groups. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter, respectively. R_g is a measure of total glucose utilization derived from the accumulation of 2-[^{3}H]deoxyglucose-6-phosphate as described in Methods. Mean±SEM (n = 5–6 in each group).

It should be noted that the elevations in FFA produced by the triglyceride plus heparin infusions range from high physiological to supraphysiological. These levels were used to expose effects that are quite small compared with the precision of estimation of the various tissue parameters. However, with regard to the inhibition of glucose utilization seen in heart and skeletal muscle, Wisneski et al. have shown in human heart in vivo that the relationship is most sensitive within the normal physiological range (7). In the basal state, our protocol produced elevations of FFA above fasting levels and therefore a proportionally larger effect might be seen with smaller FFA changes within the physiological range. Concerning the FFA-induced stimulation of glucose disposal in skeletal muscle, linear interpolation of the effects seen to be within the physiological range predicts that an elevation of FFA of 0.5 mmol/liter during the euglycemic clamp would produce an 8% increase in total glucose utilization, an 18% increase in glycogen synthesis, and a 23% increase in lipid deposition in skeletal muscle. These may be physiologically and pathologically significant effects as discussed below.

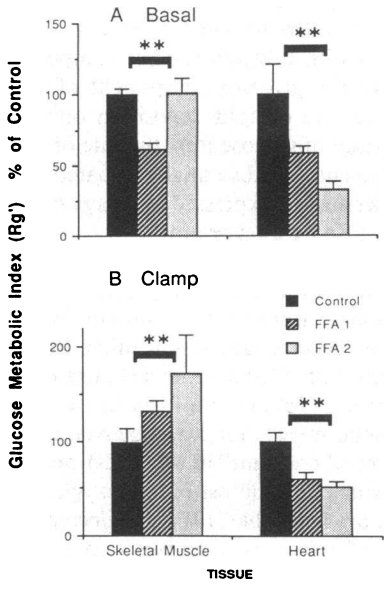

Figure 1. Glucose metabolic index (R_g) in heart and skeletal muscle under basal conditions (*A*) and during a hyperinsulinemic euglycemic clamp (*B*) in control, FFA1, and FFA2 groups expressed as the percentage of the appropriate control group±SEM. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter, respectively. The skeletal muscle data is a composite of the eight skeletal muscles sampled and was calculated as described in Methods from the individual data presented in Table III. Asterisks indicate statistically significant effects of FFA across the three groups: **P < 0.01 (ANOVA).

Skeletal muscle. Recent in vivo work has led to the conclusion that the glucose-fatty acid cycle (1) operates in skeletal muscle only under a restricted range of conditions (8, 9). The present findings suggest that inhibition of Gf (and glucose oxidation) by fatty acid oxidation can occur in skeletal muscle probably via the same mechanisms operating in the heart (1), but that this may be obscured in experimental designs that monitor net glucose utilization by an independent FFA-induced stimulation of glucose utilization directed towards storage pathways.

The mechanism responsible for the stimulation of glucose utilization and storage in skeletal muscle by FFA is not clear. Redirection of a reduced uptake towards glycogen synthesis has been explained by stimulation of glycogen synthesis due to accumulation of glucose-6-phosphate, which results from FFA-induced inhibition of Gf (1). Such a mechanism cannot explain an increase in total glucose utilization since increased glucose-6-phosphate will reduce glucose phosphorylation and hence net uptake. Clearly, an independent stimulation of the glycogen synthetic pathway, hexokinase activity, or glucose transport activity is required. We are not aware of any data supporting a stimulatory effect of FFA or their metabolites on either hexokinase or the glycogen synthetic pathway, but FFA, at physiological concentrations, have been reported to increase glucose transporter activity in isolated rat adipocytes by increasing transporter affinity for glucose (20). The magnitude of

Table IV. Skeletal Muscle Glycogen and Gf

Tissue	Basal			Clamp		
	Control	FFA1	FFA2	Control	FFA1	FFA1
Glycogen synthesis rate (*μmol/100 g per min*)*						
Soleus	1.8±0.5	1.6±0.6	3.6±1.4	7.4±1.4	13±2	13±3
Red gastrocnemius	0.7±0.1	0.6±0.2	1.5±0.4	4.8±1.5	8.4±0.8	13±3
White gastrocnemius	0.10±0.02	0.12±0.02	0.51±0.27	0.46±0.11	0.77±0.22	1.51±0.44
Glycogen mass (*μmol/g*)						
Soleus	34±3	24±3	36±4	45±5	43±4	50±4
Red gastrocnemius	36±4	35±7	45±3	42±4	35±4	53±6
White gastrocnemius	30±4	36±2	41±4	37±5	37±1	45±4
Gf (*μmol/100 g per min*)‡						
Soleus	4.8±1.4	3.5±0.2	1.9±1.1	16±2	15±2	17±2
Red gastrocnemius	2.3±0.2	1.1±0.2	1.8±0.8	10±3	7.5±0.9	7.8±1.1
White gastrocnemius	1.1±0.1	1.0±0.1	1.5±0.7	2.1±0.3	1.6±0.5	3.6±0.7

Rates of glycogen synthesis, glycogen mass, and estimated Gf in skeletal muscles under basal conditions and during a hyperinsulinemic, euglycemic clamp in control, FFA1, and FFA2 groups. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter, respectively. * Rates of glycogen synthesis in muscles were estimated from incorporation of [^{14}C]glucose into glycogen as described in Methods. ‡ Gf was calculated as the difference between R_g and the rate of glycogen synthesis. Mean±SEM (n = 5–6 in each group).

the effect was comparable to that seen here. If such a mechanism exists in skeletal muscle, it could account for the present findings. Our data from adipose tissue is consistent with the operation of such a mechanism in the basal state where glucose utilization was stimulated by elevated FFA in both white and brown depots. With elevated insulin, white adipose tissue glucose utilization was not significantly affected by elevated FFA. However, in contrast to muscle, white adipose tissue glucose utilization is almost maximally stimulated at the insulin dose used here (4) and there may be little scope for a further FFA-stimulated increase.

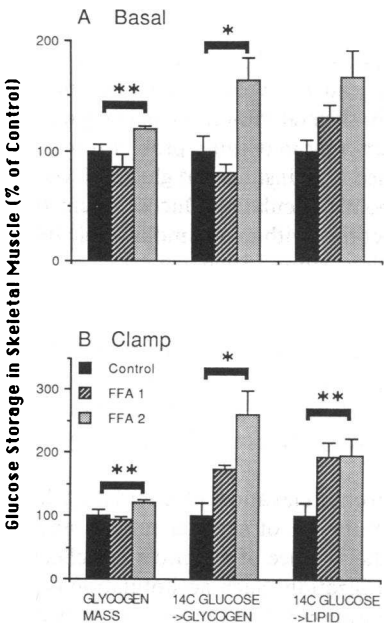

Figure 2. Glycogen mass, glycogen synthesis, and lipid deposition in skeletal muscle under basal conditions (*A*) and during a hyperinsulinemic euglycemic clamp (*B*) in control, FFA1, and FFA2 groups expressed as the percentage of the appropriate control group±SEM. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter, respectively. Rates of glycogen synthesis and lipid deposition were estimated from the incorporation of [^{14}C]-glucose into the respective products. The glycogen data is a composite of three skeletal muscles and the lipid data is a composite of four skeletal muscles calculated as described in Methods from the individual data presented in Tables IV and V. Asterisks indicate statistically significant effects of FFA across the three groups: *$P < 0.05$; **$P < 0.01$ (ANOVA).

Brown adipose tissue. Brown adipose tissue glucose utilization was suppressed by elevated FFA in the presence of elevated insulin which shows a pattern similar to that of the heart. Whether this suppression reflects reduced oxidative or lipogenic metabolism of glucose is not clear. However, the consequences of inhibition by FFA of an insulin stimulated glucose oxidation are of interest since brown adipose tissue is likely to be a major source of diet-induced thermogenesis in mammals (21). Dietary related thermogenesis is the aspect of energy expenditure that is clearly affected in rodent models of obesity (e.g., hypothalamic lesion [22] and fa/fa Zucker rat [23]) and recent well-controlled studies support the presence of a defect in the thermic effect of food in human obesity (24, 25). Conventionally, FFA are thought to be the fuel of preference and to provide most of the energy for brown adipose tissue thermogenesis under both basal and stimulated conditions (26). However, glucose utilization in brown adipose tissue is remarkably insulin-sensitive and rates of glucose utilization in the presence of insulin are extremely high and are comparable to the rates in the heart (12). If glucose is a substrate for thermogenesis in brown adipose tissue in vivo as has been argued (27), the present results showing reduced insulin-stimulated glucose utilization in brown adipose tissue in the presence of elevated FFA suggest a mechanism for impaired prandial thermogenesis in states of insulin resistance.

Whole body glucose turnover. The effects of elevated FFA on whole body glucose disposal were consistent with the individual tissue effects seen. In the basal state, the reduction in skeletal muscle R_g in the FFA1 group would result in a 6–10% reduction in R_d (assuming that muscle accounts for 15–20% of basal glucose disposal). An effect of this magnitude on R_d would not be detected using the present methods. In the clamp groups, the increases in muscle R_g predict a 10–20% increase in R_d, which is consistent with the nonsignificant elevations

Table V. Glucose Incorporation into Total Lipid in Skeletal Muscle

Tissue	Basal			Clamp		
	Control	FFA1	FFA2	Control	FFA1	FFA2
	μmol/100 g per min					
Red quadriceps	0.07±0.01	0.09±0.02	0.07±0.01	0.23±0.01	0.40±0.07	0.34±0.06
Plantaris	0.03±0.01	0.05±0.01	0.05±0.01	0.13±0.03	0.25±0.04	0.39±0.10
White quadriceps	0.02±0.01	0.02±0.01	0.03±0.01	0.09±0.01	0.15±0.03	0.13±0.02
Diaphragm	0.09±0.01	0.10±0.02	0.17±0.05	0.29±0.07	0.82±0.13	0.72±0.06

Rates of [^{14}C]glucose incorporation into total lipid in skeletal muscles under basal conditions and during a hyperinsulinemic, euglycemic clamp in control, FFA1, and FFA2 groups. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter, respectively. Mean±SEM (n = 5–6 in each group).

seen. In the FFA2 clamp group, the insulin-induced suppression of $R_{a'}$ was abolished, which was consistent with findings in humans (9). This stimulation of $R_{a'}$ in the presence of insulin is probably due to the provision of a gluconeogenic substrate in the form of glycerol derived from intravascular lipolysis (9).

There are data from humans consistent with an FFA-induced stimulation of glucose disposal under some circumstances. For example, while the major focus of Ferrannini et al. (9) was on a suppression of insulin-mediated glucose disposal by FFA, their group C (hyperglycemic, hypoinsulinemic) showed a small (12%) elevation of whole body glucose utilization with a 1.5 mmol/liter elevation of circulating FFA. More striking is the 20% increase in whole body glucose disposal associated with a 3–4 mmol/liter increase in circulating FFA described by Bevilacqua et al. (28) in obese humans during a hyperinsulinemic euglycemic clamp. While neither of these examples is statistically significant, it must be emphasized that our measure of whole body glucose disposal also did not change significantly, but the nonsignificant increases seen were consistent with the proportionally larger stimulations of individual muscle glucose uptake by elevated FFA. Thus there is reason to suppose that the present findings may apply to humans. Detection of such effects in man may require larger experimental groups or the use of techniques more selective or sensitive than measurement of whole body glucose turnover.

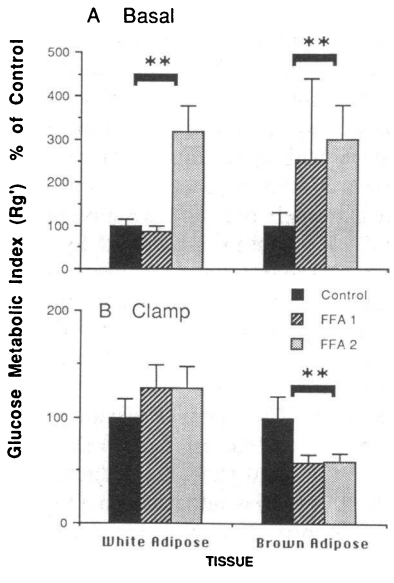

Figure 3. Glucose metabolic index ($R_{g'}$) in white and brown adipose tissue depots under basal conditions (*A*) and during a hyperinsulinemic euglycemic clamp (*B*) in control, FFA1, and FFA2 groups expressed as the percentage of the appropriate control group±SEM. The FFA1 and FFA2 groups received infusions of triglyceride plus heparin to elevate FFA to ~ 2 and 4 mmol/liter respectively. The white adipose tissue data is a composite of the two depots sampled, calculated as described in Methods from the individual data presented in Table III. Asterisks indicate statistically significant effects of FFA across the three groups: $^{**}P < 0.01$ (ANOVA).

FFA effects on glucose metabolism in normal physiology and pathology. The demonstrated FFA-induced inhibition of glucose utilization in heart (and in skeletal muscle in the basal state) is consistent with the predictions of the glucose–fatty acid cycle (1) and supports the proposed role of the process in glucose sparing and in the acute manifestation of insulin resistance in hyperlipidemic states. The physiological role of an FFA-induced stimulation of glucose uptake is a matter for conjecture. In adipose tissue, a plausible role would be the provision of glycerol-3-phosphate for triglyceride deposition in the presence of elevated FFA. The effect in muscle may have a significant physiological role in muscle triglyceride deposition and the preservation or replenishment of muscle glycogen in the presence of high circulating FFA, for example in prolonged fasting or after exercise.

Chronically elevated circulating lipids, particularly in the presence of insulin, may lead to excessive accumulation of glucose storage products in skeletal muscle. Deficient prandial suppression of FFA in particular may cause problems in combination with the associated high insulin and glucose levels. A reduced ability to metabolize circulating glucose could then develop because of competition with excess mobilizable intramuscular glycogen and lipid stores. Such mechanisms have been suggested to account in part for the in vitro insulin resistance of skeletal muscle from obese Zucker rats that have markedly elevated muscle glycogen and triglycerides (29). In this regard, it is of interest that in two models where the early development of insulin resistance or glucose intolerance has been studied, there is evidence of elevated muscle glycogen stores (30, 31).

The proposal that chronic elevation of circulating lipid leads to increased accumulation of skeletal muscle energy stores could be made in the absence of a stimulatory effect of FFA on muscle glucose utilization. Such accumulation could follow from the classical formulation of the glucose–fatty acid cycle where an FFA-induced, decreased total glucose utilization is redirected to storage pathways. However, the demonstrated stimulation of glucose utilization in skeletal muscle by FFA in some conditions may make this scenario more likely and more rapid.

In conclusion, a short term elevation of FFA clearly impairs glucose utilization in cardiac muscle (and in skeletal muscle in the basal state), which is consistent with the predictions of the glucose–fatty acid cycle (1). High FFA levels or elevated insulin levels expose a separate stimulatory effect of FFA on skeletal muscle glucose uptake. In normal physiology these effects together could result in glucose sparing and maintenance of muscle glycogen and/or lipid stores in the presence of elevated FFA. The relationship between elevated FFA and the insulin resistance of type II diabetes and obesity is potentially complex with competing acute effects of inhibition and stimulation of muscle glucose disposal, either of which could dominate or compensate for each other under different conditions. Chronic elevation of FFA could rapidly lead to the development of insulin resistance by promoting excess accumulation of glycogen and lipid stores in skeletal muscle.

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