Rapid Impairment of Skeletal Muscle Glucose Transport/Phosphorylation by Free Fatty Acids in Humans

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The initial effects of free fatty acids (FFAs) on glucose transport/phosphorylation were studied in seven healthy men in the presence of elevated (1.44 ± 0.16 mmol/l), basal (0.35 ± 0.06 mmol/l), and low (<0.01 mmol/l; control) plasma FFA concentrations (P < 0.05 between all groups) during euglycemic-hyperinsulinemic clamps. Concentrations of glucose-6-phosphate (G-6-P), inorganic phosphate (P_i), phosphocreatine, ADP, and pH in calf muscle were measured every 3.2 min for 180 min by using ^{31}P nuclear magnetic resonance spectroscopy. Rates of whole-body glucose uptake increased similarly until 140 min but thereafter declined by ~20% in the presence of basal and high FFAs (42.8 ± 3.6 and 41.6 ± 3.3 vs. control: 52.7 ± 3.3 µmol · kg^{-1} · min^{-1}, P < 0.05). The rise of intramuscular G-6-P concentrations was already blunted at 45 min of high FFA exposure (184 ± 17 vs. control: 238 ± 17 µmol/l, P = 0.008). At 180 min, G-6-P was lower in the presence of both high and basal FFAs (197 ± 21 and 213 ± 18 vs. control: 286 ± 19 µmol/l, P < 0.05). Intramuscular pH decreased by –0.013 ± 0.001 (P < 0.005) during control but increased by +0.008 ± 0.002 (P < 0.05) during high FFA exposure, while P_i rose by ~0.39 mmol/l (P < 0.005) within 70 min and then slowly decreased in all studies. In conclusion, the lack of an initial peak and the early decline of muscle G-6-P concentrations suggest that even at physiological concentrations, FFAs primarily inhibit glucose transport/phosphorylation, preceding the reduction of whole-body glucose disposal by up to 120 min in humans. *Diabetes* 48:358–364, 1999

Insulin resistance is a common feature of obesity and dyslipidemia, which predispose one to type 2 diabetes and premature cardiovascular diseases (1,2). Free fatty acids (FFAs) are considered to play a pivotal role in the pathogenesis of diabetes (2,3) and may even be involved in the early events leading to insulin resistance. In offspring of type 2 diabetic parents, plasma FFA concentrations are inversely correlated with insulin sensitivity (4,5). Moreover, nonobese normoglycemic Mexican-American offspring of type 2 diabetic parents present with diminished insulin-induced suppression of FFAs (6). However, the mechanism by which FFAs induce insulin resistance is still under debate (7).

From in vitro studies, Randle et al. (8,9) postulated that increased FFA oxidation inactivates pyruvate dehydrogenase with subsequent inhibition of phosphofructokinase (8–10). This would cause intracellular glucose-6-phosphate (G-6-P) to rise and then decrease hexokinase II activity with consequent decreased glucose uptake and glycogen synthesis. Evidence for the operation of such a mechanism in vivo has been recently provided in rats (11,12). In humans, lipid/heparin infusion inhibits carbohydrate oxidation (13–17), but neither an increase in cellular citrate levels (16) nor inhibition of pyruvate dehydrogenase could be demonstrated in muscle biopsies (18). Only a few authors also reported a reduction in insulin-sensitive whole-body glucose disposal (15,19,20), which is primarily accounted for by impaired nonoxidative glucose metabolism (16,19), suggesting FFA-induced inhibition of muscle glycogen synthesis (21). However, defects in fractional activity of glycogen synthase were observed in some (16,17,20) but not in other studies (18,22).

We have recently reported that the decrease in insulin-dependent glucose disposal is associated with a blunted increase of intramuscular G-6-P and with reduction in rates of glucose oxidation and glycogen synthesis (23). However, we could not exclude an initial transient increase in G-6-P, and the effects were observed only at high plasma FFA concentrations of ~2 mmol/l.

The aims of the present study were therefore *1*) to follow the initial time-course of intramuscular G-6-P, *2*) to correlate rates of whole-body glucose uptake and changes of muscular G-6-P concentration at fasting and postprandial plasma FFA concentrations, and *3*) to simultaneously monitor intracellular allosteric effectors of muscle glucose metabolism, such as inorganic phosphate (P_i), phosphocreatine (PCr), ADP, and pH in human calf muscle. Thus, we applied in vivo ^{31}P-nuclear magnetic resonance (NMR) spectroscopy (23,24) to continuously measure these parameters by using an NMR spectrometer of higher magnetic field strength and homogeneity to increase the sensitivity and time-resolution of G-6-P measurements. By using this improved noninvasive approach, problems of previous techniques, due to tracer methodology and handling of tissue biopsies, can be avoided (25).

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ANOVA, analysis of variance; CV, coefficient of variation; FFA, free fatty acid; G-6-P, glucose-6-phosphate; PCr, phosphocreatine; P_i, inorganic phosphate; NMR, nuclear magnetic resonance.

RESEARCH DESIGN AND METHODS

Subjects. Studies were performed in seven healthy men (age 25 ± 1 years, BMI 23.02 ± 1.30 kg/m^2, body surface area 1.99 ± 0.08 m^2) who had no family history of diabetes and who were not taking medications on a regular basis. They stopped moderate regular exercising at least 3 days before the experiments and were on an isocaloric diet (30 kcal · kg^{-1} · day^{-1}; carbohydrate/protein/fat: 60/20/20%) for 3 days and then fasted overnight for 12 h before the experiments. Written informed consent was obtained from all subjects after explanation of the nature of the studies, which were approved by the human ethics committee of the University of Vienna.

Hyperinsulinemic-euglycemic clamps. At 7:30 A.M., catheters (Vasofix; Braun, Melsungen, Germany) were placed in one antecubital vein of each arm for blood sampling (left arm) and infusions (right arm). Conditions of insulin-stimulated whole-body glucose uptake were created by performing hyperinsulinemic- (~400 pmol/l) euglycemic (~5 mmol/l) clamps for 180 min. Regular human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was administered as a primed continuous intravenous infusion (1 mU · kg^{-1} · min^{-1}). Constant fasting plasma glucose concentrations were achieved by a variable intravenous glucose infusion (1.1 mol/l). All subjects were studied under three experimental conditions: *1*) elevation of plasma FFA concentrations (~1.5 mmol/l; LIP) induced by intravenous infusion of a triglyceride emulsion (1.5 ml/min Intralipid 20%; Kabi Pharmacia, Stockholm, Sweden) combined with a bolus (250 IU) continuous intravenous infusion of heparin (0.2 IU · kg^{-1} · min^{-1}) (16,23), *2*) fasting plasma FFA concentrations (~0.5 mmol/l; BAS) induced by intravenous triglyceride infusion (1.5 ml/min) only, and *3*) insulin-suppressed plasma FFA concentrations (<0.01 mmol/l; CON) during intravenous saline infusion (1.5 ml/min). Experimental protocols were spaced by 4- to 8-week intervals during which the participants' body weights and lifestyle remained unchanged. Blood samples were drawn in 5-min intervals for monitoring of plasma glucose. For the determination of plasma insulin, FFA, and triglyceride concentrations, blood was collected every 15 or 30 min and immediately cooled, centrifuged, and stored at –80°C to avoid further lipolysis.

In vivo ^{31}P-NMR spectroscopy. Subjects were in a supine position inside an NMR spectrometer (3.0 T, 80-cm diameter bore, Medspec S300-DBX; Bruker, Ettlingen, Germany). The right lower leg was positioned so that the isocenter of the magnetic field was placed ~2 cm into the medial head of the gastrocnemius muscle to obtain ^{31}P-spectra (23,24,26) before the start (baseline) and then every 3.2 min during the clamps. ^{31}P-NMR spectra (90° radio frequency pulse, pulse length 150 µs, 4,096 data points, 32 averages, repetition time of 6 s) were obtained by using a 10-cm circular double-resonant surface coil for ^{1}H (125.6 MHz) and ^{31}P (50.8 MHz). The proton coil was used for scout imaging and shimming of the water signal. All spectra were zero filled to 32 K, apodized using either a 2-Hz exponential function for baseline spectra or a 10-Hz exponential function for difference spectra, and corrected manually for baseline and phase. Because of potential contamination with phosphomonoester resonances (α-glycerol phosphate), only part of the chemical shift range of G-6-P (2.6–2.3 ppm relative to P_i) was integrated. Intramuscular concentrations of G-6-P, P_i, and PCr were quantified by comparison of the respective resonance areas with that of β-ATP (23,24,26), assuming an ATP concentration of 5.5 mmol/l for nonexercising muscle (27,28). During the studies, changes of G-6-P, P_i, and PCr versus baseline were determined from difference spectra (Fig. 1). Measurement of G-6-P by ^{31}P-NMR spectroscopy has been validated in frozen rat muscle (29). In the present study, the precision of measurements was assessed from determination of root mean square amplitude variation due to spectral noise, as described previously (24). Average root mean square noise measured from a 1-kHz bandwidth without any resonance gives a variation (1 SD) of G-6-P concentration of 25 µmol/l muscle in a 7-min difference spectrum. In addition, variability of signal intensities due to spectrometer instability was calculated from the residual intensity of the ATP resonances in the individual difference spectra, and the subtraction error was <2%. Under these conditions, the mean basal G-6-P concentration of 118 µmol/l muscle will result in a between-scan variation of signal intensity of <2.4 µmol/l muscle. The precision of baseline muscle G-6-P measurements was assessed in three young nondiabetic subjects by comparing four consecutive 7-min spectra obtained from the same subject (14, 19, and 29 µmol/l) and was close to the predicted value of 25 µmol/l. Intracellular pH was calculated from the difference of the chemical shift between P_i and PCr, as previously described (24,30).

Analytical methods. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer II; Beckman, Fullerton, CA). Plasma insulin was measured by double antibody radioimmunoassay (Serono Diagnostics, Freiburg, Germany) with an interassay coefficient of variation (CV) of <5%. Plasma concentrations of FFAs were measured by an enzyme assay using acyl-CoA synthase and acyl-CoA oxidase with subsequent colorimetric determination of the resulting hydrogen peroxide (Wako, Richmond, VA). The inter- and intra-assay CVs were 3.6 and 2.4%, respectively. Plasma triglycerides were hydrolyzed by lipase, and the released glycerol was measured by a peroxidase-coupled colorimetric assay (31).

Calculations and statistical analysis. Whole-body glucose disposal was calculated from mean glucose infusion rates for 20-min intervals and corrected for urinary glucose loss. Statistical analyses were performed by using StatView 4.5 (Abacus Concepts, Berkeley, CA). Data are given as means ± SE. Differences between groups were evaluated by analysis of variance (ANOVA) followed by the Schéffe and the Student-Newman-Keuls post hoc tests. Changes of sequential data within experiments were compared by the paired Student's *t* test or

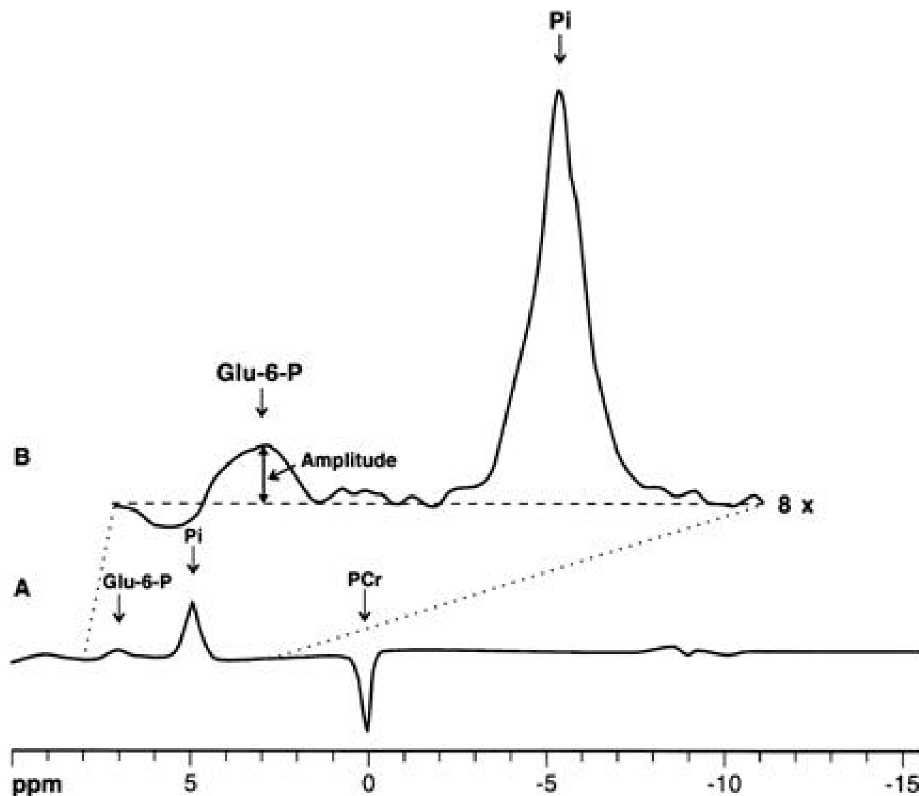

FIG. 1. Measurement of changes (Δ) in intramuscular G-6-P, P_i, and PCr in the right calf muscle of a young healthy subject during triglyceride infusion (Intralipid 20%, 1.5 ml/min) without heparin. *A*: Difference between the actual (*t* = 57–64 min) and the baseline (*t* = –5 min) ^{31}P-NMR spectrum. *B*: Determination of ΔG-6-P, ΔP_i, and ΔPCr from the respective peak amplitudes in the difference spectrum (increased in size eight times).

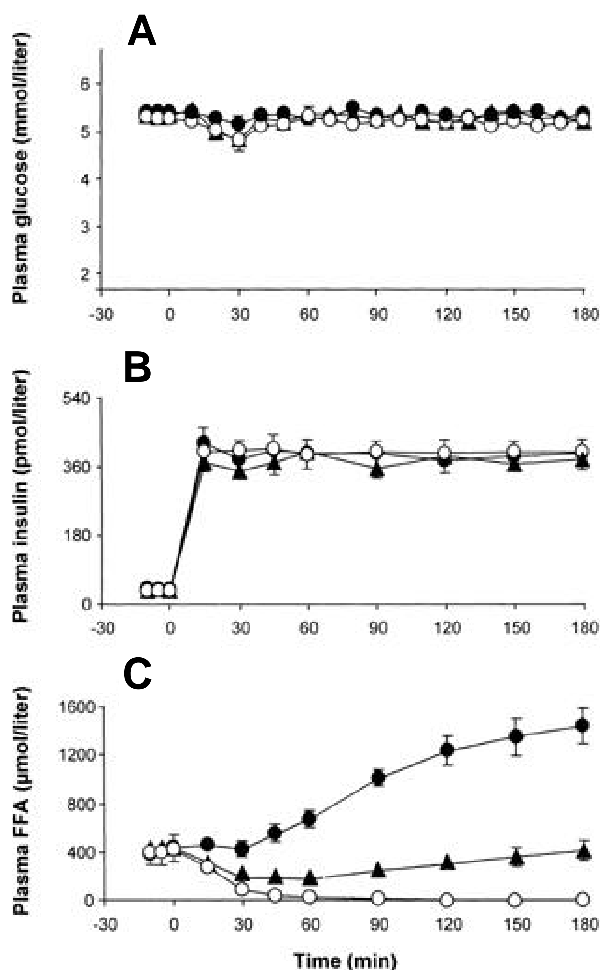

FIG. 2. Plasma concentrations of glucose (*A*), insulin (*B*), and FFAs (*C*) during infusion of saline (low FFA; CON, ○), triglycerides (basal FFA; BAS, ▲), or triglycerides plus heparin (elevated FFA; LIP, ●). Data are given as means ± SE of seven healthy subjects who underwent each of the three studies.

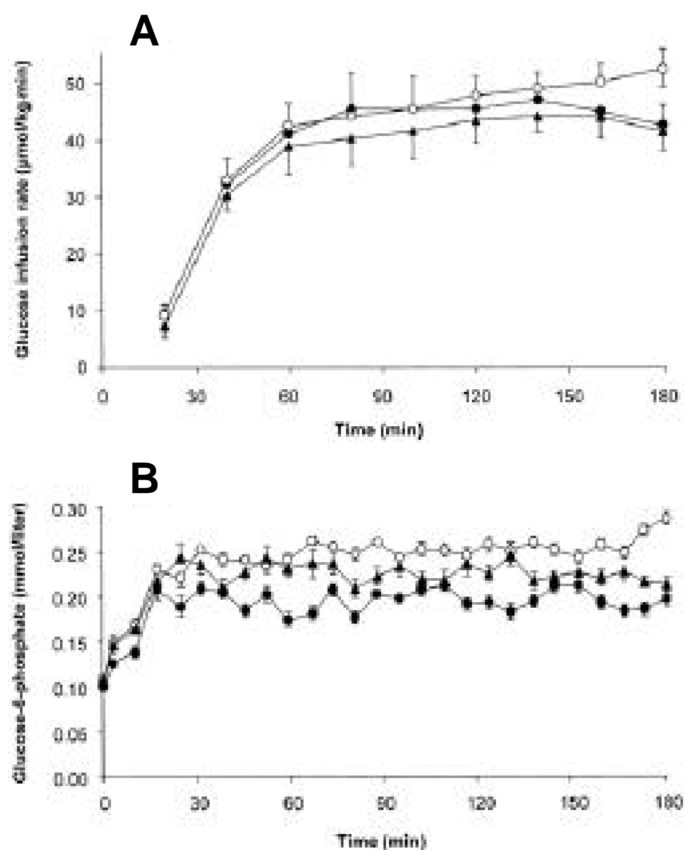

FIG. 3. Rates of whole-body glucose uptake (*A*) and intramuscular G-6-P concentrations (*B*) in the presence of low (CON, ○), basal (BAS, ▲), and elevated (LIP, ●) plasma FFA concentrations. Data are given as means ± SE of seven healthy subjects who underwent each of the three studies.

ANOVA for repeated measurements. Simple linear regression analysis was used for correlation of data. Differences were considered statistically significant at the 5% level.

RESULTS

Plasma concentrations of glucose, insulin, FFAs, and triglycerides. Fasting plasma concentrations of glucose (CON: 5.3 ± 0.0 mmol/l; BAS: 5.4 ± 0.0 mmol/l; LIP: 5.4 ± 0.0 mmol/l), FFAs (407 ± 33, 435 ± 25, and 404 ± 53 µmol/l), and insulin (34 ± 2, 38 ± 0.2, and 41 ± 3 pmol/l) were similar in all studies. During hyperinsulinemic-euglycemic clamp tests, plasma glucose concentrations did not change from baseline levels (Fig. 2*A*), while plasma insulin concentrations increased ~10-fold within 15 min and remained constant without differences between the experiments (120–180 min: 396 ± 16, 395 ± 15, and 385 ± 16 pmol/l, $P < 0.000001$ vs. baseline) (Fig. 2*B*). Plasma FFA concentrations rapidly decreased, reaching the detection limit (10 µmol/l) between 60 and 90 min of control studies ($P < 0.001$; Fig. 2*C*). During lipid infusion without heparin (BAS), plasma FFA concentrations transiently decreased to a nadir at 45 min, but they were still ~4.6-fold higher compared with control values ($P < 0.05$). Thereafter, plasma FFA levels increased toward fasting concentrations until 180 min (350 ± 65 µmol/l; NS vs. baseline, $P < 0.05$ vs. CON and LIP). In the presence of combined lipid/heparin infusion, plasma FFA concentrations started to rise at 45 min and were ~3.6-fold increased at 180 min (LIP: 1,444 ± 157 µmol/l; $P < 0.0005$ vs. baseline, $P < 0.05$ vs. CON and BAS). Plasma triglyceride concentrations were similar at baseline (CON: 84 ± 6 mg/dl; BAS: 99 ± 8 mg/dl; LIP: 84 ± 7 mg/dl) and increased during lipid infusions (BAS: 342 ± 19 mg/dl, $P < 0.05$; LIP: 260 ± 17 mg/dl, $P < 0.05$ vs. CON: 54 ± 2 mg/dl).

Whole-body glucose uptake. In control experiments, rates of whole-body glucose uptake continuously rose until the end of the study (Fig. 3*A*). During both basal and high lipid studies, rates of whole-body glucose uptake increased in parallel until 140 min only, then they dropped and were ~20% lower in the presence of basal (BAS: 41.6 ± 3.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$ vs. CON) or elevated plasma FFA concentrations (LIP: 42.8 ± 3.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$ vs. CON) than during control studies at 160–180 min (52.7 ± 3.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$).

Intramuscular G-6-P concentrations. Baseline G-6-P concentrations in human calf muscle were comparable before the start of each experiment (Table 1). Intramuscular G-6-P concentrations rose linearly within 20 min in all studies (total G-6-P at 17 min: CON: 229 ± 18 µmol/l, $P < 0.001$; BAS: 216 ± 23 µmol/l, $P = 0.01$; LIP: 207 ± 28 µmol/l, $P < 0.01$ vs. baseline)

TABLE 1
Baseline concentrations and changes (Δ) in intramuscular G-6-P, P_i, PCr, ADP, and pH determined by using noninvasive ^{31}P-NMR spectroscopy in nondiabetic men studied during hyperinsulinemic euglycemia

	Low FFAs	Basal (fasting) FFAs	Elevated FFAs
G-6-P (mg/kg muscle)			
Baseline	0.108 ± 0.004	0.107 ± 0.010	0.100 ± 0.010
50–70 min	+0.135 ± 0.008*	+0.127 ± 0.014†	+0.091 ± 0.009† ††
160–180 min	+0.157 ± 0.009¶#	+0.112 ± 0.008§‡‡	+0.090 ± 0.011§‡‡
P_i (mg/kg muscle)			
Baseline	2.382 ± 0.131	2.393 ± 0.107	2.538 ± 0.100
50–70 min	+0.351 ± 0.038‡	+0.451 ± 0.047‡	+0.358 ± 0.021‖
160–180 min	+0.218 ± 0.041*#	+0.173 ± 0.034*#	+0.141 ± 0.035**
PCr (mg/kg muscle)			
Baseline	18.837 ± 0.461	19.524 ± 0.842	18.956 ± 0.602
50–70 min	–0.501 ± 0.100‡	–0.869 ± 0.262†	–0.541 ± 0.115‡
160–180 min	–0.539 ± 0.140†	–0.647 ± 0.102§	–0.591 ± 0.082§
ADP (mg/kg muscle)			
Baseline	0.062 ± 0.001	0.059 ± 0.001	0.060 ± 0.002
50–70 min	+0.001 ± 0.000	+0.003 ± 0.001	+0.002 ± 0.001
160–180 min	+0.000 ± 0.001	+0.002 ± 0.000‖	+0.003 ± 0.001‡
pH			
Baseline	7.070 ± 0.005	7.060 ± 0.006	7.055 ± 0.003
50–70 min	–0.008 ± 0.001*	–0.002 ± 0.002	–0.001 ± 0.001
160–180 min	–0.013 ± 0.001‡	+0.003 ± 0.002	+0.008 ± 0.002 # ‡‡

Data are means ± SE of seven experiments at each FFA concentration. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.005$, §$P < 0.001$, ‖$P < 0.0005$, ¶$P < 0.00005$ vs. corresponding baseline value. #$P < 0.05$, **$P < 0.005$ vs. corresponding values between 50 and 70 min. ††$P < 0.05$ vs. low and basal FFAs. ‡‡$P < 0.05$ vs. low FFAs.

(Fig. 3*B*) without any difference between the studies. The rise of intramuscular G-6-P concentrations was blunted with as little as 45 min of high FFA exposure ($P = 0.008$ vs. CON). Until $t = 68$ min, G-6-P levels further increased in control experiments ($P < 0.05$), but they remained constant in the presence of either basal or high plasma FFA concentrations. At the end of clamps, intramuscular G-6-P concentrations had increased ~2.7-fold under control conditions ($P < 0.0005$ vs. baseline). That increase was clearly reduced in the presence of basal and high plasma FFA concentrations (total G-6-P at 180 min: 197 ± 21 and 213 ± 18 µmol/l vs. CON: 286 ± 19 µmol/l, $P < 0.05$) but was not different between the two studies (Fig. 3*B*, Table 1).

Intramuscular P_i, PCr, ADP, and pH. Intramuscular concentrations of P_i, PCr, ADP, and pH at baseline as well as changes during the clamp tests are summarized in Table 1. Baseline (fasting) concentrations of P_i, PCr, ADP, and pH were similar before the start of the clamps. Intramuscular P_i increased in parallel by ~15% within 1 h and then slowly declined in all studies (Fig. 4*A*). Independently of the plasma FFA concentration, intramuscular PCr gradually decreased within 1 h in all studies and remained stable thereafter. During lipid infusion with or without heparin, intramuscular ADP slightly but significantly increased until the end of the clamps. During control experiments, pH slightly but consistently declined until 180 min ($P < 0.005$ vs. baseline) (Fig. 4*B*). In the presence of high FFA concentrations, intracellular pH gradually increased ($P < 0.05$ vs. values between 50 and 70 min) until the end of the studies (180 min; $P < 0.05$ vs. CON).

DISCUSSION

Short-term plasma FFA elevation started to inhibit insulin-stimulated whole-body glucose uptake after 160 min. That decrease in whole-body insulin sensitivity was preceded by a blunted increase of intramuscular G-6-P, which was already noted within 1 h. An FFA-dependent accumulation of intracellular G-6-P concentration, which has been predicted by Randle's hypothesis (8,9) and observed during clamp studies in rats (11,12), could not be detected in humans. An initial transient increase of intramuscular G-6-P would be expected to allosterically inhibit glucose transport/phosphorylation. But even at improved time resolution of in vivo NMR spectroscopy to detect rapid changes of G-6-P, no such initial rise of intramuscular G-6-P was noted in the present study when compared with control experiments. This finding therefore supports the concept that an alternative or additional mechanism is operative in FFA-induced insulin resistance in humans.

As a result of its location between glucose transport/phosphorylation and glycogen synthase enzymes along the pathway of muscle glycogen synthesis, the intracellular G-6-P concentration is sensitive to the relative activities of the involved enzymes and the rate of glycolysis. Similar or decreased G-6-P concentrations in the presence of impaired nonoxidative glucose disposal argue for a defect at the steps of glucose transport/phosphorylation (24). Interestingly, impairment of G-6-P elevation was already detected at 45 min, which was previously seen only at 90 min of high FFA exposure (23). Basal (fasting) plasma FFA concentrations also induced a blunted increase in G-6-P, although of smaller extent and with a delay of more than 1 h. The changes in intramuscular G-6-P occurred more than 100 min before any reduction in whole-body glucose metabolism, which is mostly due to decreased muscle glycogen synthesis (23). It is therefore conceivable that FFAs primarily inhibit glucose transport/phosphorylation, resulting in a reduction of the

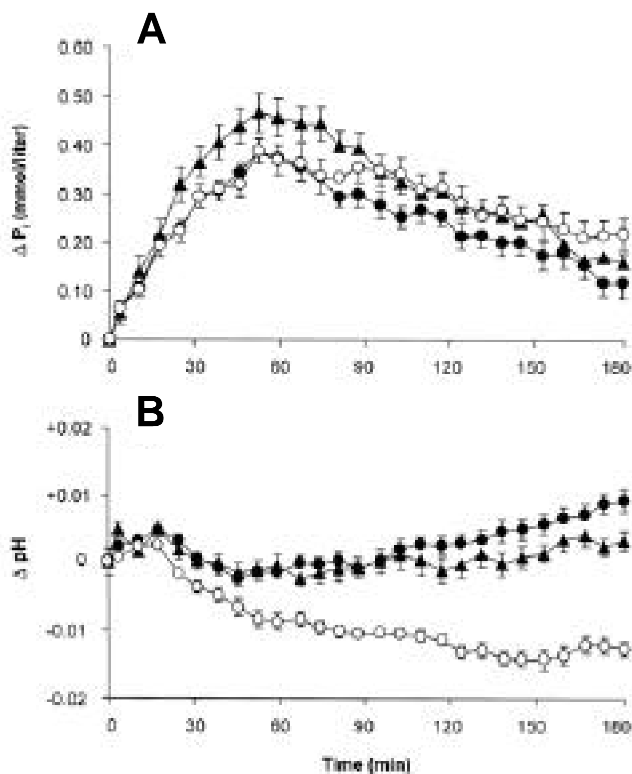

FIG. 4. Changes (Δ) in intramuscular P_i (*A*) and pH (*B*) in the presence of low (CON, ○), basal (BAS, ▲), and elevated (LIP, ●) plasma FFA concentrations. Data are given as means ± SE of seven healthy subjects who underwent each of the three studies.

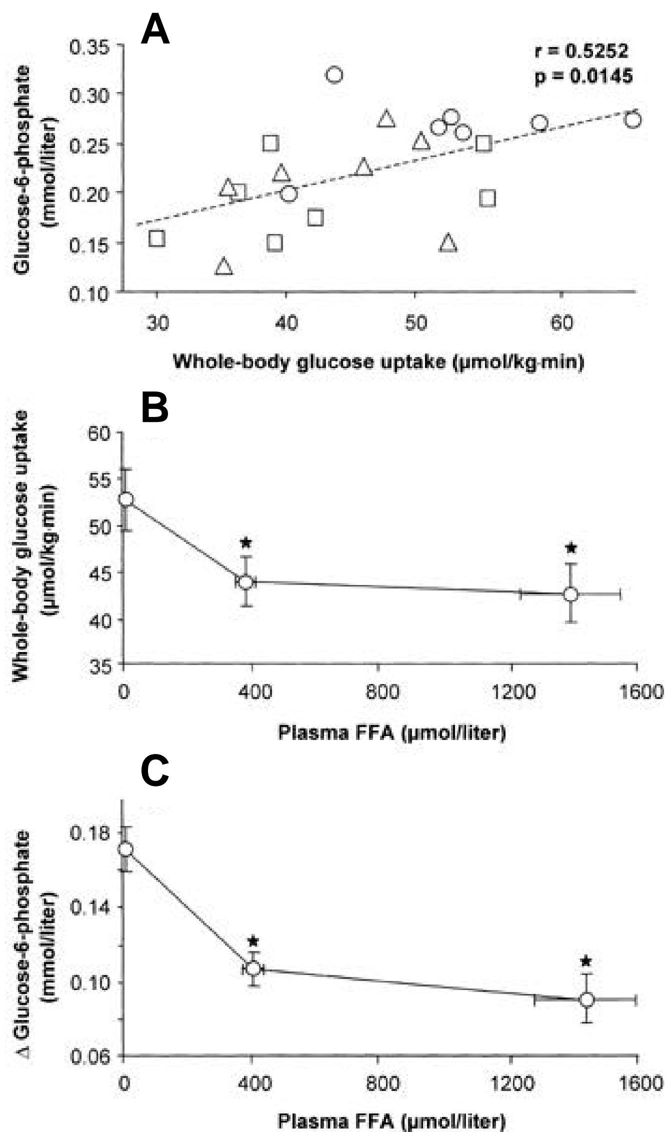

FIG. 5. *A*: Linear correlation between whole-body glucose uptake (*M* value, 160–180 min) and total intramuscular G-6-P concentration (mean G-6-P, 160–180 min) in the presence of low (○), basal (△), and elevated (□) plasma FFA concentrations. Whole-body glucose uptake (*B*) (*M* value, 160–180 min) and rise of intramuscular G-6-P over baseline (*C*) (mean ΔG-6-P, 160–180 min) at various plasma FFA concentrations. Data are given as means ± SE of seven healthy subjects who underwent all studies.

intracellular G-6-P pool. Intracellular G-6-P might subsequently gain control over the flux through glycogen synthase, since the G-6-P concentration for half-maximal stimulation of glycogen synthase is close to intramuscular G-6-P concentrations measured in vivo (~100 µmol/l) (32).

During the last 20 min of the clamp, intramuscular G-6-P was correlated with whole-body glucose uptake (Fig. 5*A*), which is in keeping with previous reports (24,25). Both whole-body glucose metabolism (Fig. 5*B*) and intramuscular G-6-P concentrations decreased with increasing plasma FFA concentrations in the physiological range (Fig. 5*C*). A small but significant elevation of plasma FFAs has been reported for offspring of parents with type 2 diabetes who are insulin resistant and at increased risk of developing diabetes (4–6). These subjects also present with an impaired increase of intramuscular G-6-P and with low whole-body glucose uptake during hyperinsulinemic-euglycemic clamp tests (26). The FFA-induced defects of glucose transport/phosphorylation are also in agreement with the metabolic alterations characteristic of human obesity (33,34).

Our results are partly in contrast to the findings of Boden et al. (17), who reported increased G-6-P at high (~750 µmol/l) plasma FFA concentrations and slightly, but not significantly, decreased G-6-P levels at lower (~550 µmol/l) plasma FFA concentrations. These differences from the present study may reside in the use of different experimental protocols, the muscle group studied, and/or the analytical methods. The present findings do not allow us to localize the defect to either glucose transport via GLUT4 or to glucose phosphorylation by hexokinase II. Although insulin has been shown to regulate hexokinase II activity or subcellular distribution (33,35), glucose transport more likely is the rate-controlling step for insulin-stimulated glucose disposal (36,37). Intrinsic activity of GLUT4 might be decreased during FFA exposure, similar to what has been reported for high-fat fed rats (38). It is notable that a decreased rate constant of ^{18}F-deoxyglucose transport was also found during insulin stimulation in skeletal muscle of obese patients (39).

Alternatively, FFAs could also decrease muscle insulin sensitivity by increasing the flux of G-6-P to the hexosamine biosynthetic pathway (40). Recently, Hawkins et al. (41) have elegantly demonstrated that the FFA-induced decrease of glucose uptake and glycogen synthesis is accompanied by an

increase of muscle UDP-*N*-acetyl-glucosamine in rats. Under comparable conditions of hyperinsulinemia-euglycemia, these authors found a similar time-course of glucose disposal, which became significantly lower at ~3 h of lipid, glucosamine, or uridine infusions. Interestingly, muscle G-6-P concentrations were increased at that time and decreased only thereafter, which is keeping with other studies in rats (11,12). Under these conditions, more G-6-P would be converted to fructose-6-phosphate and in turn result in elevation of intramuscular hexosamines, which have been shown to decrease glucose transport and GLUT4 translocation in vivo and in vitro (40,42,43). In the present study, G-6-P concentrations increased in parallel during the first ~30 min, but they were not higher during FFA exposure. Thus, it is unlikely that such a mechanism is involved in FFA-induced insulin resistance in humans, but it cannot be excluded that FFAs may stimulate enzymes involved in the hexosamine pathway, resulting in an increased flux of G-6-P to hexosamines and lower intramuscular G-6-P levels. Finally, other mechanisms, such as alteration of membrane fluidity and/or interaction with insulin binding and action (44,45), intramuscular malonyl-CoA (46), and/or impaired glucose or insulin delivery to skeletal muscle due to reduced blood flow (47), might be involved in the metabolic action of FFAs.

This study also found a small but continuous decline of intramuscular pH during the hyperinsulinemic-euglycemic control clamps. This is in contrast to the insulin-induced increase of intracellular pH in frog and rat muscle (48,49) that has been attributed to stimulation by insulin of the Na^+/H^+ exchanger, resulting in a decrease of the cytoplasmic H^+ concentration with a concomitant rise of Na^+ (50). The observed decrease of intramuscular pH during hyperinsulinemia is in excellent agreement with one previous study (51) and can be explained by an increase of the resting membrane potential and/or the rate of glycolysis and lactate production. Interestingly, intramuscular pH did not decrease but rather increased during lipid infusion. Although stimulation of membrane-associated acid extrusion systems, like the $Na^+/2HCO_3^-$ transporter, could account for that effect, FFAs may rather decrease lactate production (23) by inhibition of glycolysis (11,12,15–17), which in turn might cause pH to rise. It is notable that the observed changes in intracellular pH during FFA exposure might in part allosterically affect hexokinase activity (52).

Intramuscular P_i rose by ~15% within the first 60–90 min, which is in good agreement with previous hyperinsulinemic clamp studies (24,51). That increase could be due to increased uptake (50), presumably by using the Na^+-dependent P_i transporter (53), which is keeping with the fall of plasma P_i concentrations during hyperinsulinemic clamps (51). Alternatively, P_i may reflect an increase in oxidative ATP production (25), since the sum of P_i and G-6-P roughly equals the decline of intracellular PCr. Interestingly, closely monitoring the time-course of intramuscular P_i revealed that the increase is followed by a decrease of P_i toward baseline levels until the end of the studies. Decreased plasma P_i and/or gradual reduction of ATP production with prolonged insulin stimulation could account for a decrease of the transcellular gradient for P_i influx into skeletal muscle. Nevertheless, intramuscular P_i, PCr, and ADP were not different between the experimental protocols, indicating that allosterical effects of these compounds on glucose-metabolizing enzymes do not contribute to the FFA-induced decrease of glucose disposal.

In conclusion, plasma FFA concentrations within the physiological range cause rapid impairment of the insulin-dependent rise in intramuscular G-6-P concentrations that precedes the reduction of whole-body glucose disposal by up to 120 min. In contrast to Randle's classical hypothesis, this finding indicates that FFAs induce a defect at the glucose transport/phosphorylation step that is identical to that described in obese subjects (54) and offspring of type 2 diabetic patients (26). FFAs might therefore be involved in the early stages of insulin resistance when subjects are still normoglycemic.

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