Mechanism of Amino Acid–Induced Skeletal Muscle Insulin Resistance in Humans

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Plasma concentrations of amino acids are frequently elevated in insulin-resistant states, and a protein-enriched diet can impair glucose metabolism. This study examined effects of short-term plasma amino acid (AA) elevation on whole-body glucose disposal and cellular insulin action in skeletal muscle. Seven healthy men were studied for 5.5 h during euglycemic (5.5 mmol/l), hyperinsulinemic (430 pmol/l), fasting glucagon (65 ng/l), and growth hormone (0.4 μg/l) somatostatin clamp tests in the presence of low (~1.6 mmol/l) and increased (~4.6 mmol/l) plasma AA concentrations. Glucose turnover was measured with D-[6,6-$^2H_2$]glucose. Intramuscular concentrations of glycogen and glucose-6-phosphate (G6P) were monitored using ^{13}C and ^{31}P nuclear magnetic resonance spectroscopy, respectively. A ~2.1-fold elevation of plasma AAs reduced whole-body glucose disposal by 25% ($P < 0.01$). Rates of muscle glycogen synthesis decreased by 64% (180–315 min, 24 ± 3; control, 67 ± 10 μmol · l^{-1} · min^{-1}; $P < 0.01$), which was accompanied by a reduction in G6P starting at 130 min ($\Delta G6P_{260-300\ min}$, 18 ± 19; control, 103 ± 33 μmol/l; $P < 0.05$). In conclusion, plasma amino acid elevation induces skeletal muscle insulin resistance in humans by inhibition of glucose transport/phosphorylation, resulting in marked reduction of glycogen synthesis. *Diabetes* 51:599–605, 2002

Plasma concentrations of alanine and particularly branched-chain amino acids (AAs) are elevated in insulin-resistant states such as obesity (1,2), and high dietary protein intake impairs glucose metabolism mainly by changing the utilization of gluconeogenic precursors (3–6).

The mechanisms by which AAs could reduce skeletal muscle glucose uptake are as yet unclear. At the cellular level, availability of substrates for energy production, such as AAs and free fatty acids (FFAs), may play an important role in modulating the response to insulin (7). In vitro studies demonstrated that AAs may inhibit glucose utilization in skeletal muscle at various levels. AAs could decrease glucose oxidation by substrate competition with glucose (8,9) and/or reduce glucose uptake (10) by interaction with early steps of insulin signaling (11). Studies in humans, however, revealed controversial results. Infusion of AAs decreased forearm and whole-body glucose disposal in some (12–15), but not all (16,17), studies. Moreover, endogenous release of insulin (18) and glucagon (19) induced by plasma AA elevation might have obscured possible direct effects of AAs in those studies. Taking together all these factors, it is uncertain whether AAs directly induce skeletal muscle insulin resistance in vivo and if so, which mechanism (glucose uptake versus substrate competition) is responsible for such an effect.

This study was therefore designed to examine effects of plasma AA elevation on skeletal muscle glucose metabolism by combining isotope dilution technique with in vivo nuclear magnetic resonance (NMR) spectroscopy of gastrocnemius muscle from healthy young humans. In vivo [^{13}C]NMR spectroscopy is used to follow the time course of muscle glycogen synthesis (20), whereas [^{31}P]NMR spectroscopy monitors changes in glucose-6-phosphate (G6P) concentrations and allosteric effectors of glucose-metabolizing enzymes (21). Plasma concentrations of glucoregulatory hormones were matched between AA infusion and control studies using somatostatin/insulin/glucagon/growth hormone infusions in order to exclude indirect effects of AAs mediated by endogenous hormone release.

RESEARCH DESIGN AND METHODS

Subjects. Seven healthy male volunteers (age 27 ± 2 years, BMI 22.6 ± 0.6 kg/m^2) without family history of diabetes or dyslipidemia were included in this study. They were not glucose intolerant, suffering from conditions related to insulin resistance, or taking any medication. The 2 study days were separated by 2–12 weeks, during which their body weight and lifestyle remained unchanged. The protocol was approved by the local ethics board, and informed consent was obtained from all subjects after the nature and possible consequences of the procedures had been explained to them.

Study protocol. The participants were randomly assigned to either AA or saline infusion and crossed over to the other treatment on the second study day. They were instructed to ingest an isocaloric diet (carbohydrate/protein/fat: 60/20/20%) during the 2 days preceding the studies. After overnight fasting for 12 h, studies were begun at 6:00 A.M. (−120 min) with the insertion of catheters (Vasofix; Braun, Melsungen, Germany) into one antecubital vein of the left and one of the right arm for blood sampling and infusion, respectively. Somatostatin (UCB Pharma, Vienna, Austria) was infused at a rate of 0.1 μU · kg^{-1} · min^{-1} (−5 to 330 min) to suppress AA-induced secretion of glucoregulatory hormones such as insulin, glucagon, and growth hormone. Glucagon (0.9 ng · kg^{-1} · min^{-1}) (Novo Nordisk, Bagsvaerd, Denmark) and

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AA, amino acid; APE, atom percent excess; EGP, endogenous glucose production; FFA, free fatty acid; G6P, glucose-6-phosphate; GIR, glucose infusion rate; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; PCr, phosphocreatine; Pi, inorganic phosphate; R_a, rate of glucose appearance; R_d, rate of glucose disappearance; RIA, radioimmunoassay.

growth hormone (2.4 ng · kg^{-1} · min^{-1}) (Genotropin; Upjohn & Pharmacia, Stockholm, Sweden) were replaced (0–330 min) to maintain their fasting peripheral concentrations. Insulin (Actrapid; Novo Nordisk) was administered as primed-continuous infusion (1 mU · kg^{-1} · min^{-1}) from 0 to 330 min to create conditions of standardized hyperinsulinemia (~430 pmol/l), and plasma glucose was maintained at ~5.5 mmol/l using a variable D-glucose infusion containing 10% D-[1-^{13}C]glucose (Cambridge Isotope Laboratories, Andover, MA). Glucose turnover rates were determined using D-[6,6-$^{2}H_2$]glucose (Cambridge Isotope Laboratories) (98% enrichment; bolus, 16.7 μmol/kg; continuous infusion from –120 min to 330 min, 0.17 μmol · kg^{-1} · min^{-1}). During the clamps, plasma D-[6,6-$^{2}H_2$]glucose enrichments were kept constant using a variable glucose infusion enriched to 1.7% with D-[6,6-$^{2}H_2$]glucose (22). On one study day (AA), plasma AA concentrations were raised by infusion (0–330 min) of a balanced mixture of AAs (0.17 g · kg^{-1} · h^{-1}) (Aminoplasmal 10% without electrolytes; Braun), which is commonly used for parenteral nutrition. This solution contains the following L-AAs: isoleucine (5.1 g/l), leucine (8.9 g/l), valine (4.8 g/l), lysine (5.6 g/l), methionine (3.8 g/l), phenylalanine (5.1 g/l), tryptophan (1.8 g/l), arginine (9.2 g/l), histidine (5.2 g/l), *N*-acetyl-cysteine (0.7 g/l), proline (8.9 g/l), threonine (4.1), glutamate (4.6 g/l), serine (2.4 g/l), glycine (7.9 g/l), alanine (13,7 g/l), asparagine (3.3 g/l), ornithine (3.2 g/l), tyrosine (0.3 g/l), *N*-acetyl-tyrosine (1.3 g/l), and aspartate (1.3 g/l). During control studies, normal saline was infused at identical infusion rates.

In vivo [^{13}C] and [^{31}P]NMR spectroscopy. Intracellular concentrations of glycogen, G6P, and effectors of glucose-metabolizing enzymes in the gastrocnemius-soleus muscle were quantified noninvasively on a 3-T magnetic resonance spectrometer (Medspec S300-DBX; Bruker, Ettlingen, Germany) as described (20–24). During acquisition of spectra, subjects remained in supine position in the spectrometer with the right calf muscle positioned over a [^{13}C], [^{31}P], [^{1}H] triple-resonant circular surface coil (10 cm in diameter; Bruker). The magnetic field was shimmed on the global water signal (usual full-width half-maximum, ~35 Hz). Changes of G6P from baseline values were determined by manual processing of ^{31}P difference pulse-acquire spectra (pulse length, 150 ms; flip angle, 90° in the coil plane; repetition time, 6 s; number of averages, 64), which were acquired over 7-min intervals (0–50 min; 130–180 min; 260–300 min). Changes of inorganic phosphate (Pi) and phosphocreatine (PCr) were determined by batched time domain analysis of spectra using the MRUI software package (European Communities Project TMR/Networks ERB-FMRX-CT970160; http://carbon.uab.es/mrui/) (25). Intracellular pH and ADP concentrations were calculated as previously reported (22,23).

Intramuscular glycogen concentrations were measured at baseline and every 60 min during the clamp study from ^{13}C difference spectra obtained in duplicate with a proton-decoupled ^{13}C pulse-acquire sequence (repetition time, 400 ms; pulse length, 150 ms; flip angle, 90° in the coil plane; number of averages, 1,250). A proton decoupling pulse sequence (WALTZ 4) with peak power of 80 W was applied at the glycogen C1 proton resonance frequency during the 25.6 ms (512 data points; spectral width, 10,000 Hz) acquisition period. Power deposition was limited to 1.32 W/kg. Glycogen concentrations were quantified by integration of the C1-glycogen peak at 100.5 ppm and comparison with an external standard (20,26) after correction for the sensitive volume of the coil and coil loading.

Plasma metabolites and hormones. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Concentrations of individual plasma amino acids were measured by high-performance liquid chromatography (HPLC) as described (27,28). Plasma FFA concentrations were assayed with a microfluorimetric method (Wako Chemical, Richmond, VA). Plasma lactate concentrations were measured enzymatically (Roche, Darmstadt, Germany). Plasma immunoreactive insulin, C-peptide, glucagon, and growth hormone were measured by commercially available radioimmunoassays (RIAs) (insulin: Pharmacia, Uppsala, Sweden; C-peptide: CIS, Gif-Sur-Yvette, France; glucagon: Linco, St. Charles, MO; growth hormone: Sorin Biomedica, Saluggia, Italy). Plasma cortisol was determined following extraction and charcoal-dextran separation by RIA (29). Plasma catecholamines were analyzed by reverse-phase HPLC (30).

Gas chromatography-mass spectrometry for the determination of atom percent excess (APE) of ^{2}H and ^{13}C in glucose was performed as described (22). The glucose-pentaacetate was analyzed on a Hewlett-Packard 5890 gas chromatograph equipped with a CP-Sil5 25 m × 0.25 mm × 0.12 μm capillary column (Chrompack, Middelburg, the Netherlands) interfaced to a Hewlett-Packard 5971A Mass Selective Detector operating in the electron impact ionization mode. Selective ion monitoring was used to determine tracer enrichments in various molecular mass ion fragments of glucose. 6,6-$^{2}H_2$ (M+2) enrichments in glucose were assayed in fragments of C3-C6 with their masses of 187 and 189. 1-^{13}C (M+1) enrichments in glucose were determined from fragments of C1-C6 with their masses of 314 and 315.

Calculations. At baseline, rates of endogenous glucose production (EGP) were calculated by dividing the tracer ([6,6-$^{2}H_2$]glucose) infusion rate times tracer enrichment by the percent tracer enrichment in plasma and subtracting the tracer infusion rate (31). Rates of glucose appearance (R_a) and disappearance (R_d) during the clamp tests were calculated using Steele's non–steady-state equations (32) with EGP given as the difference between R_a and glucose infusion rates (GIRs).

Increments in glycogen concentration were calculated from the change in [1-^{13}C]glycogen and the plasma ^{13}C APE in glucose as described (20). Rates of muscle glycogen synthesis were calculated from the slope of the least-squares linear fit to the glycogen concentration curve between 180 and 315 min.

Statistics. All data are given as means ± SE. Statistical comparisons between saline and AA infusion studies were performed using the paired Student's *t* test. Data within one group were compared by repeated-measurements ANOVA and Dunnett's or Fisher's post hoc testing. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Metabolites and hormones. Fasting plasma glucose (AA, 5.34 ± 0.11; control, 5.65 ± 0.15 mmol/l), insulin (AA, 48 ± 6; control, 47 ± 6 pmol/l), C-peptide (AA, 0.55 ± 0.06; control, 0.53 ± 0.04 nmol/l), glucagon (AA, 75 ± 3; control, 75 ± 13 ng/l), and cortisol (AA, 334 ± 44; control, 372 ± 45 nmol/l) were not different between AA infusion and control studies (Fig. 1). During the clamp tests, plasma glucose remained at baseline and was not different between the studies (Fig. 1*A*). Plasma insulin increased similarly to ~430 pmol/l (Fig. 1*B*), whereas plasma C-peptide was equally suppressed in both studies (Fig. 1*C*). Plasma glucagon was not different between the two studies (Fig. 1*D*). Fasting plasma growth hormone concentrations varied during the baseline period (−120 min: AA, 0.49 ± 0.24; control, 0.22 ± 0.06 μg/l; NS; 0 min: AA, 0.65 ± 0.18; control, 1.16 ± 0.87 μg/l; NS), likely because of its spontaneous pulsatile secretion pattern (33). During the clamp tests, plasma growth hormone was comparable between the two studies (Fig. 1*E*). Plasma cortisol concentrations were similar between the two studies (Fig. 1*F*). Plasma concentrations of epinephrine (0 min: AA, 80 ± 12; control, 111 ± 36 pmol/l; 330 min: AA, 84 ± 12; control, 84 ± 16 pmol/l) and norepinephrine (0 min: AA, 1.19 ± 0.08; control, 1.25 ± 0.18 nmol/l; 330 min: AA, 1.23 ± 0.21; control, 1.39 ± 0.38 nmol/l) neither changed from baseline values nor differed between the two studies. Plasma FFA concentrations were comparable at baseline (AA, 397 ± 66; control, 332 ± 31 μmol/l) and similarly fell to ~20 μmol/l in both studies from 60 min on. Plasma lactate concentrations were similar at baseline (AA, 0.92 ± 0.2; control, 0.78 ± 0.08 mmol/l) and gradually increased during insulin infusion in both studies (AA, 1.15 ± 0.09; control, 1.19 ± 0.09 mmol/l; NS).

Fasting plasma AA concentrations were comparable in the two studies (Fig. 2*A*; Table 1). During AA infusion, the total plasma amino acid concentration increased by ~2.1-fold, whereas it decreased by ~19% during control studies (Fig. 2*A*). Plasma concentrations of individual AAs at baseline and during steady-state conditions are presented in Table 1. Except for glutamate, all AAs contributed to the rise in plasma AA concentrations observed during AA infusion. Whereas most AAs decreased during control studies, glutamine, histidine, glycine, and alanine remained at their basal fasting levels.

Glucose turnover. EGP was comparable between the two studies at baseline (AA, 10.5 ± 0.4; control,11.0 ± 0.4 μmol · kg^{-1} · min^{-1}) and close to zero during the clamp

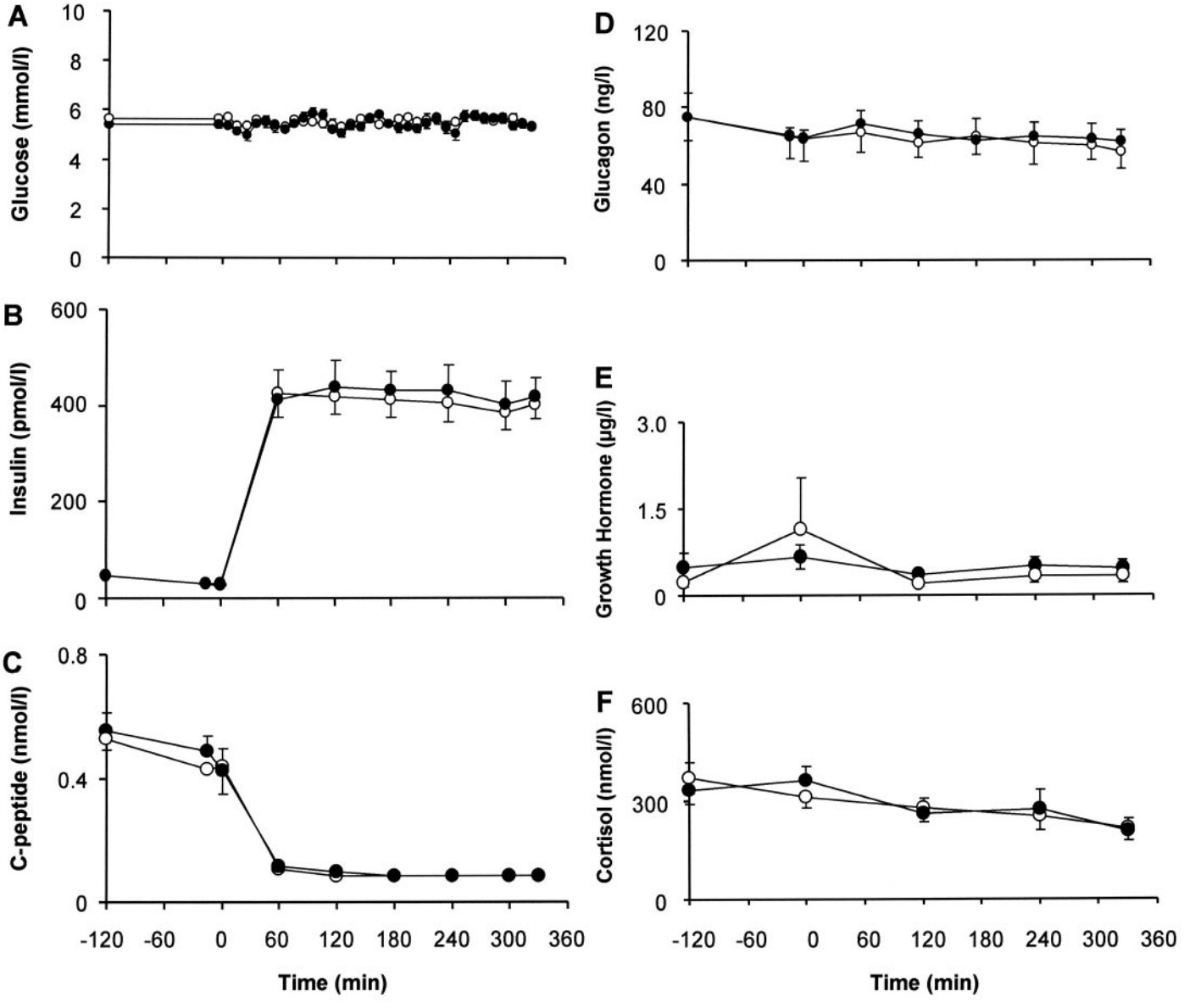

FIG. 1. Plasma concentrations of glucose (*A*), insulin (*B*), C-peptide (*C*), glucagon (*D*), growth hormone (*E*), and cortisol (*F*) during saline (control, ○) and AA (●) infusion. Data are given as means ± SE of seven healthy subjects who underwent both clamp tests.

tests (Table 2). GIRs required to maintain euglycemia were ~24% ($P < 0.01$) lower during AA infusion than control studies from 90 min on (Fig. 2*B*). At steady state (270–330 min), the reduction in GIR (~25%) was similar to the decrease of tracer-determined R_d (~21%) during AA infusion (Table 2).

Intramuscular glucose metabolism. At baseline, skeletal muscle glycogen concentrations were comparable (AA, 70 ± 4; control, 67 ± 2 mmol/l). The insulin-stimulated increase in glycogen concentrations was lower ($P < 0.05$) at the end of plasma AA elevation (Fig. 2*D*). Plasma [^{13}C]glucose APE remained constant from 180 min on and did not differ between the studies (Fig. 3*A*). This made it possible to calculate net rates of glycogen synthesis, which were ~64% lower ($P < 0.01$) during AA infusion compared with control studies (Fig. 3*B*).

Intramuscular G6P concentrations were identical at baseline (AA, 0.10 ± 0.01; control, 0.10 ± 0.02 mmol/l). In parallel to the reduction of GIRs, the insulin-induced rise in G6P was lower ($P < 0.05$) during AA infusion from 130 min onward (Fig. 2*C*). Glycogen synthetic rate as well as insulin-induced rise in G6P was decreased during AA infusion in each of the seven participants (Fig. 4*A* and *B*).

Intramuscular Pi, ADP, PCr, and pH at baseline and during the clamp tests are summarized in Table 3. Concentrations of Pi, PCr, ADP, and pH were comparable at baseline. During control studies, only intramuscular Pi transiently increased and was higher than during AA infusion between 150 and 180 min. Intracellular concentrations of ADP, PCr, and pH were not different between the two studies.

DISCUSSION

This study demonstrates that short-term elevation of plasma AAs induces skeletal muscle insulin resistance in healthy humans. At steady state, the reduction of whole-body glucose disposal by ~25% along with complete

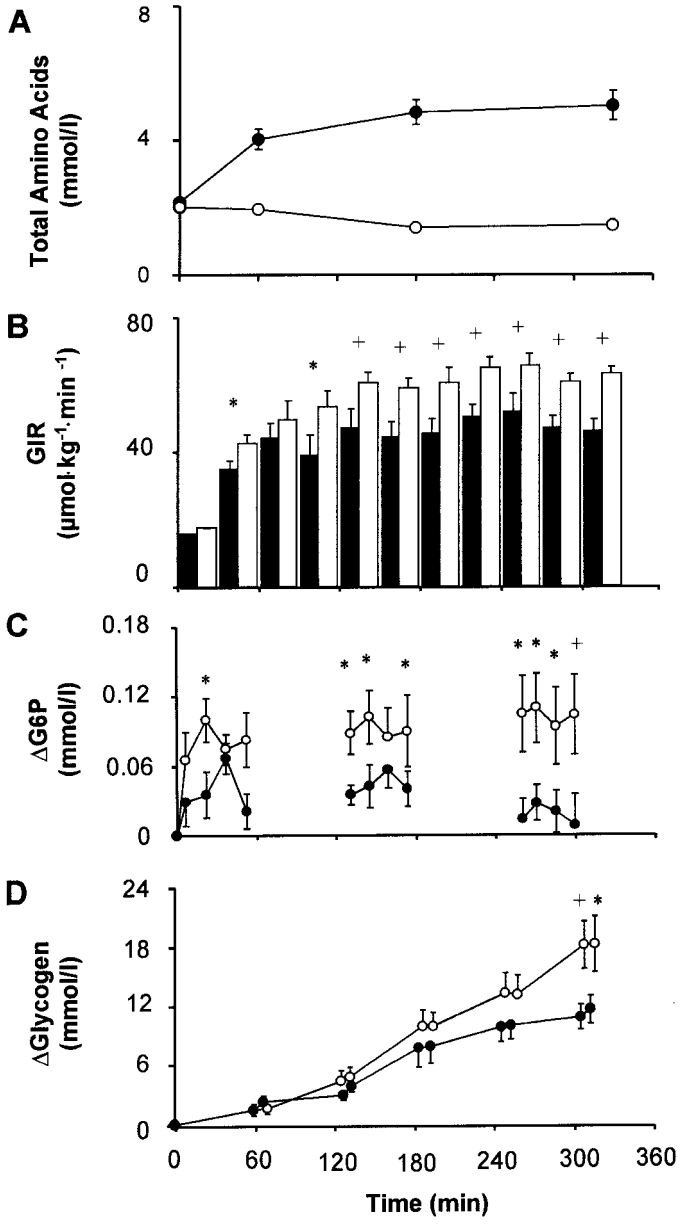

FIG. 2. Plasma concentrations of total AAs (*A*), GIRs (*B*), increments in intramuscular G6P (*C*), and increments in glycogen concentrations (*D*) during saline (control, □ and ○) and AA (■ and ●) infusion. Data are given as means ± SE of seven healthy subjects who underwent both clamp tests. *$P < 0.05$, +$P < 0.01$ vs. AA.

suppression of EGP indicates diminished peripheral glucose uptake during plasma AA elevation. This finding is in contrast to a previous report (16), which concluded that the decrease in GIR during AA infusion was entirely attributable to increased EGP due to a combined elevation of plasma AA and glucagon concentrations. However, in that study, plasma insulin concentrations were up to twofold higher during AA infusion than during control studies, which likely counteracted the AA-dependent inhibition of glucose uptake.

Consistent with the findings of the present study, previous reports indicated that AAs might induce insulin resistance in human skeletal muscle (12–15). However, the mechanism responsible for impaired glucose uptake in vivo was not elucidated. In vitro studies showed that the availability of certain nutrients may play an important role in determining the cellular response to insulin stimulation. From studies in isolated rat muscle preparations, Randle et al. (34) concluded that FFAs reduce glucose uptake by substrate competition between FFAs and glucose for mitochondrial oxidation. Increased FFA oxidation would cause elevation of the intramitochondrial acetyl-CoA/CoA and NADH/NAD^+ ratios, which decreases the activities of

TABLE 1
Plasma concentrations of individual AAs (μmol/l) at baseline (0 min) and at the end of AA and saline infusion (330 min)

AAs	Time (min)	AA	Control
Alanine	0	284 ± 23	245 ± 24
	330	916 ± 60*	265 ± 7†
Arginine	0	79 ± 4	74 ± 6
	330	278 ± 22*	44 ± 3†‡
Asparagine	0	43 ± 1	38 ± 2
	330	121 ± 10*	25 ± 1†‡
Citrulline	0	35 ± 1	34 ± 2
	330	54 ± 4†	17 ± 1†‡
Glutamine	0	520 ± 30	476 ± 50
	330	738 ± 54‡	393 ± 29*
Glutamate	0	58 ± 18	64 ± 16
	330	62 ± 12	48 ± 19*
Glycine	0	210 ± 7	192 ± 16
	330	714 ± 76*	167 ± 13‖
Histidine	0	91 ± 4	81 ± 7
	330	259 ± 23†	70 ± 5†
Isoleucine	0	56 ± 3	61 ± 4
	330	127 ± 15‡	12 ± 1†‡
Leucine	0	113 ± 5	106 ± 8
	330	259 ± 27*	43 ± 2†*
Methionine	0	36 ± 2	31 ± 2†*
	330	194 ± 20*	14 ± 1†*
Phenylalanine	0	47 ± 2	42 ± 3
	330	140 ± 13	25 ± 1†‡
Serine	0	116 ± 7	96 ± 7¶
	330	181 ± 10‡	68 ± 3†‡
Taurine	0	36 ± 2	32 ± 2
	330	42 ± 3§	29 ± 1‖‡
Threonine	0	107 ± 3	91 ± 6
	330	266 ± 21*	62 ± 3†‡
Tryptophan	0	71 ± 5	63 ± 4
	330	117 ± 10*	37 ± 3†*
Tyrosine	0	51 ± 2	44 ± 2
	330	36 ± 2‡	22 ± 2†*
Valine	0	197 ± 24	196 ± 15
	330	317 ± 34‡	115 ± 6‖‡
Branched-chain AAs	0	366 ± 27	353 ± 29
	330	703 ± 75*	142 ± 30†‡

Data are means ± SE of seven healthy subjects who underwent both somatostatin insulin glucagon growth hormone clamp tests. †$P <$ 0.001 vs. AA, ‖$P < 0.01$, ¶$P < 0.05$. *$P < 0.001$ vs. corresponding baseline value, ‡$P < 0.01$, §$P < 0.05$.

TABLE 2
Tracer-determined rates of R_d and EGP during steady-state conditions (270–330 min) of AA and saline infusion

	AA	Control
R_d (μmol · kg^{-1} · min^{-1})	47.3 ± 4.4*	60.0 ± 2.6
EGP (μmol · kg^{-1} · min^{-1})	−0.7 ± 0.9	−1.6 ± 1.0

Data are given means ± SE of seven healthy subjects who underwent both somatostatin insulin glucagon growth hormone clamp tests. *$P < 0.01$ vs. saline infusion.

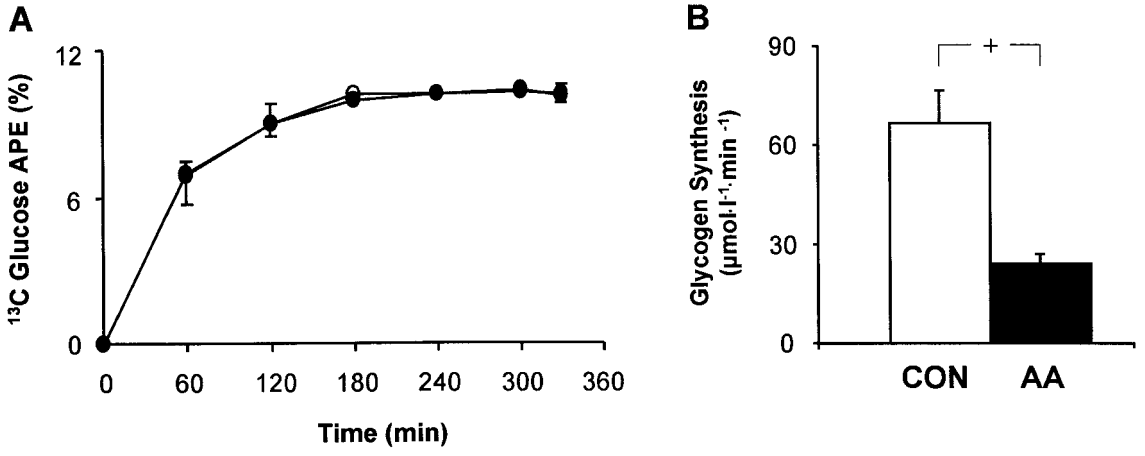

FIG. 3. Time course of plasma [^{13}C]glucose APE (*A*) and rates of skeletal muscle glycogen synthesis calculated from the glycogen concentration curves between 180 and 315 min (*B*) during saline (CON, □ and ○) and amino acid (AA, ■ and ●) infusion. Data are given as means ± SE of seven healthy subjects who underwent both clamp tests. +P < 0.01 vs. AA.

pyruvate dehydrogenase and phosphofructokinase. The subsequent reduction in glycolysis would give rise to intracellular G6P, which then decreases muscular glucose uptake by allosteric inhibition of hexokinase II. Because glycogen synthase is also under the allosteric control of G6P, high intramuscular concentrations of G6P would be expected to increase glycogen synthesis (34,35). Similar to FFAs, ketogenic AAs can be metabolized to acetyl-CoA and further oxidized by the tricarboxylic acid cycle. Therefore, substrate competition between AAs and glucose for mitochondrial oxidation might occur. Indeed AAs, particularly the branched-chain amino acids (valine, leucine, and isoleucine), decrease glucose oxidation by inhibition of pyruvate dehydrogenase secondary to a rise of the acetyl-CoA/CoA and NADH/NAD$^+$ ratios in isolated rat skeletal muscle and liver cells (8,9,36).

To examine the mechanism by which AAs caused the impairment in insulin-stimulated whole-body glucose uptake, rates of skeletal muscle glycogen synthesis and intramuscular concentrations of G6P were measured. The rate of glycogen synthesis was reduced by ~64% (P < 0.01) in the presence of plasma AA elevation. Because glycogen synthesis in skeletal muscle accounts for almost all glucose disposal under insulin-stimulated conditions (20), the observed decrease in rates of glycogen synthesis most likely accounted for the AA-induced reduction in whole-body glucose uptake. This decrease in skeletal muscle glycogen synthesis could have resulted from a reduction in glycogen synthase activity and/or inhibition of glucose transport/phosphorylation. G6P is an intermediate between glycogen synthesis and glucose transport/phos-

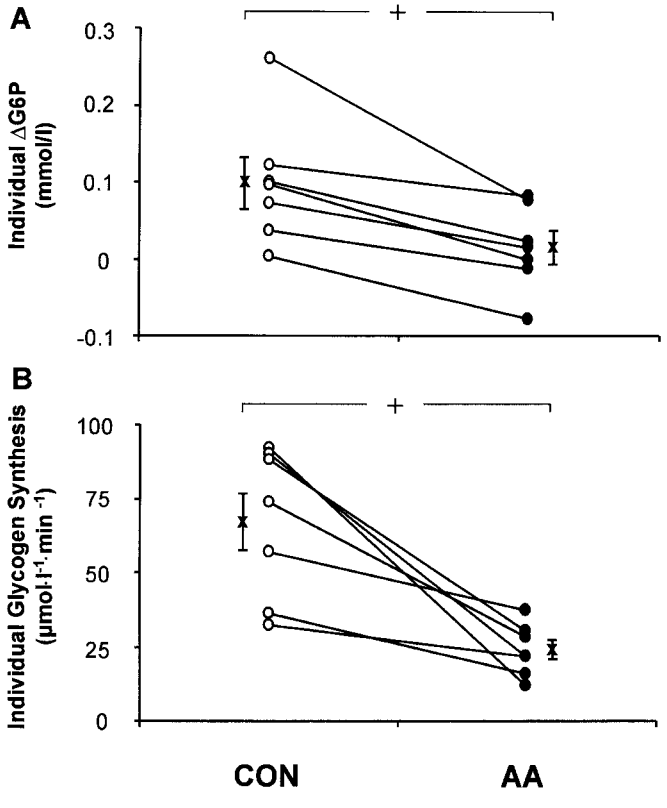

FIG. 4. Individual increments in intramuscular G6P (ΔG6P between 286 and 300 min) (*A*) and individual rates of skeletal muscle glycogen synthesis (180 and 315 min) (*B*) during saline (CON, ○) and AA (●) infusion. Individual data for each of seven healthy subjects who underwent both clamp tests and the corresponding means ± SE are presented. +P < 0.01 vs. AA.

TABLE 3
Baseline and steady-state concentrations of intramuscular Pi, ADP, PCr, and pH determined noninvasively using [^{31}P] NMR spectroscopy during AA and saline infusion

	Baseline	150–180 min	270–300 min
Pi (mmol/l)			
AA	2.92 ± 0.09	3.12 ± 0.12	2.99 ± 0.08
Control	3.16 ± 0.08	3.62 ± 0.27*	3.15 ± 0.18
ADP (μmol/l)			
AA	58 ± 2	57 ± 2	59 ± 1
Control	60 ± 2	57 ± 4	57 ± 3
PCr (mmol/l)			
AA	19.50 ± 0.33	19.19 ± 0.59	19.06 ± 0.53
Control	18.95 ± 0.41	19.59 ± 0.57	19.22 ± 0.68
pH			
AA	7.063 ± 0.007	7.037 ± 0.012	7.048 ± 0.009
Control	7.052 ± 0.008	7.050 ± 0.020	7.035 ± 0.008

Data are means ± SEM of results from six healthy subjects who underwent both somatostatin insulin glucagon growth hormone clamp tests. *P < 0.05 vs. baseline value, vs. value between 270 and 300 min, and vs. AA.

phorylation. Therefore, any isolated decline in the rate of net glycogen synthesis relative to glucose transport/phosphorylation would be expected to result in a rise of the G6P concentration (24). Furthermore, any reduction in glycolysis secondary to a possible AA-induced inhibition of pyruvate dehydrogenase would similarly give rise to G6P. In contrast to these assumptions, intramuscular G6P concentrations were lower during AA infusion than saline infusion studies, suggesting a direct inhibitory effect of AA on glucose transport/phosphorylation. Because intramuscular pH, PCr, and ADP did not change, allosteric effects of these mediators on glucose-metabolizing enzymes can be excluded. The small and transient increase in Pi during control studies is in line with previous observations (22,23). If anything, the lower intracellular concentrations of Pi, which is an allosteric inhibitor of hexokinase, observed during AA infusion would serve to underestimate the inhibitory effect of AA on glucose transport/phosphorylation (22).

Taken together, our data do not support the concept of substrate competition between AA and glucose. Likewise, recent in vitro studies showed that AA can interact with early steps of insulin signaling critical for glucose transport, resulting in impaired insulin-stimulated tyrosine phosphorylation of insulin receptor substrates with subsequent inhibition of insulin-stimulated phosphatidylinositol 3-kinase (11). Inhibition of insulin signaling could then impair insulin-stimulated glucose transport (10) and consecutively glycogen synthesis in skeletal muscle. Such mechanism of action is in line with that reported for insulin resistance typical for experimental hyperlipidemia (22,26,37), obesity (38), and type 2 diabetes (39).

Insulin-resistant states are also associated with increased plasma AA concentrations of particularly branched-chain AAs (1,2). Furthermore, a protein-enriched diet may impair glucose utilization (3–6). Thus, AAs might play a role in the development of insulin resistance typical for obesity and type 2 diabetes (20,39). The most marked increments after protein meals were observed for branched-chain AAs (leucine, isoleucine, and valine), which remained elevated for up to 8 h (40,41). From the present results, one cannot discern whether a single AA or a certain combination of AAs was responsible. Previous studies in vivo and in vitro (8,11–15,36), however, indicated that the branched-chain AAs, which are preferentially taken up by skeletal muscle (42), might be most important for the regulation of peripheral glucose metabolism.

Some limitations must be considered. First, the present study compared hyperaminoacidemic with moderately hypoaminoacidemic conditions. Total plasma AA concentrations achieved during AA infusion are comparable with those seen after ingestion of a large protein meal (40), whereas the moderate insulin-induced decrease in plasma AA of the control study is similar to what has been observed after a carbohydrate-rich meal (43). This suggests that short-term changes in plasma AAs within the physiologic range suffice to modify skeletal muscle glucose metabolism. Of note, the insulin-induced hypoaminoacidemia with low plasma concentrations of branched-chain AAs could have affected glucose kinetics and muscle glucose metabolites in control experiments of this and other studies (44). Thus, it cannot be ruled out that the differences observed between hyperaminoacidemic and hypoaminoacidemic conditions might be attenuated were fasting plasma AA concentrations present during the control study. Nevertheless, skeletal muscle G6P and glycogen concentrations previously reported for control conditions are in line with those of the present study (26,37). The lower rates of glycogen synthesis observed in this study could be due to the combined somatostatin/insulin/glucagon/growth hormone infusions, which have not been used in previous, otherwise comparable, studies (20,26,37,38).

Second, the present study was performed in the presence of fasting peripheral concentrations of the major glucoregulatory hormones such as insulin, glucagon, growth hormone, cortisol, and catecholamines. Particularly, during AA infusion, a rise in plasma glucagon was prevented to exclude its possible effects on isotopic equilibration and EGP, which may obscure the detection of direct AA action on skeletal muscle glucose disposal. It is of note that administration of protein or AAs will give rise to glucagon secretion, and hyperglucagonemia is frequently present in type 2 diabetic patients (45), which is different from the hormonal environment created in the present study. Nevertheless, peripheral effects of glucagon are unlikely, because glucagon receptors could not be demonstrated in skeletal muscle (46). In the present study, growth hormone was replaced by continuous infusion, which abolished its physiological pulsatile secretion pattern. Physiological growth hormone delivery is required for its stimulatory effects on adipose tissue lipolysis (47) and skeletal muscle protein synthesis (48) and could therefore alter the availability of circulating FFAs and intracellular AAs. Although the absence of growth hormone pulsatility could have affected our results, it is of note that dosage and mode of growth hormone infusion were identical during the two parts of the present study.

Finally, recent studies suggest that premenopausal women can be protected from nutrient-dependent insulin resistance as induced by FFAs (49,50). Because the present study included only male subjects, it cannot be ruled out that such sex differences also hold true for AA-induced insulin resistance.

In conclusion, in contrast to classical concepts of substrate competition between AAs and glucose for mitochondrial oxidation, which would lead to inhibition of pyruvate dehydrogenase, we found that elevation of plasma AAs to postprandial concentrations causes insulin resistance by direct inhibition of muscle glucose transport and/or phosphorylation with subsequent reduction in rates of glycogen synthesis. Thus, AAs might play a relevant role in the modulation of peripheral insulin sensitivity and contribute to insulin resistance observed in type 2 diabetic patients.

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