

Free Fatty Acids Induce Peripheral Insulin Resistance Without Increasing Muscle Hexosamine Pathway Product Levels in Rats

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To evaluate the role of the hexosamine biosynthesis pathway (HBP) in fat-induced insulin resistance, we examined whether fat-induced insulin resistance is additive to that induced by increased HBP flux via glucosamine infusion and, if so, whether such additive effects correlate with muscle HBP product levels. Prolonged hyperinsulinemic (~550 pmol/l) euglycemic clamps were conducted in conscious overnight-fasted rats. After the initial 150 min to attain steady-state insulin action, rats received an additional infusion of saline, Intralipid, glucosamine, or Intralipid and glucosamine ($n = 8$ or 9 for each) for 330 min. At the conclusion of clamps, skeletal muscles (soleus, extensor digitorum longus, and tibialis anterior) were taken for the measurement of HBP product levels. Intralipid and glucosamine infusions decreased insulin-stimulated glucose uptake (R_d) by 38 and 28%, respectively. When the infusions were combined, insulin-stimulated R_d decreased 47%, significantly more than with Intralipid or glucosamine alone ($P < 0.05$). The glucosamine-induced insulin resistance was associated with four- to fivefold increases in muscle HBP product levels. In contrast, the Intralipid-induced insulin resistance was accompanied by absolutely no increase in HBP product levels in all of the muscles examined. Also, when infused with glucosamine, Intralipid decreased insulin action below that with glucosamine alone without changing HBP product levels. In a separate study, short-term (50 and 180 min) Intralipid infusion also failed to increase muscle HBP product levels. In conclusion, increased availability of plasma free fatty acids induces peripheral insulin resistance without increasing HBP product levels in skeletal muscle. *Diabetes* 50:418–424, 2001

Insulin resistance is a major characteristic of type 2 diabetes and obesity (1–3). Although insulin resistance is well characterized, the mechanisms by which insulin resistance develops remain largely unknown. Skeletal muscle is responsible for the majority of peripheral insulin action to promote glucose utilization (4). In this tissue, insulin resistance (i.e., decrease in insulin-mediated glucose uptake [R_d]) is associated with impaired insulin actions on both glucose transport and intracellular glucose metabolism (5–7). Researchers have debated as to which of these defects is the primary defect responsible for the development of insulin resistance or type 2 diabetes (8–11). We have recently put forth and tested the hypothesis that during the development of insulin resistance in skeletal muscle, impairment of intracellular glucose metabolism precedes and causes impairment of insulin's action on glucose transport/uptake (12,13). We demonstrated that metabolic impairment (suppression of glycolysis) preceded insulin resistance during high-fat feeding in rats (12) and that suppression of intracellular glucose metabolism (glycolysis or glycogen synthesis) in skeletal muscle caused development of insulin resistance (13). We also reported that metabolic changes preceded the development of insulin resistance during growth hormone infusion (14). Taken together, these data support the hypothesis that impaired glucose metabolism is the primary event leading to the development of insulin resistance and/or type 2 diabetes.

One potential mechanism by which impaired glucose metabolism leads to impairment of insulin's action on R_d involves the hexosamine biosynthesis pathway (HBP) (12,15). The HBP is a minor glucose metabolic pathway converting fructose-6-phosphate (F-6-P) to nucleotide hexosamines that serve as essential substrates for protein glycosylation. Marshall et al. (16,17) discovered that the HBP is involved in the downregulation of insulin's action on glucose transport in cultured fat cells exposed to high glucose and insulin concentrations. A series of experiments by these investigators demonstrated that increased substrate flux through the HBP results in decreased insulin action on glucose transport. Subsequently, several groups (18–20) have demonstrated that an infusion of glucosamine, which increases the HBP flux, decreases insulin-mediated R_d in vivo by decreasing insulin's action on GLUT4 translocation in skeletal muscle (20). The HBP has been implicated mainly in hyperglycemia-induced insulin resistance ("glucose toxicity" [21]). Excessive glucose flux into cells with hyperglycemia would increase glucose-6-phosphate (G-6-P) and F-6-P levels

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dpm, disintegrations per minute; EDL, extensor digitorum longus; F-6-P, fructose-6-phosphate; FFA, free fatty acid; G-6-P, glucose-6-phosphate; GFAT, glutamine F-6-P amidotransferase; GINF, glucose infusion rate; HBP, hexosamine biosynthesis pathway; HGO, hepatic glucose output; HPLC, high-performance liquid chromatography; R_d , glucose uptake; TBS, tetrabutylammonium sulfate; UDP-Gal, UDP-galactose; UDP-GalNAc, uridinediphospho-N-acetylgalactosamine; UDP-Glc, UDP-glucose; UDP-GlcNAc, uridinediphospho-N-acetylglucosamine.

and consequently increase the HBP flux by mass action (22). However, similar changes (i.e., increased G-6-P/F-6-P levels and HBP flux) may occur with normal glucose influx at euglycemia if intracellular glucose metabolism is impaired (12,15). Thus, the HBP may be involved not only in hyperglycemia-induced insulin resistance but also in the development of insulin resistance at euglycemia with metabolic impairment (12,13). To support this concept, Hawkins et al. (15) reported that fat (Intralipid)-induced insulin resistance was accompanied by two- to threefold increases in muscle HBP product levels. These data support the notion that suppression of glycolysis during fat infusion may increase muscle G-6-P/F-6-P levels and HBP flux to result in insulin resistance. However, these observations were made with maximally effective insulin concentrations (~3,000 pmol/l), and it is unknown whether similar changes also occur at physiological insulin concentrations.

The present study was designed to further address the role of the HBP in fat-induced insulin resistance. We examined whether fat infusion (or increased plasma free fatty acid [FFA] levels) at physiological insulin levels increases HBP flux in skeletal muscle, as indicated by HBP product levels. In addition, we examined whether fat-induced insulin resistance is additive to that induced by increased HBP flux via glucosamine infusion and, if so, whether such additive effects correlate with muscle HBP product levels.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats weighing 275–300 g were obtained from Simonsen (Gilroy, CA) and studied at least 6 days after arrival. Animals were housed under controlled temperature ($22 \pm 2^\circ\text{C}$) and lighting (12 h of light, 0600–1800 h; 12 h of dark, 1800–0600 h) and were given free access to water and a standard rat diet. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California.

Catheterization. At least 4 days before the experiment, the animals were placed in individual cages with wire floors. The distal one-third of each rat's tail was drawn through a hole placed low on the side of the cage and secured there with a rubber stopper (12–14). This arrangement was required to protect tail blood vessel catheters during experiments. Animals were free to move about and were allowed unrestricted access to food and water. Two tail-vein infusion catheters were placed the day before the experiment, and one tail-artery blood sampling catheter was placed at least 3 h before the start of insulin infusion (i.e., ~0700 h). Catheters were placed percutaneously during local anesthesia with lidocaine while rats were restrained in a towel. The animals were returned to their cages after catheter placement with their tails secured as described above and were free to move about during the experiments. Patency of the arterial catheter was maintained by a slow (0.016 ml/min) infusion of heparinized saline (10 U/ml).

Experimental protocols. Two separate studies were performed in normal rats after an overnight fast (food was removed at 1700 h on the day before the experiment, and experiments were started at ~1100 h).

Study 1: Effects of Intralipid and/or glucosamine infusions on insulin-stimulated glucose fluxes and muscle HBP product levels.

Hyperinsulinemic-euglycemic clamp was conducted for 480 min with a continuous infusion of porcine insulin (Novo Nordisk, Princeton, NJ) at a rate of $22 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to raise plasma insulin within a physiological range. Blood samples (30 μl) were collected at 10- to 20-min intervals for the immediate measurement of plasma glucose, and 20% dextrose was infused at variable rates to maintain plasma glucose at basal concentrations (~5.6 mmol/l). After the initial 150-min clamp (control period), the clamps were continued with a constant infusion of saline ($n = 8$), Intralipid (Liposyn II [Abbott, North Chicago]; triglyceride emulsion, 20% wt/vol; 0.9 ml/h) and heparin (40 U/h with 10 U as a priming bolus; $n = 9$), glucosamine (30 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $n = 8$), or Intralipid and glucosamine together ($n = 8$) during the remaining 330 min (treatment period). To estimate insulin-stimulated whole-body glucose fluxes, we infused [$3\text{-}^3\text{H}$]glucose (high-performance liquid chromatography [HPLC]-purified; Du Pont, Boston, MA) at a rate of 0.2 $\mu\text{Ci}/\text{min}$ throughout the clamps. Blood samples for the measurement of plasma ^3H -glucose (60 μl) were taken every 10 min during the last 30 min of the control and the treatment periods.

Additional blood samples (20 or 60 μl) were taken at 0, 10, 30, 90, 150, 160, 180, 240, 360, and 480 min for the determination of plasma FFA and/or insulin concentrations. At the end of clamps, animals were anesthetized with pentobarbital sodium injection. Within 5 min, three muscles (soleus, tibialis anterior, and extensor digitorum longus [EDL]) were taken from each hindlimb for measurements of HBP products. Each muscle, once exposed, was dissected out within 2 s, frozen immediately using liquid N_2 -cooled aluminum blocks, and stored at -80°C for later analysis. Glucose and insulin infusions were continued to prevent any perturbation of glucose metabolism during the muscle sampling procedure.

Study 2: Effects of short Intralipid infusions on muscle HBP product levels. This study was carried out to examine the effects of short-term (50 and 180 min vs. 330 min in study 1) Intralipid infusion on muscle HBP product levels. For this, we used muscles collected from an independent study in which experimental conditions were identical to those of study 1, except for the rate and duration of Intralipid infusion; the Intralipid infusion rate was 0.75 ml/h, slightly lower than the rate in study 1 (i.e., 0.9 ml/h), and muscles were collected 50 and 180 min after the start of Intralipid infusion.

Analysis. Plasma glucose was analyzed during the clamps using 10 μl plasma by a glucose oxidase method on a Beckman glucose analyzer II (Beckman, Fullerton, CA). Plasma insulin was measured by radioimmunoassay using a kit from Linco Research (St. Charles, MO). Plasma FFA was determined using an acyl-CoA oxidase-based colorimetric kit (Wako Pure Chemical Industries, Osaka, Japan). For the determination of plasma ^3H -glucose, plasma was deproteinized with ZnSO_4 and $\text{Ba}(\text{OH})_2$, dried to remove $^3\text{H}_2\text{O}$, resuspended in water, and counted in scintillation fluid (Ready Safe; Beckman) on a Beckman scintillation counter.

Muscle contents of HBP products (uridinediphospho-*N*-acetylglucosamine [UDP-GlcNAc] and uridinediphospho-*N*-acetylgalactosamine [UDP-GalNAc]) were measured using two sequential chromatographic separations with ultraviolet detection basically as described by Rossetti and colleagues (15,23). Frozen muscles were homogenized in three volumes of ice-cold 0.3 mol/l perchloric acid and centrifuged for 5 min at $3,000g$ at 4°C . The supernatant was then mixed with two volumes of freon (1:4 triethylamine:1,1,2 trichlorotrifluoroethane) and centrifuged for 5 min at $3,000g$ at 4°C (24). The aqueous phase, cleared of perchloric acid, was mixed with a small volume of 150 mmol/l KH_2PO_4 (pH 2.5) to have a final concentration of 10 mmol/l. A small amount of tritiated UDP-GlcNAc was added to determine the recovery of muscle UDP-GlcNAc in the subsequent chromatographic procedures. The tissue extract was then run through a 1-ml-strong anion-exchange column (Supelco LC-SAX; Supelco, Bellefonte, PA) for partial purification of HBP products (23). The column was washed with 2 ml of 10 mmol/l KH_2PO_4 followed by 1 ml of 50 mmol/l KH_2PO_4 . HBP products were eluted with 150 mmol/l KH_2PO_4 into five separate fractions of 0.2- to 1.0-ml volumes. UDP-GlcNAc and UDP-GalNAc coelute with UDP-glucose (UDP-Glc) and UDP-galactose (UDP-Gal) from the column. Two of these fractions with highest concentrations were combined and injected into HPLC for separation of UDP-sugars. HPLC analysis was carried out on a Beckman HPLC system (Beckman, Fullerton, CA) using a reverse-phase, ion-pairing isocratic method with two LC18T reverse-phase columns (Supelco) connected in series (23). The columns were equilibrated in mobile phase buffer (125 mmol/l KH_2PO_4 , 5 mmol/l tetrabutylammonium sulfate [TBS], adjusted to pH 6.5 with 125 mmol/l K_2HPO_4 , 5 mmol/l TBS buffer) for 1 h at 1 ml/min before each injection. Samples were run isocratically at 1 ml/min for 35 min with 100% mobile phase buffer followed by 15 min of 60% methanol gradient.

Calculations. Rates of total glucose appearance and whole-body R_i were determined as the ratio of the [$3\text{-}^3\text{H}$]glucose infusion rate (disintegrations per minute [dpm] per minute) to the specific activity of plasma glucose (dpm per micromole) during the final 30 min of the control and treatment periods. Hepatic glucose output (HGO) was determined by subtracting the glucose infusion rate (GINF) from the total glucose appearance.

Statistical analysis. Data are expressed as means \pm SE. The significance of the differences in mean values among different treatment groups was evaluated using the one-way analysis of variance, followed by ad hoc analysis using Tukey's test. The significance of the effects of treatment within the groups was evaluated using the paired *t* test. $P < 0.05$ was considered statistically significant.

RESULTS

Study 1: Effects of Intralipid and/or glucosamine infusions on insulin-stimulated glucose fluxes and muscle HBP product levels. In this study, insulin was infused at a constant rate ($22 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) for 480 min to raise plasma insulin within a physiological range, and plasma glucose was

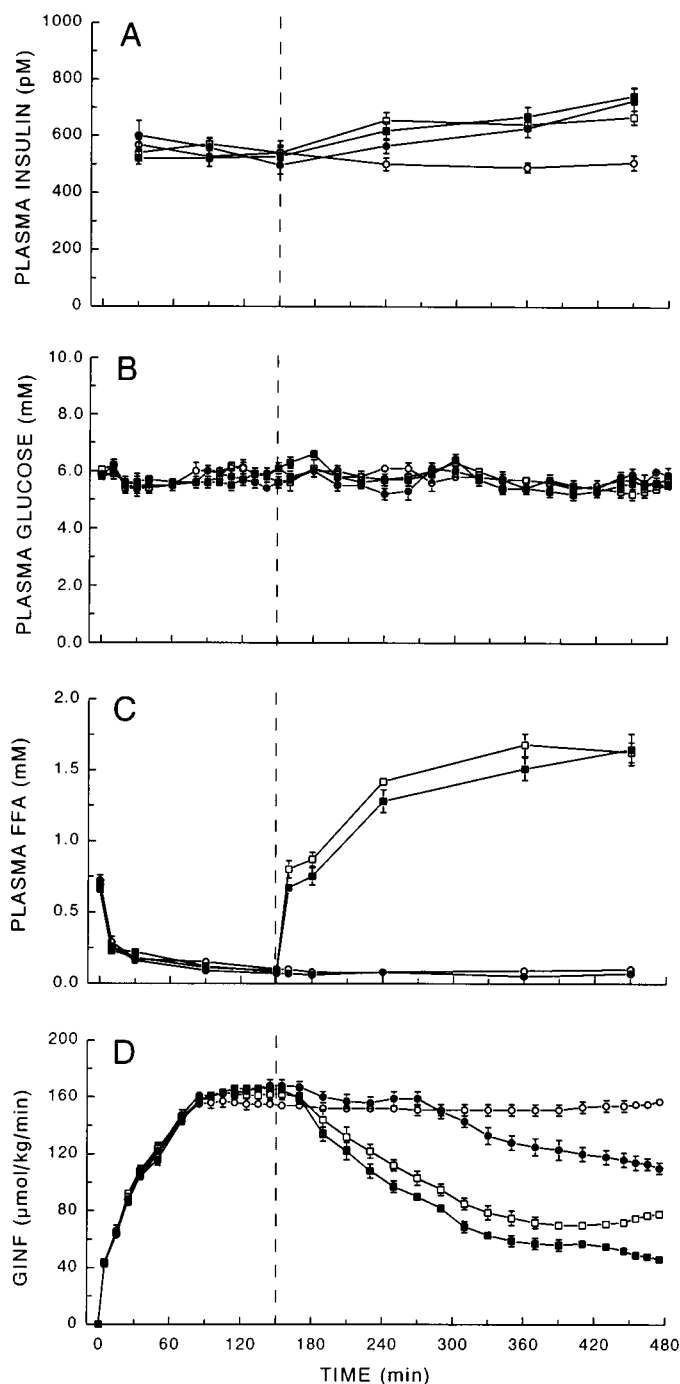


FIG. 1. Plasma insulin (A), glucose (B), and FFA (C) concentrations and GINF (D) during the 480-min hyperinsulinemic-euglycemic clamp with an infusion of saline (○), Intralipid (□), glucosamine (●), or Intralipid and glucosamine (■) that was initiated at 150 min as indicated by broken lines. Values are means ± SE for eight or nine experiments.

clamped at basal levels. After the initial 150-min control clamp period, the clamp was continued with an additional infusion of saline (control), Intralipid, glucosamine, or Intralipid and glucosamine for the remaining 330-min treatment period.

Fasting plasma glucose (~5.9 mmol/l) and FFA (~0.70 mmol/l) concentrations were similar among the four experimental groups. Plasma insulin was raised to and maintained at ~550 pmol/l during the control period (0–150 min; Fig. 1A).

During the treatment period (150–480 min), plasma insulin was not significantly altered with the saline infusion but was increased 20–30% with the individual and the combined infusions of Intralipid and glucosamine ($P < 0.05$). Plasma glucose was clamped at ~5.6 mmol/l, similar to basal levels, in all groups throughout the experiments (Fig. 1B). Plasma FFA concentrations decreased similarly in all groups during the control period (Fig. 1C). During the treatment period, plasma FFA remained suppressed with the saline and the glucosamine infusions but were raised to levels (~1.5 mmol/l) above the basal levels with the infusion of Intralipid (alone or combined with glucosamine infusion). GINFs required to maintain plasma glucose increased rapidly during the initial 90 min and reached steady-state levels during the control period (Fig. 1D). During the treatment period, GINFs were constant with saline infusion (i.e., control group) but decreased with the Intralipid and/or glucosamine infusions, with more rapid and profound effects observed with the Intralipid infusions.

GINFs decreased 52 and 34% at the end (final 30 min) of the Intralipid and the glucosamine infusions, respectively ($P < 0.05$ vs. control period; Fig. 2A). When Intralipid and glucosamine infusions were combined, GINFs decreased further (i.e., 72%; $P < 0.05$ vs. the decreases with the Intralipid or glucosamine alone). Thus, the effects of Intralipid and glucosamine infusions to reduce GINFs were additive. Similarly, insulin-stimulated whole-body R_d decreased 38 ± 2 and $28 \pm 3\%$ with the Intralipid and the glucosamine infusions, respectively ($P < 0.05$ vs. control period; Fig. 2B), and further decreased when the infusions were combined (i.e., $47 \pm 1\%$; $P < 0.05$ vs. the decreases with the Intralipid or glucosamine alone). HGO was completely suppressed in all groups during the control period and with the saline and the glucosamine infusions during the treatment period. In contrast, HGO was not completely suppressed by insulin in the Intralipid-infused groups (i.e., with elevated plasma FFA levels). Hepatic insulin resistance, reduced ability of insulin to suppress HGO, was more severe when Intralipid was infused together with glucosamine ($P < 0.05$; 38 ± 3 vs. $22 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with Intralipid alone; Fig. 2C). Thus, glucosamine potentiated FFA induction of hepatic insulin resistance.

The levels of HBP products (i.e., UDP-GlcNAc and UDP-GalNAc) in skeletal muscles (soleus, EDL, and tibialis anterior) were increased by four- to fivefold with the glucosamine infusion, as expected (Table 1). UDP-hexoses (i.e., UDP-Glc and UDP-Gal) showed a tendency to decrease with the glucosamine infusion. In contrast to the dramatic increases with the glucosamine infusion, muscle HBP product levels were not altered by the Intralipid infusion. Thus, the 30–40% decreases in insulin-stimulated R_d with the Intralipid infusion were accompanied by absolutely no change in muscle HBP product levels (Fig. 3). Also, when infused with glucosamine, Intralipid decreased insulin-mediated R_d below that with glucosamine alone without changing HBP product levels. These data indicate that the HBP was not responsible for fat-induced insulin resistance under our experimental conditions. UDP-glucose levels were increased with the Intralipid infusion in soleus but not in the other muscles. This increase may represent a type I error, since the increase was largely due to two soleus samples in the Intralipid infusion group, of which UDP-glucose levels were significantly higher than the rest of the group.

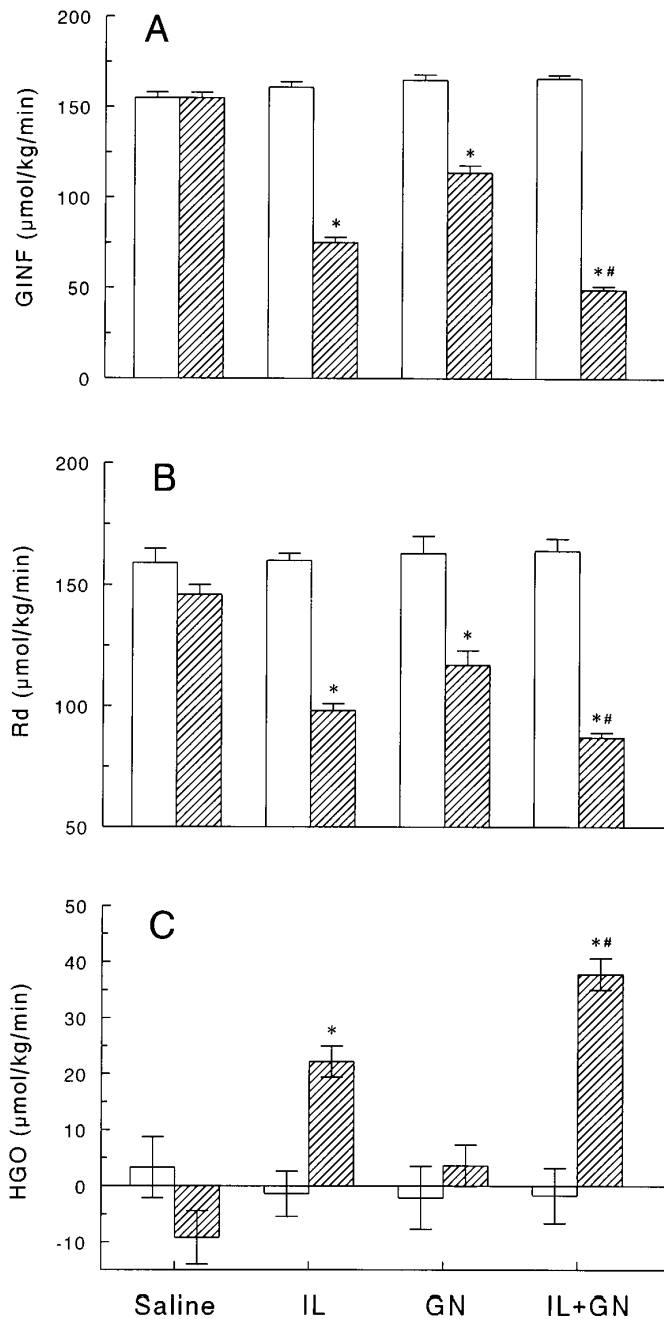


FIG. 2. GINF (A), insulin-stimulated R_d (B), and HGO (C) during the final 30 min of the control (120–150 min [□]) and the treatment (450–480 min [▨]) periods in the saline, Intralipid (IL), glucosamine (GN), and Intralipid + glucosamine (IL+GN) groups. Values are means \pm SE for eight or nine experiments. * $P < 0.05$ vs. control period; # $P < 0.05$ vs. glucosamine or Intralipid alone.

Study 2: Effects of short intralipid infusions on muscle HBP product levels. In the study above, the effects of Intralipid infusion on muscle HBP product levels were studied after a prolonged (330 min) infusion. It is possible that HBP product levels increased during an earlier period of the Intralipid infusion but returned to control levels when insulin resistance was fully developed after the prolonged infusion. To test this possibility, we examined muscle HBP product levels on muscles collected after a 50- or 180-min Intralipid infu-

sion. For this, we used muscles collected from an independent study in which experimental conditions were identical to those in study 1, except for the rate and the duration of Intralipid infusion; the infusion rate was 0.75 ml/h in this study, instead of 0.9 ml/h as in study 1. Intralipid infusion at this lower rate decreased insulin-stimulated R_d by ~25% within 180 min (data not shown). However, this effect of Intralipid on insulin-stimulated R_d was accompanied by no significant changes in HBP product levels in muscles collected at 50 and 180 min after the start of Intralipid infusion (Fig. 4). These data further support the notion that Intralipid infusion induces peripheral insulin resistance without increasing muscle HBP product levels.

DISCUSSION

To evaluate the role of the HBP in the development of insulin resistance with metabolic impairment, we examined 1) whether fat infusion (that suppresses glycolysis) increases muscle HBP product levels during physiological insulin stimulation, 2) whether fat-induced insulin resistance is additive to that induced by increased HBP flux (via glucosamine infusion), and if so, 3) whether such additive effects correlate with HBP product levels (study 1). As expected, glucosamine-induced insulin resistance was associated with four- to five-fold increases in muscle HBP product levels (18). In contrast, fat-induced insulin resistance was accompanied by absolutely no increase in muscle HBP product levels, despite the fact that fat-induced insulin resistance was greater in magnitude than that induced with glucosamine (Fig. 3). In addition, although the effects of fat and glucosamine infusions on insulin action were additive, these additive effects did not correlate with muscle HBP product levels. Furthermore, fat infusion for short periods (50 or 180 min vs. 330 min in study 1) also failed to increase muscle HBP product levels (study 2), excluding the possibility that fat infusion induced insulin resistance by transiently increasing muscle HBP flux during an early period. Thus, the present data strongly indicate that fat infusion (or elevated plasma FFA levels) induced insulin resistance without increasing HBP product levels under our experimental conditions.

Our results are contrary to the findings of Hawkins et al. (15) that indicated that fat infusion significantly (two- to threefold) increased muscle HBP product levels. This increase was shown to be similar in magnitude to those seen during the development of insulin resistance of similar magnitude with agents that increase HBP flux (i.e., glucosamine, glucose, and uridine). The reason for this apparent discrepancy between the two studies is unclear. Fat infusion rates and plasma FFA levels during fat infusion were similar and therefore cannot explain the discrepancy. Also, although different muscles were examined in these studies, the discrepancy cannot be attributed to the differences in muscle fiber type, since our finding was consistently observed in several muscles (soleus, EDL, and tibialis anterior) with different fiber compositions. However, there are other major differences in experimental conditions between the studies, and the discrepancy might arise from these differences. First of all, the study of Hawkins et al. was carried out at maximally effective insulin concentrations (~3,000 pmol/l), whereas the present study was carried out at physiological (~550 pmol/l) insulin concentrations. The effect of fat infusion (i.e., metabolic suppression) to increase muscle G-6-P (and its mass action to

TABLE 1

Concentrations of UDP-hexoses in soleus, EDL, and tibialis anterior muscles at the end of saline, Intralipid, glucosamine, and Intralipid + glucosamine infusions

Group	n	UDP-Gal (nmol/g)	UDP-Glc (nmol/g)	UDP-GalNAc (nmol/g)	UDP-GlcNAc (nmol/g)
Soleus					
Saline	7	7.2 ± 0.5	19.9 ± 0.9	12.5 ± 1.3	35.4 ± 2.5
Intralipid	7	9.5 ± 1.3	28.4 ± 3.5*	14.2 ± 1.3	39.8 ± 5.7
Glucosamine	6	4.3 ± 0.3	13.1 ± 1.1	32.1 ± 2.9*	183.8 ± 13.2*
IL + GN	6	5.6 ± 0.8	16.9 ± 1.1	33.2 ± 2.7*	194.2 ± 11.6*
EDL					
Saline	7	8.8 ± 1.0	19.1 ± 1.4	8.7 ± 1.0	25.1 ± 1.8
Intralipid	7	8.7 ± 0.9	20.1 ± 1.6	9.3 ± 1.2	27.6 ± 2.3
Glucosamine	6	7.5 ± 1.4	17.3 ± 1.4	17.1 ± 2.5*	109.1 ± 10.7*
IL + GN	6	7.0 ± 0.8	16.0 ± 0.9	17.2 ± 1.5*	111.6 ± 6.8*
Tibialis anterior					
Saline	7	7.4 ± 0.4	16.6 ± 0.9	7.3 ± 0.7	21.9 ± 0.8
Intralipid	7	7.7 ± 0.5	14.1 ± 1.4	6.4 ± 0.6	19.2 ± 1.3
Glucosamine	6	5.4 ± 0.7	12.9 ± 0.8	17.0 ± 1.0*	112.3 ± 6.4*
IL + GN	6	5.9 ± 0.5	13.4 ± 1.2	16.2 ± 1.6*	107.3 ± 5.6*

**P* < 0.05 vs. saline. IL + GN, Intralipid and glucosamine.

increase F-6-P and HBP flux) could have been greater in the Hawkins et al. study because of greater R_d into muscle at maximally effective insulin concentrations. To support this idea, our previous study (25) showed that muscle G-6-P levels increased during fat infusion to levels that were significantly (40–50%) higher at maximal than at physiological insulin levels. Thus, it may be possible that fat infusion significantly increases HBP flux at maximal but not at physiological insulin concentrations. However, our preliminary data do not support this possibility (data not shown), and this issue remains unresolved and merits further investigation. Another difference between the two studies was the fasting state; 6-h-fasted rats were used in the study of Hawkins et al., whereas overnight-fasted rats were used in the present study. Nelson et al. (24) showed that the activity of glutamine F-6-P amidotransferase (GFAT), the rate-limiting enzyme of the HBP, in rat skeletal muscle decreased by 30% after an 18-h fast. These data suggest the possibility—though unlikely—that the lack of the effect of fat infusion on muscle HBP product levels in the present study might be due to decreased GFAT activity in muscles of overnight-fasted animals. Finally, it may be worthwhile to point out that Sprague-Dawley rats

were used in the study by Hawkins et al., whereas Wistar rats were used in the present study. There is evidence for differential regulation of glucose metabolism between the two rat strains. For example, different effects of aging (or growth) were observed on insulin sensitivity of glucose transport, lactate production, and glycogen synthesis (26). It would be extremely interesting if the discrepancy regarding the role of HBP in fat-induced insulin resistance arose from the strain difference. Whatever the reason for the discrepancy, the present data indicate that there was a mechanism independent of HBP product levels that induced insulin resistance in skeletal muscle with increased availability of plasma FFA.

The role of the HBP in the regulation of insulin action has been extensively studied since the discovery of Marshall et al. (16,17) that the HBP is involved in the downregulation of insulin action on glucose transport in cultured fat cells exposed to high glucose and insulin concentrations. Increasing HBP flux via glucosamine infusion/treatment has been shown to induce insulin resistance, accompanied by impairment of insulin action on GLUT4 translocation in insulin-sensitive cells in vivo (18–20) and in vitro (22,27). In addition, overexpression of GFAT in muscles of transgenic mice (28)

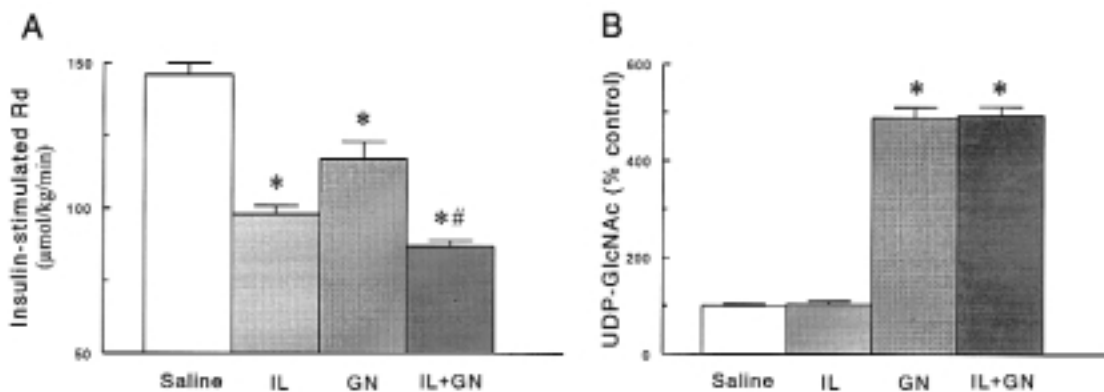


FIG. 3. Effects of Intralipid and/or glucosamine infusions on insulin-stimulated R_d (A) and skeletal muscle UDP-GlcNAc concentrations (B), measured at the end of the 330-min infusion of saline, Intralipid (IL), glucosamine (GN), or Intralipid and glucosamine. A: Values are means ± SE for eight or nine experiments. B: Data are expressed as percent of control (saline) values to combine the data from different types of muscles (Table 1). **P* < 0.05 vs. saline; #*P* < 0.05 vs. glucosamine or Intralipid alone.

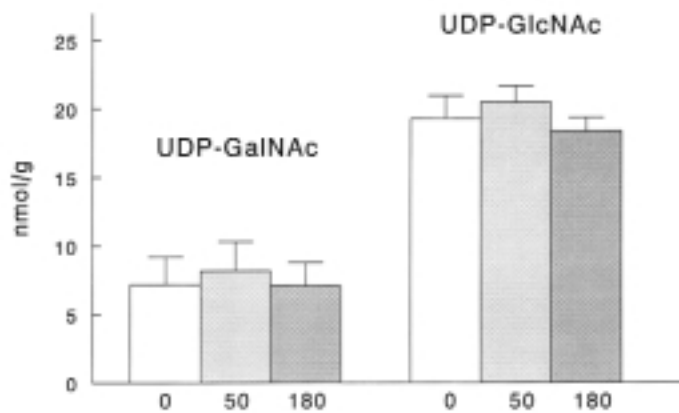


FIG. 4. Effects of short (50 and 180 min) Intralipid infusions on skeletal muscle (tibialis anterior) HBP product concentrations. Intralipid infusion was initiated after the 150-min control clamp period, and “0 min” refers to muscles collected at the end of the control period. Values are means \pm SE for five experiments.

or in Rat-1 fibroblasts (29) resulted in impaired insulin action on glucose uptake/transport. These studies have established that increased HBP flux leads to impairment of insulin action on glucose transport in insulin-sensitive cells. The HBP appears to be involved in the development of insulin resistance secondary to hyperglycemia (22,30). However, whether the HBP is also involved in the development of insulin resistance at euglycemia under pathophysiological conditions (e.g., increased plasma FFA levels) has not been rigorously studied. Because insulin resistance develops long before the frank onset of type 2 diabetes (31), this issue may be a critical one in evaluating the role of the HBP in the pathogenesis of type 2 diabetes. HBP product levels and GFAT activity have been shown to be altered in various metabolic states characterized by altered insulin action, including fasting (24), calorie restriction (32), diabetes (22,30), obesity (33), and growth hormone deficiency (34). In addition, GFAT activity, measured in cultured human muscle cells, was inversely correlated with insulin-stimulated R_d in vivo in normal subjects (35). Although these data are consistent with the role of the HBP in the regulation of insulin action at euglycemia, the causal relationship in the association between GFAT activity/HBP products and insulin action remains to be tested.

The HBP has been proposed to serve as a negative feedback control mechanism that senses hyperglycemia or excessive glucose influx to adjust insulin's action on glucose entry (17). To support this idea, it has been demonstrated that hyperglycemia (22) or overexpression of GLUT1 in skeletal muscle of transgenic mice (36) results in insulin resistance associated with increased HBP product levels. Regarding this issue, an important factor in the development of insulin resistance may be the magnitude and/or duration of enhanced R_d into muscle rather than plasma glucose concentration per se. Theoretically, the role of the HBP can be extended to the sensing of the balance between glucose influx and glucose metabolism. G-6-P and F-6-P can be increased not only by increased glucose flux into cells at hyperglycemia but also by suppressed glucose metabolism (e.g., glycolysis) with normal glucose flux into cells at euglycemia, as previously demonstrated (13,15). On the basis of this reasoning, we previously speculated on the potential role

of the HBP as part of the mechanism by which metabolic impairment leads to insulin resistance (12). This intriguing possibility was supported by the study of Hawkins et al. (15) but not by the present study. It is unclear whether this discrepancy arose from the difference in the magnitude of glucose flux into muscle, which would have been greater in the Hawkins et al. study because of the higher insulin levels, as discussed in the second paragraph of the DISCUSSION section. Further studies are required to resolve this discrepancy and, more important, to address the issue of whether the HBP plays a role in the acute or chronic development of insulin resistance at euglycemia under various pathophysiological conditions.

We measured HBP product levels as an indicator of HBP flux as in other studies (15,22,24). This would be a reasonable approach if cellular utilization of HBP products is constant or unaltered under the experimental conditions studied. There is little information available on the intracellular kinetics of HBP products, and it is unknown whether cellular utilization of HBP products is subject to regulation by increased FFA levels. Because we cannot exclude the possibility that the Intralipid infusion increased cellular utilization of HBP products (and masked its possible effect to increase HBP flux), our finding of the lack of Intralipid effect on HBP product levels may not be taken as directly indicating a lack of Intralipid effect on HBP flux. However, a significant effect of Intralipid on cellular utilization of HBP products is unlikely, because Intralipid also failed to change HBP product levels raised by glucosamine. Thus, it may be reasonable to conclude that the Intralipid infusion did not increase substrate flux through the HBP under our experimental conditions.

Robinson et al. (22) suggested that substrate flux through the HBP should be critically dependent on the level of F-6-P, based on the finding that the apparent K_m for F-6-P of GFAT from muscle (2.4 mmol/l) was considerably greater than cellular F-6-P levels. Our previous study (13) showed that muscle G-6-P levels increased \sim 40% in soleus and EDL muscles during an early phase of Intralipid infusion under the conditions similar to those of the present study. Assuming that there were similar increases in F-6-P level, these data, taken together with the present data, suggest that the HBP—an important metabolic pathway that would affect glycosylation (and thus function) of various cellular proteins—may not be significantly altered by fluctuations in substrate levels and may be regulated rather tightly by other factors. This concept is supported by the finding of Castle et al. (37) that three- to fivefold increases in G-6-P level, induced by increased glycogenolysis with amylin, failed to increase HBP product levels in perfused rat hindlimb muscles.

In summary, increased availability of plasma FFA at physiological insulin levels induced marked peripheral insulin resistance in overnight-fasted rats, which was accompanied by no change in skeletal muscle HBP product levels. In addition, the FFA-induced insulin resistance was additive to that induced with increased HBP flux via glucosamine infusion, but the additive effects of FFA and glucosamine could not be explained by muscle HBP product levels. Taken together, these data indicate that the HBP was not involved in fat-induced insulin resistance in overnight-fasted rats and that there must be another mechanism by which suppression of glucose metabolism (i.e., glycolysis with fat infusion) leads to impaired insulin action on R_d .

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