

Lipid Infusion Impairs Physiologic Insulin-Mediated Capillary Recruitment and Muscle Glucose Uptake In Vivo

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Infusion of triglycerides and heparin causes insulin resistance in muscle. Because the vascular actions of insulin, particularly capillary recruitment, may contribute to the increase in glucose uptake by skeletal muscle, we investigated the effects of Intralipid/heparin infusion on the hemodynamic actions of insulin during clamp conditions. Saline or 10% Intralipid/heparin (33 U/ml) was infused into anesthetized rats at 20 μ l/min for 6 h. At 4 h into the saline infusion, a 2-h hyperinsulinemic (3 $\text{mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)-euglycemic clamp was conducted (Ins group). At 4 h into the lipid infusion, a 2-h saline control (Lip group) or 2-h hyperinsulinemic-euglycemic clamp (Lip + Ins group) was conducted. Arterial blood pressure, heart rate, femoral blood flow (FBF), hindleg vascular resistance, glucose infusion rate (GIR), hindleg glucose uptake (HGU), and muscle 2-deoxyglucose uptake ($\text{R}'\text{g}$) were measured. Capillary recruitment, as measured by metabolism of infused 1-methylxanthine (1-MX), was also assessed. When compared with either Lip or Lip + Ins, Ins had no effect on arterial blood pressure, heart rate, FBF, or vascular resistance but increased GIR, HGU, and $\text{R}'\text{g}$ of soleus, plantaris, extensor digitorum longus, and gastrocnemius red muscles and hindlimb 1-MX metabolism. GIR, HGU, and $\text{R}'\text{g}$ of soleus, plantaris, gastrocnemius red, and the combined muscles and 1-MX metabolism were less in Lip + Ins than in Ins rats. HGU correlated closely with hindleg capillary recruitment ($r = 0.86$, $P < 0.001$) but not total hindleg blood flow. In conclusion, acute elevation of plasma free fatty acids blocks insulin-mediated glucose uptake and capillary recruitment. *Diabetes* 51:1138–1145, 2002

Skeletal muscle accounts for ~80% of the insulin-mediated glucose uptake (IMGU) after a meal (1) and becomes resistant to insulin in type 2 diabetes (2), hypertension (3), obesity (4), and severe forms of stress (5). However, the mechanisms by which muscle insulin resistance develops are not fully understood. One factor that may be central is lipid availability.

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1-MX, 1-methylxanthine; 2DG, 2-deoxyglucose; eNOS, endothelial nitric oxide synthase; FBF, femoral arterial blood flow; HGU, hindleg glucose uptake; IMGU, insulin-mediated glucose uptake; PKC θ , protein kinase C θ ; $\text{R}'\text{g}$, muscle 2DG uptake; TNF, tumor necrosis factor; XO, xanthine oxidase.

For example, insulin sensitivity in humans is reduced in obesity (4) and especially when individuals possess an abnormally high proportion of abdominal fat (6). In addition, lipid infusion in humans (7,8) and in rats (9) gives rise to insulin resistance in muscle. Furthermore, genetically obese Zucker rats have elevated plasma levels of free fatty acids and show marked muscle insulin resistance (10), as do rats fed high-fat diets (11,12), although the type of fatty acid appears to be critical: oils of fish origin are protective against the effects of animal fat diets (13).

Explanations to account for the link between lipid availability and muscle insulin resistance have until recently focused on the Randle glucose–fatty acid cycle (14), in which fatty acid metabolites collectively contribute to the inhibition of oxidative and nonoxidative glucose metabolism. Recently, there have been a number of studies (15–17) suggesting that fatty acids or their metabolites inhibit insulin signaling at points preceding activation of glycogen synthase or glucose transport.

Insulin in vivo has hemodynamic effects in addition to its well-described direct metabolic action on muscle. Although the precise role of the hemodynamic effects are unknown, it is possible that they contribute to the overall metabolic response by enhancing access for both insulin and glucose to muscle (18–20). There appear to be two components to insulin's hemodynamic actions, one involving an increase in bulk flow and another involving capillary recruitment. A number of laboratories have reported an effect of insulin to increase bulk blood flow to muscles (18,21–26) and that this effect is impaired in states of insulin resistance (18) and when lipid is infused in otherwise normally responsive subjects (27). However, the role of the increase in total blood flow mediated by insulin is controversial. There have been claims that insulin-mediated changes in total blood flow relate poorly to muscle glucose uptake under several circumstances, including insulin dose and time course (19). In addition, there have been studies where total flow changes persist when glucose uptake is inhibited (28). Also, most vasodilators that augment total blood flow to the limbs do not enhance insulin action, nor do they overcome insulin resistance (29–31).

Because of techniques unique to our laboratories, we have been the first to report a direct effect of insulin to increase capillary recruitment (or nutritive flow) within skeletal muscle of anesthetized rats (20) and the forearm of humans (32). Insulin-mediated capillary recruitment appears to be independent of changes in bulk blood flow

to the limb in both rats and humans, particularly if physiologic levels of insulin are used (33). Measurement of capillary exposure (or nutritive flow) in anesthetized rats was assessed using 1-methylxanthine (1-MX) metabolism. Hindlimb metabolism of this infused substrate targeted for capillary endothelial xanthine oxidase (XO) was shown to increase in the presence of insulin (20). In addition, if α -methyl serotonin, an agent that prevented capillary recruitment in the hindlimb, was administered (34), or if tumor necrosis factor (TNF)- α was infused (35), the ability of insulin to increase either total blood flow or capillary recruitment in the hindlimb was markedly impaired, and IMGU was blocked by 50–60%.

Because there appears to be a link between insulin-mediated capillary recruitment and IMGU in muscle, it was considered important to explore a model of insulin resistance in which insulin-mediated muscle glucose uptake is impaired. Thus, the aim of these experiments was to assess whether fatty acid-induced muscle insulin resistance was accompanied by impaired capillary recruitment at physiologic insulin.

RESEARCH DESIGN AND METHODS

Animals. Male rats of the hooded Wistar strain weighing 249 ± 1 g were raised on a commercial diet (Gibsons, Hobart, Australia) containing 21.4% protein, 4.6% lipid, 68% carbohydrate, and 6% crude fiber with added vitamins and minerals together with water ad libitum. Housing was at a constant temperature of $21 \pm 1^\circ\text{C}$ in a 12-h light/dark cycle. All procedures and experiments were approved by the University of Tasmania Animal Ethics Committee.

Surgery. Details were essentially as described previously (35). In brief, rats were anesthetized using pentobarbital (Nembutal; 50 mg/kg body wt) and had polyethylene cannulas (PE-50; Intramedic, Balsta, Sweden) surgically implanted into the carotid artery for arterial sampling and measurement of blood pressure (pressure transducer Transpac IV; Abbott Critical Care Systems, Salt Lake City, UT) and into both jugular veins for continuous administration of anesthetic and other intravenous infusions. A tracheotomy tube was inserted, and the animal was allowed to spontaneously breathe room air throughout the course of the experiment. Small incisions (1.5 cm) were made in the skin overlaying the femoral vessels of both legs, and the femoral artery was separated from the femoral vein and saphenous nerve. The epigastric vessels were then ligated, and an ultrasonic flow probe (VB series 0.5 mm; Transonic Systems, Ithaca, NY) was positioned around the femoral artery of the right leg just distal to the rectus abdominis muscle. The cavity in the leg surrounding the flow probe was filled with lubricating jelly (H-R; Mohawk Medical Supply, Utica, NY) to provide acoustic coupling to the probe. The probe was then connected to the flow meter (Model T106 ultrasonic volume flow meter; Transonic Systems). This in turn was interfaced with an IBM-compatible computer that acquired the data (at a sampling frequency of 100 Hz) for femoral blood flow (FBF), heart rate, and blood pressure using WINDAQ data acquisition software (Dataq Instruments, Akron, OH). The surgical procedure generally lasted ~ 30 min, and then the animals were maintained under anesthesia for the duration of the experiment using a continual infusion of pentobarbital via the left jugular cannula, which was varied to maintain the arterial blood pressure at ~ 105 mmHg. The femoral vein of the left leg was used for venous sampling, using an insulin syringe with an attached 29G needle (Becton Dickinson, Franklin Lakes, NJ). A duplicate venous sample was taken only on completion of the experiment (360 min) to prevent alteration of the blood flow from the hindlimb due to sampling and to minimize the effects of blood loss. The body temperature was maintained using a water-jacketed platform and a heating lamp positioned above the rat.

Experimental procedures. A 60-min equilibration period was allowed after surgery so that leg blood flow and blood pressure could become stable and constant. Rats were then assigned to protocol A, B, or C (Fig. 1). Protocol A (Ins) involved 6-h infusion of saline with a 2-h euglycemic clamp ($3 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) (Humulin R; Eli Lilly, Indianapolis, IN) commenced 4 h into the saline infusion. Protocol B (Lip) involved an initial bolus dose of heparin (10 U) before a 6-h infusion of 10% Intralipid/heparin (33 U/ml) at $20 \mu\text{l}/\text{min}$ with a 2-h saline infusion commenced 4 h into the lipid infusion. Protocol C (Lip + Ins) was identical to protocol B except that a 2-h euglycemic clamp replaced the 2-h saline. Volumes were matched between all three protocols. As in previous

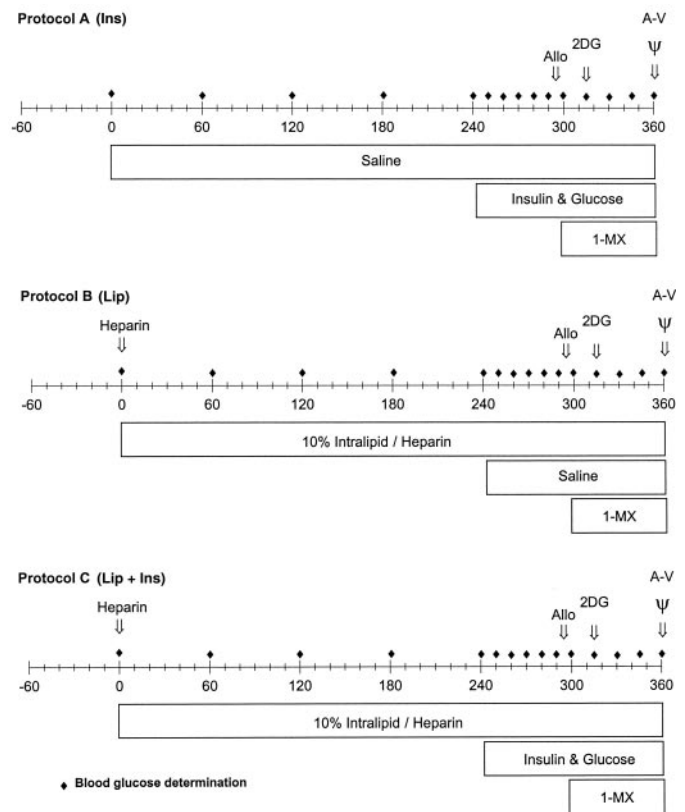


FIG. 1. Study design. In two protocols (A and C), the euglycemic-hyperinsulinemic ($3 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) clamp was commenced 4 h after the start of saline (protocol A [Ins]) or Intralipid/heparin (protocol C [Lip + Ins]) infusion. During protocol B (Lip), saline replaced insulin at 4 h. Duplicate arterial and femoral venous plasma samples were collected at 360 min, as indicated by $\psi \downarrow$, for high-performance liquid chromatography analysis and plasma glucose determinations. Heparin, allopurinol (Allo), and [^3H]2DG were injected as indicated by \downarrow at 0, 295, and 315 min, respectively. Arterial samples were also taken for monitoring plasma [^3H]2DG radioactivity at 5, 10, 15, 30, and 45 min after injection (not shown). Arterial samples for glucose determinations are indicated by \diamond ; venous infusions are indicated by the bars. A-V, arteriovenous.

similar studies (20,34,35)—and because 1-MX (Sigma-Aldrich, St. Louis, MO) clearance was very rapid—it was necessary to partially inhibit the activity of XO, particularly in nonmuscle tissues. To do this, an injection of a specific XO inhibitor, allopurinol ($10 \mu\text{mol}/\text{kg}$) (36), was administered as a bolus dose 5 min before commencing the 1-MX infusion ($0.5 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) (Fig. 1). This allowed constant arterial concentrations of 1-MX to be maintained throughout the experiment.

As shown in Fig. 1, a $50\text{-}\mu\text{Ci}$ bolus of [^3H]2-deoxyglucose ([^3H]2DG) (specific activity $44.0 \text{ Ci}/\text{mmol}$; Amersham Pharmacia Biotech, Little Chalfont, UK) in saline was administered 45 min before completion of the experiment. Plasma samples ($20 \mu\text{l}$) were collected at 5, 10, 15, 30, and 45 min to determine plasma clearance of the radioactivity and for the calculation of muscle 2DG uptake ($R'g$). At the conclusion of the experiment, the soleus, plantaris, extensor digitorum longus, gastrocnemius red and white, and tibialis muscles were removed, clamp-frozen in liquid nitrogen, and stored at -20°C until assayed for [^3H]2DG uptake.

The total blood volume drawn from the animals before the final arterial and venous samples did not exceed 1.5 ml and was easily compensated by the volume of fluid infused.

All other procedures were as described previously (35), including analytical methods for glucose, 1-MX, allopurinol, and oxypurinol assays. Free fatty acids were assayed using a NEFA C Colorimetric Kit (Wako, Richmond, VA). **2DG uptake assay.** The frozen muscles were ground under liquid nitrogen and homogenized using an Ultra Turrax (IKA, Staufen, Germany). Free and phosphorylated [^3H]2DG were separated by ion exchange chromatography using an anion exchange resin (AG1-X8) (37,38). Biodegradable Counting Scintillant (Amersham) was added to each radioactive sample, and radioactivity was determined using a scintillation counter (LS3801; Beckman, Fuller-

ton, CA). From this measurement, a knowledge of plasma glucose, and the time course of plasma 2DG disappearance, $R'g$, which reflects glucose uptake into the muscle, was calculated ($\mu\text{g} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ wet wt of muscle) as previously described by others (37,38).

XO activity. XO was assayed on gastrocnemius red muscle that had been frozen in liquid nitrogen at the end of each experiment and stored at -20°C . Powdered muscle (~ 200 mg) was homogenized in 2 ml homogenizing buffer (50 mmol/l Na_2HPO_4 , 0.1 mmol/l Na_2EDTA , 4 mmol/l dithiothreitol, and 0.5 mg/ml trypsin inhibitor, pH 7.4) and centrifuged for 5 min at $13,000g$. The supernatant (1 ml) was passed through a PD-10 desalting column (Amersham Pharmacia Biotech). Fractions were collected (30 s) and tested for protein content using the Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA). Protein fractions (100 μl) were incubated with 0.9 ml homogenizing buffer and 100 $\mu\text{mol/l}$ xanthine for 1 h at 37°C . The reaction was stopped by the addition of 250 μl assay mix to 50 μl of 2 mol/l perchloric acid. The amount of uric acid formed from xanthine was determined by high-performance liquid chromatography using a Purosphere (Merck, Darmstadt, Germany) 5 $\mu\text{mol/l}$ C18 250×4.6 mm column with isocratic separation in 50 mmol/l $\text{NH}_4\text{H}_2\text{PO}_4$ buffer (pH 3.5) at 1 ml/min. XO activity is expressed as $\text{pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein.

Data analysis. All data are expressed as means \pm SE. Mean FBF, mean heart rate, and mean arterial blood pressure were calculated from 5-s subsamples of the data, representing ~ 500 flow and pressure measurements every 15 min. Vascular resistance in the hindleg was calculated as mean arterial blood pressure in millimeters of mercury divided by FBF in milliliters per minute and expressed as resistance units. Hindleg glucose uptake (HGU) was calculated from arteriovenous glucose difference and multiplied by FBF and expressed as $\mu\text{mol/min}$. 1-MX disappearance was calculated from arteriovenous plasma 1-MX difference and multiplied by FBF (corrected for the volume accessible to 1-MX, 0.871, determined from plasma concentrations obtained after additions of standard 1-MX to whole rat blood) and expressed as nmol/min .

Statistical analysis. To ascertain differences between treatment groups at the end of the experiments (i.e., HGU, $R'g$, and 1-MX metabolism) one-way ANOVA was used. Statistical differences between treatment groups for repeated measures throughout the 6 h (i.e., blood pressure, heart rate, FBF, vascular resistance, blood glucose, and glucose infusion rate) were determined using two-way repeated-measures ANOVA. All comparisons were made using the Student-Newman-Keuls method. The correlation between 1-MX disappearance and HGU was determined by linear regression. All tests were performed using the SigmaStat statistical program (SPSS, Chicago, IL). Significance was determined by P values < 0.05 .

RESULTS

Hemodynamic effects. Figure 2 shows the arterial blood pressure, heart rate, FBF, and hindleg vascular resistance for the three different protocols. Although there was a trend for the FBF of Lip + Ins rats to be less than that of Ins rats at the closing stages of the clamp (340–360 min), the difference was not significant. There was no significant difference between Ins, Lip, and Lip + Ins rats for the other three parameters (blood pressure, heart rate, or vascular resistance).

Glucose metabolism. Blood glucose levels for the three groups are shown in Fig. 3. Blood glucose was unadjusted during the first 4 h (0–240 min), and in general, it remained constant at ~ 5 mmol/l. However, there was some variability for Intralipid/heparin-treated rats, and for the Lip group, there was a small transient decrease at 120 min followed by a recovery, so that at 240 min all three groups were similar. In addition, there was a rise in the blood glucose level in the Lip rats at the 360-min time point; at that time, glucose was significantly higher than in the Ins and Lip + Ins groups. The Fig. 3 inset shows glucose infusion rate (GIR) to maintain euglycemia for the Ins and Lip + Ins groups. For the Ins group, the GIR increased from 0 to 9.0 ± 0.3 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at 250 min (10 min) to 11.9 ± 0.6 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at 360 min (120 min), whereas the GIR for Lip + Ins decreased from ~ 9.0 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at 250 min to 1.5 ± 0.6 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at

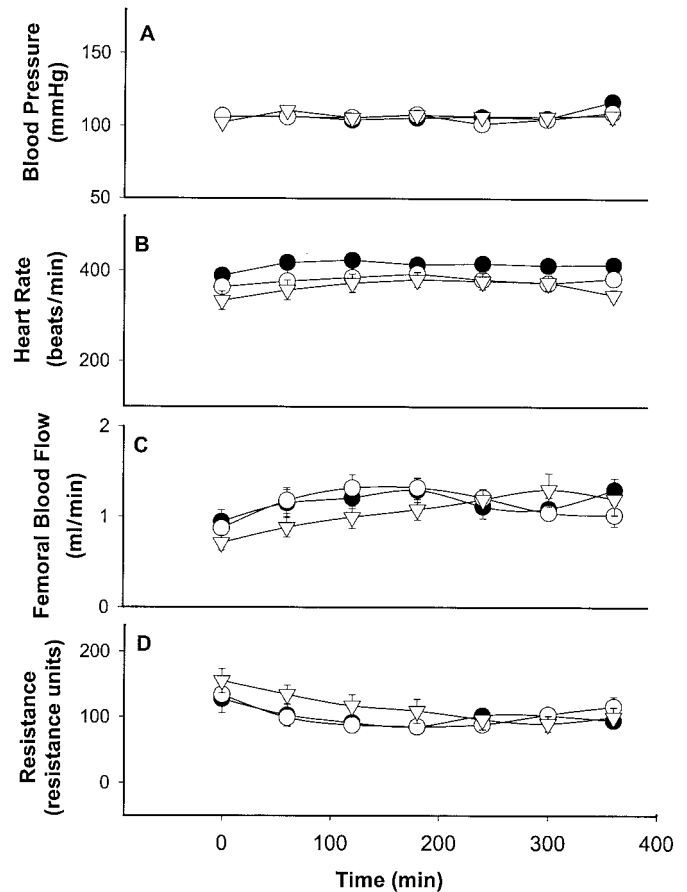


FIG. 2. Mean arterial blood pressure, heart rate, FBF, and hindleg vascular resistance throughout the experiments. Data were collected from 5-s subsamples as indicated in the text. Mean values are shown at each hour. ●, Ins; ○, Lip + Ins; ▽, Lip.

360 min. The differences in GIRs to maintain euglycemia became significant as early as 20 min after commencement of the insulin infusion.

Measurements for arterial plasma free fatty acids were taken immediately before the commencement of the 2-h insulin clamp. Four-hour infusion of 10% Intralipid/heparin

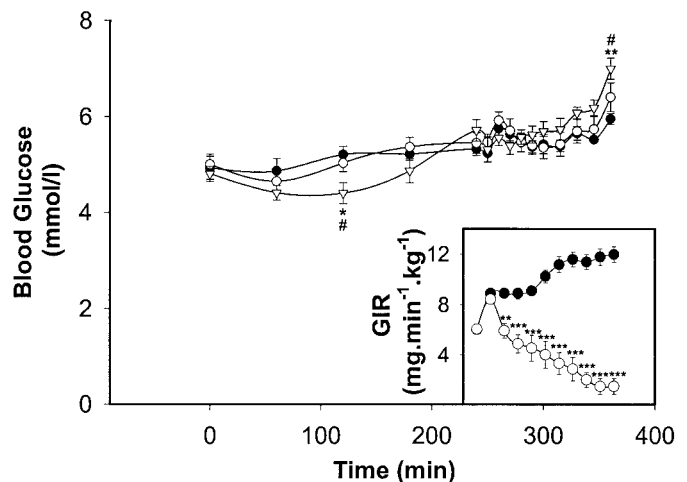


FIG. 3. Arterial blood glucose and GIR (inset) for Ins (●), Lip + Ins (○), and Lip (▽). Values are means \pm SE. Significant differences from Ins: * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; significant differences from Lip + Ins: # $P \leq 0.05$.

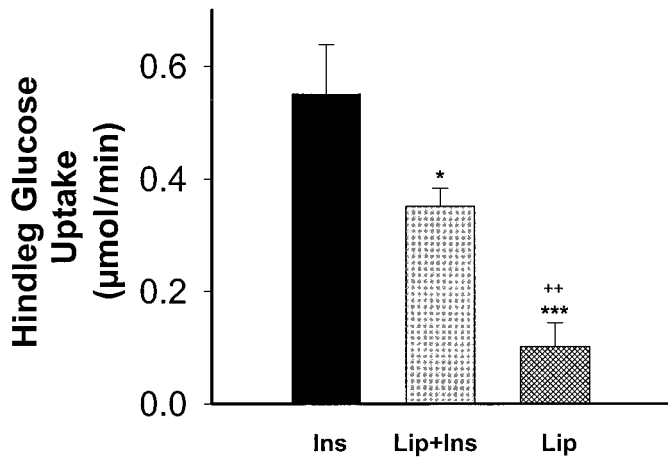


FIG. 4. HGU for Ins, Lip + Ins, and Lip. Values are means \pm SE. Significant differences from Ins: * $P \leq 0.05$, *** $P \leq 0.001$; significant differences from Lip + Ins: ++ $P \leq 0.01$.

significantly increased arterial plasma free fatty acids to 3.9 ± 0.6 mmol/l ($P < 0.001$) in Lip + Ins animals and to 5.3 ± 0.6 mmol/l in Lip animals ($P < 0.001$) compared with animals receiving no lipid infusion (0.3 ± 0.1 mmol/l). There was no significant difference between the free fatty acid levels in the two groups receiving Intralipid/heparin.

HGU values for Ins, Lip + Ins, and Lip groups are shown in Fig. 4. Infusion of Intralipid/heparin alone (Lip) for 6 h did not affect HGU, and the value determined at the end of the experiment was similar to saline (basal) values previously reported at the end of 2 h (35). However, the combination of Intralipid/heparin for 4 h before and during the 2 h clamp significantly reduced the stimulatory effect of insulin from a net of 0.45 to 0.25 $\mu\text{mol}/\text{min}$, an inhibition of 44%.

[^3H]2DG uptake. [^3H]2DG was administered for the final 45 min of each experiment. Figure 5A shows uptake values for soleus, plantaris, extensor digitorum longus, gastrocnemius red and white, and tibialis muscles removed at the completion of the experiment. Values for soleus and plantaris muscles were 7.9 ± 0.7 and 4.9 ± 0.5 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, respectively, following infusion of Intralipid/heparin alone (Lip) for 6 h. Insulin infusion alone (Ins) increased the values for R'g in soleus (3.2-fold), plantaris (2.4-fold), extensor digitorum longus (2-fold), and gastrocnemius red (2.4-fold) compared with Lip (Fig. 5). Notwithstanding the presence of the lipid in the control situation, which may have influenced the rate of basal 2DG uptake, the magnitude of stimulation by insulin at $3 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ was less than previously reported for the higher dose of $10 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, 6.5-fold for soleus and 7.8-fold for plantaris muscles (35). Intralipid/heparin (Lip + Ins) infusion markedly inhibited the stimulatory effect of insulin in three of the six muscles (soleus, plantaris, and gastrocnemius red). In addition, Fig. 5B shows that Lip + Ins significantly inhibited R'g for the combined muscles (aggregated on proportional weight) from a net stimulatory effect by insulin of 6.4 to 2.0 $\mu\text{g} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$, an inhibition of 69%.

1-MX metabolism. No significant difference was found between the experimental groups in arterial plasma concentrations of 1-MX (Fig. 6A) or oxypurinol, the metabolite of allopurinol and inhibitor of XO (Ins, 3.5 ± 0.5 $\mu\text{mol}/\text{l}$; Ins + Lip, 4.0 ± 1.2 $\mu\text{mol}/\text{l}$; Lip, 4.0 ± 0.7 $\mu\text{mol}/\text{l}$).

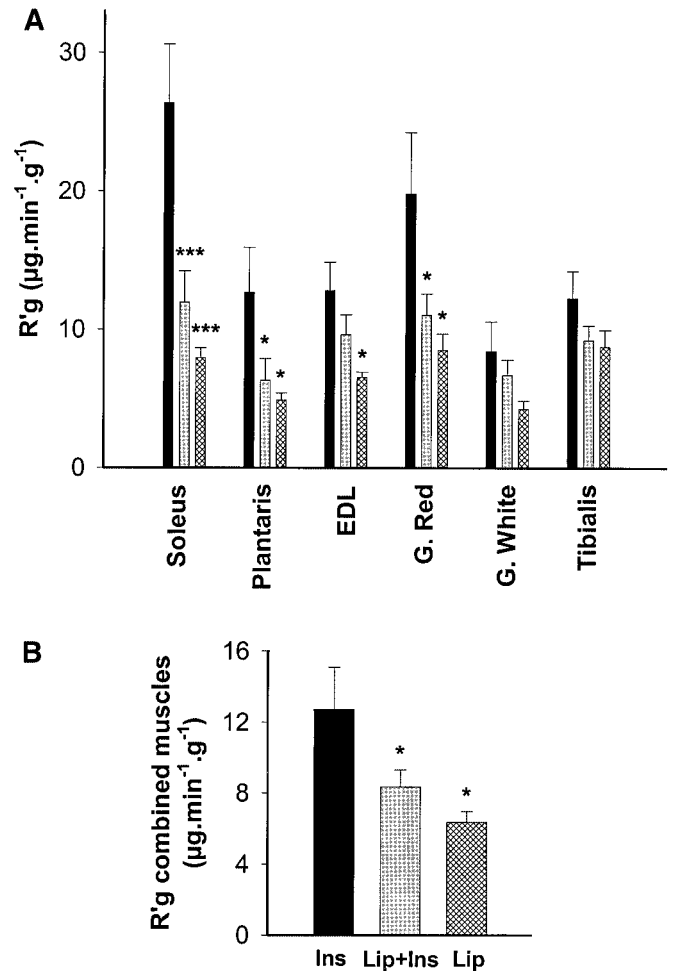


FIG. 5. [^3H]2DG uptake values for individual muscles (A) and for the combination of the six muscles adjusted for differences in weight (B). Conditions were Ins (black bars), Lip + Ins (gray bars), and Lip (hatched bars). Values are means \pm SE. Significant differences from Ins: * $P < 0.05$, *** $P \leq 0.001$. EDL, extensor digitorum longus; G., gastrocnemius.

Insulin infusion alone (Ins) significantly increased hindleg 1-MX metabolism relative to Lip ($P < 0.01$) (Fig. 6C). However, the increase in 1-MX metabolism with insulin was inhibited with Intralipid/heparin infusion ($P < 0.05$). Total muscle XO activity was no different in Ins, Lip + Ins, and Lip groups (76.1 ± 5 , 74.0 ± 9 , and 70.9 ± 8 pmol $\cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein, respectively), indicating an alteration in enzyme exposure rather than activity.

When individual data for HGU were plotted against corresponding values for 1-MX disappearance and analyzed by linear regression, a positive correlation was noted (Fig. 7A; $r = 0.6$, $P = 0.014$). There was no correlation between HGU and FBF (Fig. 7C) or between FBF and 1-MX metabolism (Fig. 7B).

DISCUSSION

The main finding emerging from this study was that Intralipid/heparin caused a marked decrease in insulin-mediated capillary recruitment in muscle. This was evident as an inhibition of 65% of the insulin-mediated metabolism of infused 1-MX and occurred in conjunction with insulin resistance at the whole-body level characterized by diminished GIR and an equally marked reduction

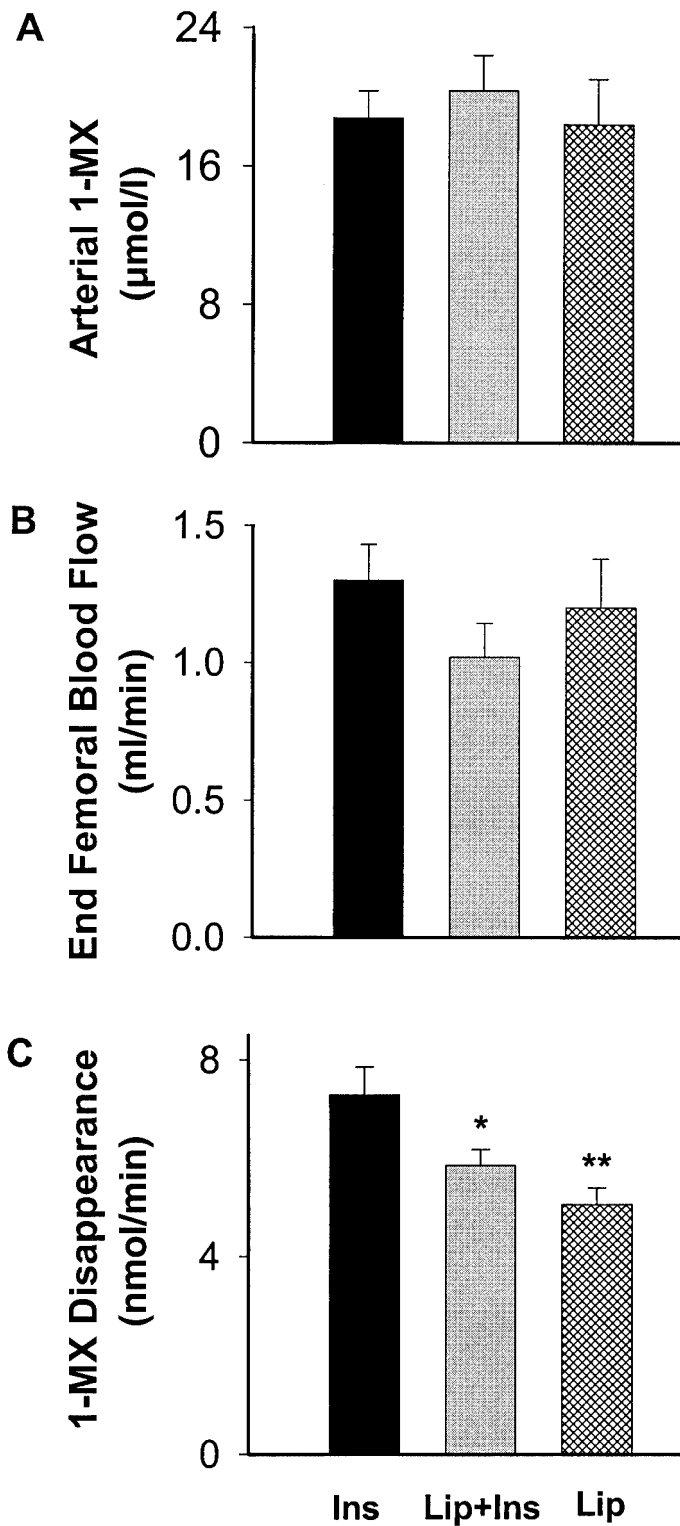


FIG. 6. Arterial 1-MX (A), end arterial femoral blood flow (B), and hindleg 1-MX disappearance (C) values of Ins, Lip + Ins, and Lip. Values are means \pm SE. Significant differences from Ins: * $P \leq 0.05$, ** $P \leq 0.01$.

in insulin-mediated metabolic changes in the hindleg. These included an inhibition of 45% of the IMGU by the hindleg and an inhibition of 69% of the insulin-mediated uptake of 2DG by muscles of the lower leg. The reductions in insulin-mediated changes occurred at physiologic insulin levels (33) and a total exposure to Intralipid/heparin for

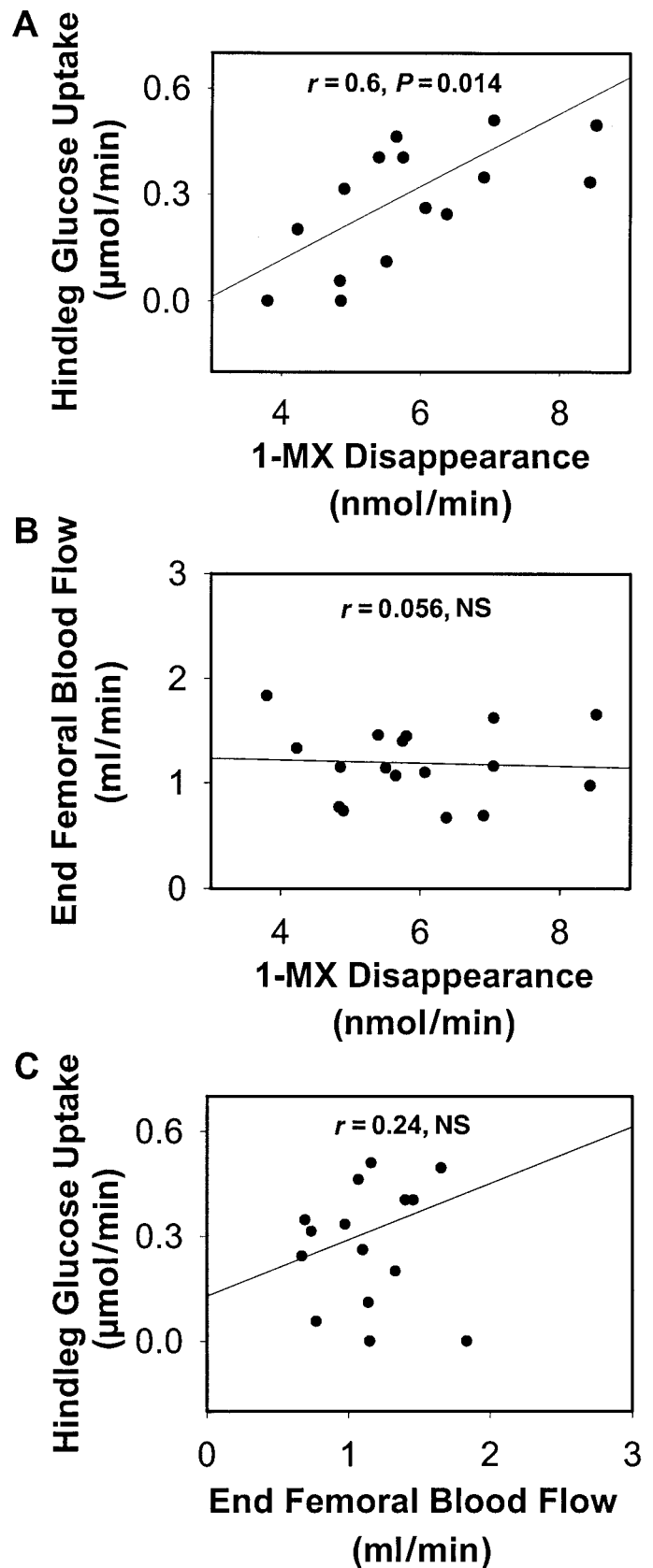


FIG. 7. Relationships between HGU and 1-MX disappearance (A), end femoral arterial blood flow and 1-MX disappearance (B), and HGU and end femoral arterial blood flow (C). Individual values for $n = 15$ rats are shown.

6 h that included 2 h during the hyperinsulinemic-euglycemic clamp. This impairment of insulin-mediated capillary recruitment by Intralipid/heparin is the second hemodynamic defect of insulin to be reported: impaired bulk leg blood flow in humans treated with Intralipid/heparin has been described (39).

A second finding to emerge from this study was the positive correlation between HGU and 1-MX metabolism (Fig. 7A). This contrasted with HGU and femoral arterial blood flow, where there was no significant correlation. The absence of a correlation between HGU and FBF resulted from the absence of a difference for FBF between any of the three treatment protocols, Ins, Lip, or Lip + Ins, despite individual animal differences for HGU and 1-MX metabolism.

The present study represents another instance in which we have documented a close relationship between glucose uptake and capillary recruitment under hyperinsulinemic-euglycemic clamp conditions. Previously, this relationship has been described for α -methyl serotonin (34), TNF- α (35), and exercise-trained rats (40). A recurring correlation between IMGU and insulin-mediated capillary recruitment implies but does not necessarily prove causality. In one of the prior studies, we reported a marked inhibition of IMGU when insulin-mediated capillary recruitment was blocked pharmacologically by a peripherally acting serotonergic agonist, α -methyl serotonin (34). This intervention was based on experiments using the isolated pump-perfused rat hindleg in which the vasoconstrictors α -methyl serotonin or serotonin were found to redirect flow from the nutritive to the nonnutritive route (41). Indirect evidence that serotonergic agonist-mediated insulin resistance did not result from a direct effect on muscle glucose uptake and metabolism was obtained using isolated incubated muscle preparations (42), where the vascular distribution of insulin and glucose is not involved. In that preparation, serotonin had no effect on IMGU (42). Thus, it would appear likely that IMGU becomes partly inhibited by denying access for insulin and glucose to regions of the muscles of the perfused hindleg or the hindleg *in vivo*. Similarly, an increase in nutritive blood flow, whether mediated in perfused muscle by vasoconstrictors (43) or *in vivo* by insulin (20), leads to increased muscle glucose uptake.

However, even if insulin-mediated capillary recruitment contributes in part to the increase in muscle glucose uptake by this hormone *in vivo* by acting to enhance access for itself and glucose, no information emerges from the present or previous studies (20,34,35) as to mechanism. Given that an increase in bulk blood flow is not involved, increased capillary (nutritive) flow must then result from a redistribution of flow from the nonnutritive route. That being so, a net vasodilation of sites controlling entry of blood to the nutritive route mediated by insulin is likely. We know that insulin-mediated capillary recruitment is nitric oxide (NO) dependent (44), and there are a number of possible mechanisms to account for the effect. First, insulin may act at insulin receptors on endothelial cells to produce NO, which in turn permeates adjacent vascular smooth muscle cells to lower the vascular tone of precapillary sphincters. In favor of this mechanism is that this process is NO dependent and is thus consistent with

our preliminary data (44) that endothelial NO synthase (eNOS) knockout mice are insulin resistant (45), and there are data that TNF- α interrupts insulin signaling to eNOS in this cell type (46); against this mechanism is the fact that to do so insulin must have access to the sites it is going to dilate. This requires that some flow occur before insulin arrives at these sites. Also against this possibility is the preliminary report that vascular endothelial insulin receptor knockout mice are not insulin resistant (47). Second, insulin may act at insulin receptors on the vascular smooth muscle cells (48) to cause vasorelaxation. Third, insulin may act at insulin receptors on skeletal muscle to activate glucose transport and metabolism to produce a metabolite (e.g., adenosine) that permeates adjacent tissue to react with appropriate receptors on endothelial and/or vascular smooth muscle cells to result in vasorelaxation—as for example, during exercise. This mechanism would not necessarily be NO dependent but would be inhibited by agents that inhibit muscle glucose metabolism. As a variant of this third mechanism, a form of NOS is activated in skeletal muscle independently of glucose metabolism. NO could then permeate neighboring tissue as above. Regardless, all mechanisms will be sensitive to inhibitors of insulin signaling.

From the present study, it is clear that elevated plasma free fatty acids resulting from the infusion of Intralipid/heparin led to an inhibition of insulin-mediated capillary recruitment and glucose uptake in muscle. The question now arises as to how this occurred. Until recently, the focus has been on the metabolic effects of the fatty acids on muscle glucose metabolism. In 1963, Randle et al. (14) proposed the notion of competition between glucose and free fatty acids as oxidative fuel sources in muscle (glucose-fatty acid hypothesis). Despite a number of studies (49–51) examining the effect of fatty acids on skeletal muscle glucose metabolism, it is still controversial whether insulin resistance in diabetes and obesity result from increased plasma fatty acids. A number of sites in glucose metabolism have been reported to be inhibited by fatty acids, and it is generally agreed that raising free fatty acids increases fatty acid oxidation at the expense of carbohydrate oxidation. There have been independent reports that glycolysis (52) and glycogen synthesis (53) are inhibited. Some found inhibitory effects on glucose uptake (54,55) but most did not (56–59). A complication in identifying the precise site or sites is that the effects of fatty acids on insulin-stimulated glucose metabolism are time dependent (60,61). Roden et al. (61) claim that the key event is glucose transport. Noninvasive nuclear magnetic resonance imaging of stable isotope-enriched fuels in the muscle of healthy humans showed that the reduction in glycogen synthesis by elevated plasma free fatty acids was preceded by a decrease of muscle glucose 6-phosphate concentrations starting at ~ 1.5 h (61). However, this could not be explained by changes in the amount of insulin-regulatable glucose transporter protein in either oxidative or glycolytic muscle (62). In view of this result, recent studies have focused on insulin signaling. A particularly valuable approach has been to assess signaling in muscle *in vivo*. With this approach, Griffin et al. (17) reported a decrease in IRS-1-associated phosphatidylinositol 3-kinase activity and a blunting in insulin-stimulated

IRS-1 phosphorylation. Because these effects were accompanied by a marked increase in protein kinase C θ (PKC θ) activity, Griffin et al. (17) propose that activation of PKC θ is responsible for the decrease in insulin signaling, glucose transport, and dependent metabolism, including glycolysis, glucose oxidation, and glycogen metabolism.

If capillary recruitment is a consequence of glucose metabolism and mediated by a generated product, then impairment of glucose metabolism by fatty acids acting via PKC θ would be expected to curtail the component of 1-MX metabolism increased by insulin. However, if we assume insulin signaling mechanisms are similar in most tissues, then impairment of insulin signaling in vascular tissue by any of the mechanisms discussed above could also inhibit insulin-mediated capillary recruitment. On balance, existing data would favor the latter direct mechanism to affect vascular tissue. There are some data that help this argument. Lind et al. (63) have demonstrated impairment in endothelial function by free fatty acids in the human forearm in terms of a reduced response to local intra-arterial methacholine relative to nitroprusside. In addition, Steinberg et al. (39) have shown that free fatty acid elevation impairs insulin-mediated vasodilation and NO production in the legs of insulin-sensitive subjects undergoing euglycemic-hyperinsulinemic (40 mU \cdot m $^{-2}$ \cdot min $^{-1}$) clamps.

In conclusion, elevation of plasma free fatty acid levels for 6 h during a hyperinsulinemic-euglycemic clamp at physiologic insulin leads to whole-body insulin resistance with impaired insulin-mediated muscle glucose uptake and capillary recruitment. We propose that the fatty acids induce an impairment of insulin signaling in vascular tissue. The reduction of insulin-mediated muscle glucose uptake may be due in part to reduced access for insulin and glucose.

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