

Experimental Hyperlipidemia Dramatically Reduces Access of Insulin to Canine Skeletal Muscle

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A complex sequence of steps is required for insulin to cause glucose uptake. Impairment of any one of these steps can contribute to insulin resistance. We observed the effect of insulin resistance induced by hyperlipidemia on the dynamics of insulin injected into skeletal muscle. Basal insulin euglycemic clamps (0.2mU/min/kg) with or without lipid infusions (20% at 1.5 ml/min) were done on anesthetized dogs. Sequential insulin doses were administered by intramuscular injection directly into the vastus medialis of one hindlimb, using the contralateral leg for comparison. Intramuscular insulin injection in normal animals caused a clear dose-dependent increment in interstitial insulin levels, as well as dose-dependent increase in leg glucose uptake. In a second group of animals, lipid was infused before and during intramuscular insulin injection to cause systemic increase in free fatty acids (FFAs). In sharp contrast, systemic lipid infusion caused insulin resistance, indicated by reduced glucose infusion required to maintain euglycemia, and prevented injection-induced increase in lymphatic insulin and leg glucose uptake observed without lipid. The injected insulin was instead detected in the venous outflow from the leg. Lipid infusion caused intramuscular insulin to be diverted from interstitium into the capillary circulation, preventing a rise in interstitial insulin and any increase in local leg glucose uptake. The diversion of insulin from the interstitium under hyperlipidemic conditions may play a role in the insulin resistance observed coincident with elevated nocturnal FFAs as is observed in obesity.

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INTRODUCTION

Insulin resistance is a risk factor for various diseases, including type 2 diabetes, cardiovascular disease, hypertension, and cancer (1). The mechanisms causing insulin resistance continue to be debated (2). Most research currently focuses on cellular defects and signaling abnormalities (3). But, there are other factors that enhance insulin-mediated glucose disposal. For skeletal muscle and adipose tissue, insulin must first cross the capillary endothelial barrier, and enter the interstitial space. Once in interstitium, insulin can bind to insulin receptors to initiate signaling cascades, causing GLUT4 translocation and glucose uptake (4,5). In insulin-resistant states including type 2 diabetes, there is a reduction in skeletal muscle insulin signaling (5–7). Intracellular lipids in muscle and liver can interfere with oxidative phosphorylation in the mitochondria, activating kinases that reduce insulin signaling and glucose transport (8). Although reduction in insulin signaling may be a cause of insulin resistance, it could also result from reduced access of insulin to the interstitial space and insulin receptors (9).

In fact, we have presented evidence that experimental obesity due to high-fat diet reduces access of insulin to skeletal muscle (10) possibly due to changes in capillary recruitment (11) or sluggish transport of insulin across the capillary endothelium (12). Definitive studies showing that insulin resistance is associated with a decrease in the hormone's ability to access insulin-sensitive tissues are lacking.

Many studies examining the effects of free fatty acids (FFAs) on insulin action involve insulin resistance secondary to acute hyperlipidemia caused by infusion of lipid. The latter raises plasma FFAs, which inhibit insulin signaling (13) and impair insulin-mediated glucose transport in skeletal muscle (14,15), possibly secondary to impaired insulin signaling (16,17). Also, increased intramyocellular lipid content has been associated with the development of insulin resistance (3,18). We suggest that lipid-mediated cellular defects in insulin action may be secondary to insulin's failure to access skeletal muscle during lipid infusion.

Even in normal individuals, glucose uptake is delayed after insulin infusion, becoming significantly elevated only 30 min

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after the beginning of the hyperinsulinemic euglycemic clamp, and does not reach steady state for several hours. Changes in capillary recruitment occur much earlier than increased glucose disposal; from 7 min, reaching a peak at ~20 min (ref. 19). The insulin action delay may be due to sluggish appearance of insulin in the interstitial space, delaying the binding of insulin to myocytes (20). Data in healthy canines indicate delivery of insulin to the interstitial space is temporally limiting (21); whether this is due to transendothelial transport delay or changes in distribution of blood flow that supplies muscle is not clear.

Here, we used the intramuscular injection of insulin to investigate the correlation between interstitial insulin concentrations and local glucose uptake in skeletal muscle. Insulin was injected into a hindlimb muscle under hyperlipidemia induced by lipid infusion. If lipid causes insulin resistance at the receptor or signaling level, there would be a delay in local glucose uptake even with immediate appearance of interstitial insulin, such that for the same amount of interstitial insulin, less glucose uptake occurs. In effect, the myocytes would become resistant at a cellular level. However, if interstitial insulin causes local glucose uptake to a comparable extent with or without lipid infusion, resistance caused by lipid is upstream of the interstitial space, and the issue is delivery to the interstitial space. The present data argues that hyperlipidemia induced by lipid infusion causes insulin resistance by limiting the access of insulin to the interstitial space.

METHODS AND PROCEDURES

Animals

One acute glucose clamp experiment was performed on each of eight anesthetized 1-year-old male mongrel dogs (29.6 ± 1.6 kg lipid, 31.3 ± 1.3 kg control). Large animals were necessary to allow dynamic collection of skeletal muscle lymph exudate, which mirrors interstitial fluid. For ethical reasons, it is mandatory to utilize a minimal number of animals in acute experiments such as these. Therefore we utilized control experiments, previously published using the identical experimental design but in which lipid was not infused ($n = 13$) (ref. 21). Control studies used a similar protocol and overlapped the time frame of lipid studies, so we did not consider it prudent to repeat identical previously published control experiments for this article, and previous data are shown here.

Before experiments animals were housed in the University of Southern California Medical School Vivarium under controlled conditions (12 h light:12 h dark) and fed standard chow (49% carbohydrate, 25% protein, 9% fat; Alfred Mills, Chicago, IL) once per day. Dogs in good health as determined by visual observation, body weight, hematocrit, and body temperature were used for experiments. Protocols were approved by the USC Institutional Animal Care and Use Committee, and were in accordance with the Principles of laboratory animal care suggested by the National Institutes of Health.

Surgery

Animals were fasted 15 h before the morning of the experiment at 0600 hours and preanesthetized with acepromazine maleate (Prom-Ace, 0.22 mg/kg; Auco, Fort Dodge, IA) and atropine sulfate (0.11 ml/kg; Western Medical, Arcadia, CA). Anesthesia was induced with sodium pentobarbital (0.44 ml/kg; Western Medical) and maintained with inhalant isoflurane (Western Medical). Dogs were placed on heating pads to maintain body temperature. Indwelling catheters were implanted in the right jugular vein for a continuous saline drip (~1 l for the first 60 min of surgery and a slow drip thereafter) and the left carotid artery for sampling and blood pressure monitoring (model #90603A;

Space Labs, Issaquah, WA). Intracatheters were inserted into the left cephalic vein for variable glucose infusion and the right cephalic vein for insulin, somatostatin and lipid infusion. Indwelling catheters were placed into both right and left femoral arteries and veins for blood sampling. Perivascular ultrasonic flowprobes (2-mm diameter; Transonic, Ithaca, NY) were placed around right and left femoral arteries proximal to the femoral catheter for measuring blood flow rates. Left and right hindlimb lymphatic vessels were cannulated by placing polyethylene catheters (PE10) into the afferent lymphatic vessels of the deep inguinal lymph node. Blood pressure, heart rate, O₂ saturation, and CO₂ were monitored continuously. At the conclusion of these experiments, animals were euthanized with an overdose of sodium pentobarbital (Eutha-6, 65 mg/kg; Western Medical).

Clamps

Immediately after starting surgical procedures, a basal insulin euglycemic clamp was started ($t = -240$ min). Simultaneous systemic infusions of somatostatin (1 μ g/min/kg; Bachem, Basel, Switzerland) and basal insulin replacement (0.2 mU/min/kg; Novo Nordisk, Bagsvaerd, Denmark) were continued for the remainder of the study (Figure 1). Exogenous 20% glucose was infused at variable rates to maintain euglycemia at the basal level based on online glucose measurements from the injected leg's (INJ) femoral artery plasma (established before insulin infusion) throughout the entire experimental period. At least 60 min after insulin infusion commenced, a bolus of heparin was given (250 mU), immediately followed by a lipid infusion containing heparin (20% Liposyn with 16 mU/ml heparin, 1.5 ml/min). Samples were taken simultaneously from the right and left femoral arteries and veins. Left and right hindlimb lymph vessels were sampled by gently massaging the hindlimb distal to the site of catheterization from 2 min before to 2 min after each blood sample.

Intramuscular insulin

At times 0, 60, 120, 180, and 240 min, porcine insulin (0.3, 0.5, 0.7, 1, and 3 U) was injected into the vastus medialis of the quadriceps femoris with two 30-gauge needles inserted ~1 cm apart with a volume of 0.5 ml per syringe (Figure 1a), given at ~0.6 ml/min. Simultaneous injection enabled a larger area of distribution of insulin throughout the muscle, and subsequent injections were administered to a similar area as the original injection for consistency between administrations. Volume and rate of injection was matched for each dose to ensure similar tissue damage (if any) among doses. For individual experiments, right or left hindlimb was chosen at random for injections (INJ) while the noninjected

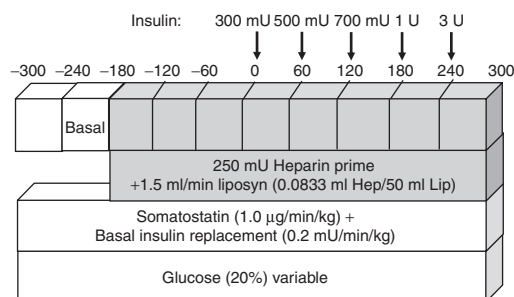


Figure 1 Experimental protocol used throughout the study. Time in minutes is shown across the top. Somatostatin was infused with basal insulin replacement; glucose was infused at a variable rate to maintain euglycemia. In lipid experiments, 180 min before the first injection, a priming heparin injection was given, and lipid infusion was initiated. Samples were taken periodically to ensure euglycemia was maintained. Every 60 min from time 0, an insulin injection was given, with doses of 300 mU, 500 mU, 700 mU, 1 U, and 3 U. Samples were taken every 10 min during from time 0–300 min from the femoral artery and vein, as well as lymph, of INJ and NINJ legs. INJ, injected; NINJ, noninjected.

contralateral limb (NINJ) was used for comparison. Plasma and lymph samples were taken every 10 min after each injection.

Assays

Plasma and lymph samples were collected in microtubes that were precoated with lithium–heparin (Becton Dickinson, Franklin Lakes, NJ) and assayed for insulin and glucose. Plasma tubes also contained 50 μ l 20 g/l EDTA (Sigma Chemicals, St Louis, MO) providing <5% dilution of sample. Blood samples were centrifuged immediately; the supernatant was transferred and stored at -20°C until further assay. INJ femoral artery plasma samples were immediately assayed for glucose with an YSI 2700 auto-analyzer (Yellow Springs Instrument, Yellow Springs, OH) before freezing at -20°C . Lymph samples were stored at -20°C after sampling. Samples were assayed for insulin by enzyme-linked immunosorbent assay, and glucose with an YSI 2700 auto-analyzer.

Calculations

We calculated “net” insulin or local glucose uptake as INJ minus NINJ values. Glucose uptake across each limb was calculated based on Fick’s principle: $\text{LGU} = (G_A - G_V) \times \text{BF}$, where LGU, local glucose uptake; G_A , arterial glucose; G_V , venous glucose; BF, femoral blood flow.

Statistical analyses

All experimental data are expressed as the means \pm s.e., and interpolated where necessary. Two-way repeated measures ANOVAs were performed on all groups; once a significant difference was detected, comparisons made within each group (control or lipid) used paired *t*-tests, and control and lipid groups were compared using unpaired *t*-tests. Significance was assumed at $P \leq 0.05$.

RESULTS

Glucose clamp stability

Artery glucose was successfully clamped at each animal’s basal glucose concentration (99.1 ± 2.0 mg/dl) as shown in **Figure 2a**, and was not different from studies without lipid infusion. The infusion rate required to maintain euglycemia was significantly reduced in the presence of lipid (compared to control data (21), two-way repeated measures ANOVA, **Figure 2b**).

Blood pressure and blood flow

Vital signs were monitored and maintained throughout the experiments, with an average systolic blood pressure of 124.5 ± 1.0 mm Hg, diastolic pressure of 57.0 ± 0.4 mm Hg, and pulse of 109.7 ± 0.4 beats per minute, all of which are significantly different from control values (108.7 ± 0.4 mm Hg, 47.6 ± 0.7 mm Hg, 112.7 ± 0.5 beats per minute, respectively). Blood flow comparing INJ to NINJ legs was not significantly different at basal (INJ 171.6 ± 21.04 ml/min; NINJ 172.0 ± 16.17 ml/min, $P = 0.622$) and was unaffected by lipid infusion (control basal: INJ 165.4 ± 19.1 ml/min; NINJ 160.0 ± 13.9 ml/min, $P = 0.780$) or insulin injections, even at the highest dose (INJ 153.7 ± 17.4 ml/min, $P = 0.155$). Thus, blood flow changes are not implicated in effects of lipid or insulin.

Basal glucose and insulin

Confirming previous results (21,22), basal lymph glucose levels tended higher (+10%) than plasma glucose levels (**Table 1**), though this apparent difference was not significant. Other studies show interstitial glucose is lower than plasma (e.g., ref. 23); it is possible that the sampling mechanism may

be responsible for this difference. Lymph insulin was half that of arterial plasma (**Table 1**) as previously reported (21,22). When compared to results for experiments without lipid infusion, there were no differences between basal glucose or insulin levels.

Arterial insulin

Basal arterial insulin was 14.1 ± 1.4 mU/l in both legs. There was a small rise in arterial insulin with the intramuscular injections; this was the same for both limbs (**Figure 3a**). This increase was presumably due to a portion of the injected insulin exiting through the venous circulation and being redistributed through the systemic circulation. There was no significant difference in arterial insulin between INJ and NINJ or between lipid experiments and controls at any time point during the experiments.

Venous insulin

Direct, stepwise injection of insulin only into one leg caused an increase in the INJ vein insulin, which was not observed in the contralateral NINJ (**Figure 3b**). Subsequent insulin injections induced a further rise in venous insulin in the INJ leg, which was not observed in the NINJ leg. There was a significant difference between INJ vein insulin in control and lipid studies. The washout of insulin in the femoral vein accounted

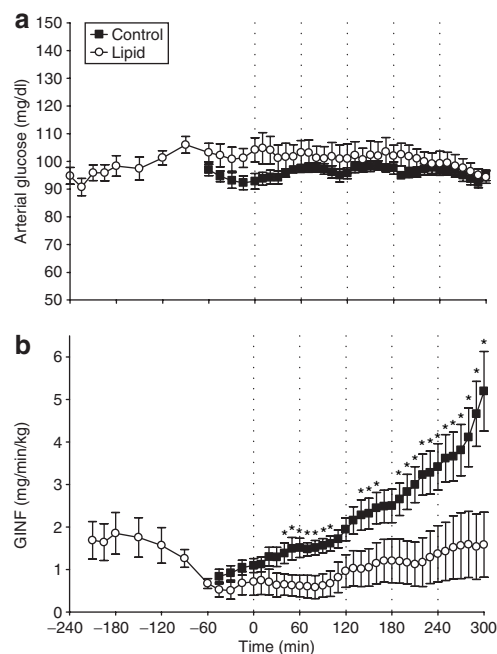


Figure 2 (a) Femoral artery glucose was maintained throughout the experiment by modifying the (b) glucose infusion rate (GINF). Dotted lines represent an intramuscular insulin injection of increasing concentration, in control (black filled symbols) and lipid (white symbols) experiments as described in the methods. Lipid infusion began at -180 min, and basal insulin replacement occurred throughout the experiment; unpublished data show that lipid causes insulin resistance in 2–3 h. Two-way repeated measures ANOVA demonstrated no difference in arterial glucose, but a significant difference in GINF between groups; these were then analyzed by unpaired *t*-test at each time point.

Table 1 Average insulin and glucose concentration at basal

| | Control | | Lipid | |
|-----------------|---------------|---------------|---------------|---------------|
| | INJ | NINJ | INJ | NINJ |
| Insulin (mU/l) | | | | |
| Artery | 11.86 ± 0.12 | 12.15 ± 0.29 | 13.62 ± 1.26 | 13.38 ± 1.09 |
| Vein | 11.32 ± 0.57 | 11.36 ± 0.21 | 13.31 ± 1.13 | 12.32 ± 1.24 |
| Lymph | 6.04 ± 0.6 | 5.53 ± 0.18 | 6.33 ± 0.57 | 7.72 ± 0.72 |
| Glucose (mg/dl) | | | | |
| Artery | 93.66 ± 1.87 | 93.3 ± 1.52 | 103.04 ± 3.51 | 100.27 ± 3.83 |
| Vein | 94.18 ± 1.87 | 93.11 ± 1.83 | 101.06 ± 3.34 | 102.25 ± 5.49 |
| Lymph | 108.01 ± 1.87 | 105.18 ± 3.46 | 115.55 ± 4.63 | 108.47 ± 6.34 |

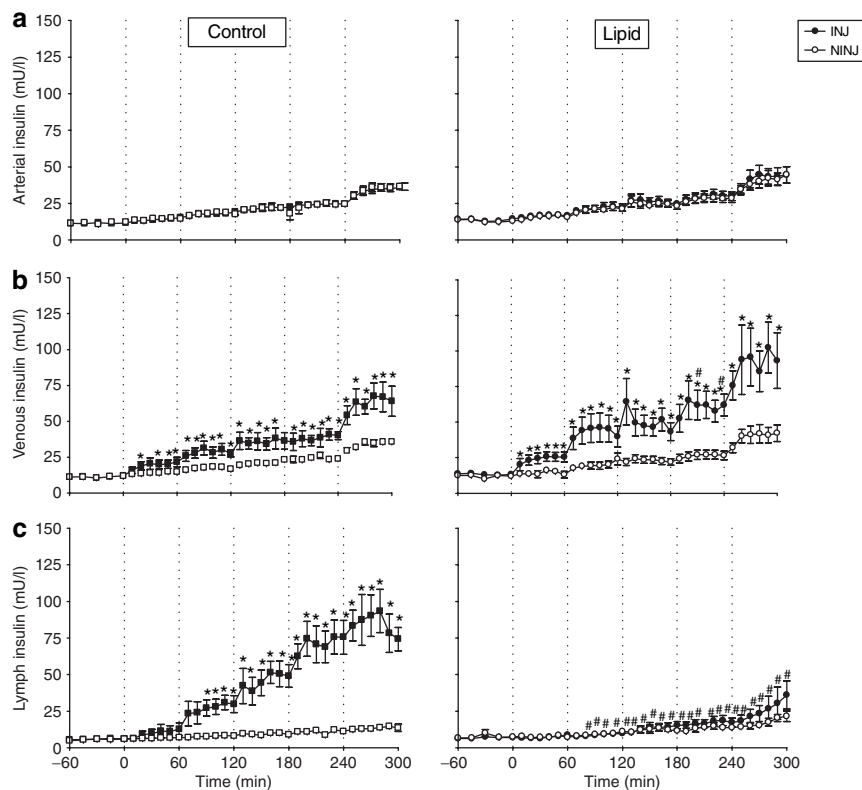


Figure 3 (a) Femoral artery, (b) femoral vein, (c) and lymph insulin concentration throughout experiment for control (left) and lipid (right) in INJ (black symbols) and NINJ (white symbols) legs after insulin injection (dotted lines). There was no significant difference in arterial insulin as determined by two-way repeated measures ANOVA, though there were significant differences between vein and lymph insulin concentrations. *Significantly different from corresponding NINJ leg at same time point with paired *t*-test; #significantly different from control INJ leg, using unpaired *t*-test. INJ, injected; NINJ, noninjected.

for $24.87 \pm 6.82\%$ of the total injected insulin (compared to $17.15 \pm 5.19\%$ in controls, no significance).

Lymph insulin

Controls showed a dose-dependent increase in interstitial concentrations following local insulin injection. In stark contrast, there was no significant difference between INJ and NINJ legs with lipid infusion, and control and lipid INJ legs were significantly different (Figure 3c). There was a trend for increasing lymph insulin with time, likely due to the corresponding increase in arterial insulin supply (Figure 3a). Hyperlipidemia

prevented the appearance of locally injected insulin in the sampled interstitium. The washout of insulin in the lymph accounted for $0.02 \pm 0.02\%$ of the total injected insulin (compared to $0.20 \pm 0.03\%$ in controls, $P < 0.001$). The total amount of injected insulin remaining in the muscle and unaccounted for in vein or lymph is $74.9 \pm 6.8\%$ in lipid, and $81.9 \pm 4.9\%$ in control (no significance).

Insulin-mediated glucose uptake

Without lipid, insulin injection increased glucose uptake substantially in INJ, but not NINJ. In contrast, and consistent

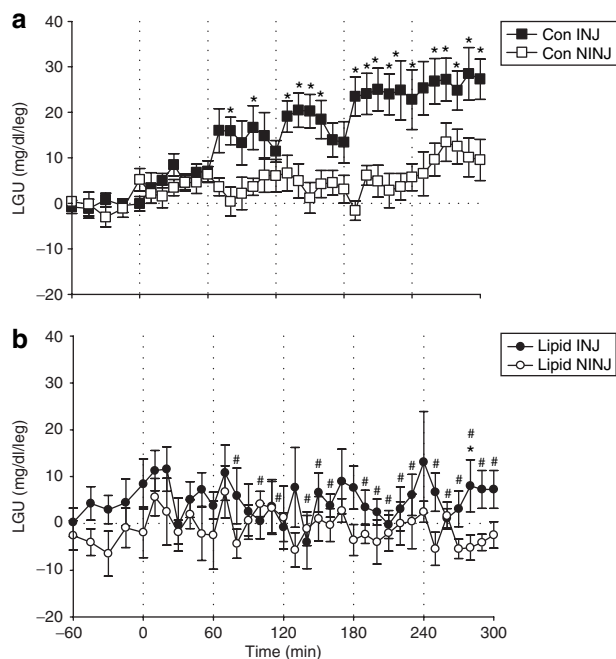


Figure 4 Local glucose uptake calculated from arteriovenous difference across the INJ (black symbols) and NINJ (white symbols) leg for (a) control and (b) lipid dogs. The insulin injections (indicated by dotted lines) had no effect on local glucose uptake in INJ when compared to NINJ in the lipid experiments, though significantly increased in control experiments. *, significantly different from corresponding NINJ leg at same time point with paired *t*-test; #, significantly different from control INJ leg, using unpaired *t*-test. INJ, injected; NINJ, noninjected.

with failure of interstitial insulin to rise, glucose uptake in INJ, with hyperlipidemia, did not show any significant increment compared to the contralateral leg (NINJ) (Figure 4). Glucose uptake was significantly higher in INJ controls than with lipid. Lipid prevented insulin-mediated glucose uptake (Figure 4).

DISCUSSION

In vivo, secreted insulin must traverse a series of hurdles before it can stimulate glucose uptake into skeletal muscle cells. After release from the pancreas, insulin is distributed through the blood to the capillaries, where it can cross the endothelial barrier to access the interstitial space. Once diffused throughout the interstitial space, it can bind to insulin receptors on skeletal muscle to cause GLUT4 translocation and glucose uptake. This final step of receptor binding and signaling has been well characterized (24), and other studies have observed the effect of delivery of insulin and glucose in the form of local changes in blood flow and distribution (9,11,19), but further characterization of the transport is limited. The novel technique in this study uses a direct intramuscular delivery of insulin, which does not require transport from the blood into the interstitial space, and lymph vessel cannulation allows an indication of the interstitial insulin concentrations.

In healthy animals, intramuscular insulin injections lead to dose-dependent rapid increases in interstitial insulin, which results in proportional dose-dependent rapid increases in local glucose uptake (21). In stark contrast, the present results show

that hyperlipidemia due to systematic lipid infusion causes the insulin injected into muscle to divert into the venous circulation, preventing a significant local elevation of interstitial insulin. Also, there is no local glucose uptake during lipid infusion, which would be expected based on the lack of increased insulin in the interstitial space. The venous washout of insulin is on the average greater in the lipid study shown here than in control studies, though the difference is not significant (21). Inclusion of lipid prevents a significant rise in interstitial insulin after an intramuscular injection.

Our results show that lipid significantly lowers insulin sensitivity as measured by glucose infusion rate. While the rate of lipid infusion we use is comparable to that used in previous studies in adults (13,14,18,25,26), it is worth noting that there would be significant weight differences between dogs and adult men, although body weights were not reported in the human studies. However, other studies in rats have used 2.6–5 ml/h/kg (refs. 15–17), which is comparable to the rate of infusion we have used here (~3 ml/h/kg). It would be interesting to know the FFA levels in this study, but the anesthesia and heparin included in the lipid infusion would alter lipolysis, making comparisons to healthy controls difficult.

The mechanism by which fat causes insulin resistance is unclear, as reviewed in Krebs and Roden (6). Much of the effect of fat to cause insulin resistance appears to correlate well with intracellular accumulation of lipid (3,6,26), possibly leading to mitochondrial dysfunction and insulin resistance (27) although there can be exceptions (reviewed in ref. 6). Fat also alters vascular function and inhibits insulin-mediated capillary recruitment (28). Previous studies observed that palmitate can inhibit insulin-mediated glucose uptake at a cellular level, suggesting that lipid has a direct effect on the cell (29,30). What is most surprising and interesting in this study is that we have shown that lipid prevents the rise in interstitial insulin, suggesting myocytes are not even exposed to increased insulin. This mechanism may be as important or even more important than lipid-induced downstream signaling impairment (16,17), intramyocellular fat accumulation (3,18), or mitochondrial dysfunction (27).

In vivo studies in healthy individuals have demonstrated that insulin signaling and GLUT4 translocation occur within 10 min of insulin binding to its receptor (20), a finding supported by our studies (21). Typically, plasma insulin increases before lymph insulin and insulin receptor tyrosine kinase activation at the myocytes, yet there was a close temporal relationship between insulin receptor activation and glucose disposal (20). Therefore, the delay in insulin action in healthy individuals is due to some prereceptor step: delivery by blood, transendothelial transport, or diffusion within the interstitial space. Our data support this, as glucose uptake is increased within 10 min of an interstitial injection (21).

Here, insulin is administered into the intramuscular space, producing a local high concentration compared to the basal levels in the plasma and surrounding interstitium, yet insulin never appears in the lymph. Although there is no significant differences between the amounts of injected insulin remaining

in the muscle in either study, lymph measures demonstrate that the myocytes in general are not exposed to insulin in lipid studies, whereas a larger proportion of the myocytes are able to react to insulin in controls. The location of the insulin before it appears in the venous blood is not known, but it may involve transport through the endothelial cells; in fact Wang *et al.* (31) showed that insulin is sequestered in the endothelial cells.

It is possible that lipid infusion somehow alters the ability of the lymph vessel to sample the interstitial space, whereby the insulin is in the interstitial space, but is not detected in lymph. However, basal lymph insulin is unaffected by lipid and even seems to respond to slight changes in arterial insulin consistent with control experiments (Figure 3). This suggests that lymph reflects systemic insulin, although there is an apparent failure to reflect the acute local injection. Another method of measuring interstitial insulin concentrations may confirm or clarify these results; however, microdialysis, a common method of sampling interstitial fluid, would necessitate that the injections are performed at a constant site in relation to the position of the microdialysis probe. This would be difficult to reproduce between injections and between animals, and would likely provide very inconsistent results. Preliminary studies injecting a higher concentration of insulin have shown that insulin can still appear in the lymph, and therefore can be elevated in the interstitial space even with lipid infusion.

Changes in the permeability of the endothelial wall may be responsible for insulin's movement into the blood, as previous studies have shown that permeability is increased in various insulin-resistant states (32,33). However, in the fructose-fed hypertensive rat model, which begins to develop glucose intolerance and elevated plasma insulin, there is a decrease in permeability (34). These studies focus on the movement from plasma to the interstitial space, but an increased permeability may work both ways, causing the insulin to move from an area of high concentration (in this case, the interstitium) to low (plasma). This theory does not account for the location of insulin after injection and before appearance in the vein.

An alternative explanation for the present results is that insulin-mediated capillary recruitment is impaired with lipid, preventing the dispersion of insulin through the muscle to cause glucose uptake. It is interesting that in healthy situations, the injection of insulin into the intramuscular space is able to significantly increase the lymph insulin concentration (21), when the injected area is only a small section of the entire muscle sampled by lymph. It is perhaps more likely that the insulin is removed into the surrounding capillaries and redistributed to the local muscle, where the lymph can reflect it more efficiently, and more glucose uptake is induced. This would effectively increase the volume through which the insulin is distributed. As there is no delay in diffusion through the interstitium (21), capillary recruitment would rapidly facilitate the dispersal of the insulin injection to a larger area (19). Lipid reduces capillary recruitment (27), and the insulin injected into the muscle is restricted to the area of injection, without further dissemination through the muscle. This would cause a local high concentration of insulin, which may not be directly

sampled by lymph. Whether or not glucose uptake is occurring in this area is difficult to determine, as insulin may cause local glucose uptake, but at too small an amount to detect across the leg. A method to measure capillary recruitment in this dynamic state is currently being developed. Also, vasoactive agents that increase basal capillary recruitment with lipid will determine whether the injected insulin is then able to disperse further into the muscle, and be detected in lymph.

In conclusion, we show that when insulin resistance is induced by lipid infusion, insulin injected into the muscle appears in the vein, but not lymph. This differs from control studies without lipid infusion, where there was a significant elevation of insulin in the lymph (21). Here, insulin resistance occurs because the myocytes are not exposed to insulin; care must be taken to ensure that results suggesting resistance is due to downstream signaling impairment, intramyocellular fat accumulation, or mitochondrial dysfunction are not in fact due to insulin's inability to access the tissue (27). This may have implications on the use of intramuscular injections of insulin as obese diabetic patients that who high circulating lipid concentrations will respond differently to lean individuals. The reason for this change is unknown, although future experiments will aim to determine the mechanism of the change induced by lipid and more physiological plasma lipid concentrations may help to understand this result.

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DISCLOSURE

The authors declared no conflict of interest.

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