

Diet-Induced Obesity Prevents Interstitial Dispersion of Insulin in Skeletal Muscle

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OBJECTIVE—Obesity causes insulin resistance, which has been interpreted as reduced downstream insulin signaling. However, changes in access of insulin to sensitive tissues such as skeletal muscle may also play a role. Insulin injected directly into skeletal muscle diffuses rapidly through the interstitial space to cause glucose uptake. When insulin resistance is induced by exogenous lipid infusion, this interstitial diffusion process is curtailed. Thus, the possibility exists that hyperlipidemia, such as that seen during obesity, may inhibit insulin action to muscle cells and exacerbate insulin resistance. Here we asked whether interstitial insulin diffusion is reduced in physiological obesity induced by a high-fat diet (HFD).

RESEARCH DESIGN AND METHODS—Dogs were fed a regular diet (lean) or one supplemented with bacon grease for 9–12 weeks (HFD). Basal insulin ($0.2 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) euglycemic clamps were performed on fat-fed animals ($n = 6$). During clamps performed under anesthesia, five sequential doses of insulin were injected into the vastus medialis of one hind limb (INJ); the contralateral limb (NINJ) served as a control.

RESULTS—INJ lymph insulin showed an increase above NINJ in lean animals, but no change in HFD-fed animals. Muscle glucose uptake observed in lean animals did not occur in HFD-fed animals.

CONCLUSIONS—Insulin resistance induced by HFD caused a failure of intramuscularly injected insulin to diffuse through the interstitial space and failure to cause glucose uptake, compared with normal animals. High-fat feeding prevents the appearance of injected insulin in the interstitial space, thus reducing binding to skeletal muscle cells and glucose uptake. *Diabetes* 59:619–626, 2010

Insulin resistance is associated with a number of diseases, including cardiovascular disease, hypertension (1), cancer (2), and obesity (1,3), as well as type 2 diabetes. Much research into insulin resistance has focused on insulin signaling, suggesting that target cell (e.g., skeletal muscle) response to insulin is the primary defect. Most recently, mitochondrial dysfunction (4,5) and intracellular fat accumulation (6) have been proposed as primary causes of insulin resistance. Despite these efforts, there is no definitive conclusion as to the primary cause of cellular insulin resistance.

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There are a number of loci where insulin action can be altered, including the direct effect of inflammatory cytokines to attenuate distribution of blood flow and glucose uptake (7), and direct effects of plasma free fatty acids (FFAs) to impede insulin signaling in the cell (4) and/or reduce capillary recruitment (8). To act, insulin must cross the capillary endothelium, traverse the interstitial fluid compartment, and thence access and bind to insulin receptors. A reduction in insulin's ability to diffuse through the interstitial space in lipid-induced insulin resistance (9) suggests that access to sensitive tissues (such as skeletal muscle) may be important in addition to cellular insulin resistance. In insulin-resistant situations, it is therefore important to examine whether insulin can access cells prior to stimulating cell signaling.

A high-fat diet (HFD), which induces obesity and leads to deposition of fat in tissues (10,11), does not always increase basal FFAs in humans or canines (12,13); but large elevations of FFAs can be observed at night in canines (13) and humans (14). Given the uncertainty regarding the effect of HFDs on FFA levels in the circulation, it is not yet known whether the effects of lipid infusion on insulin action can be extrapolated to explain how an HFD per se would affect insulin action and the ability of insulin to diffuse through skeletal muscle.

Previous studies in our laboratory have shown that insulin injected directly into healthy muscle is disseminated throughout the interstitial space, where the hormone accesses myocytes and stimulates glucose uptake (15). In a model of insulin resistance where plasma FFAs were raised by lipid infusion, injected insulin was surprisingly not detected in the interstitial space (lymph) of skeletal muscle, supporting absence of elevated interstitial insulin. Under this condition there was little stimulation of glucose disposal (9). This latter result suggests that high plasma FFAs may prevent insulin's access to skeletal muscle, resulting in insulin resistance. It cannot be concluded whether this is a direct effect of elevated plasma FFAs, or of acute insulin resistance induced by lipid infusion. Therefore, it was important to examine whether lipid elevated physiologically by an HFD would similarly limit insulin's access to muscle cells. For example, in dogs fed a control diet, insulin stimulates the extravascular distribution of insulin without effects on vascular distribution volume (16), suggesting that insulin can increase its own access to insulin-sensitive tissues. This effect was lost after induction of insulin resistance by an HFD (17), possibly due to an alteration in the diffusion of insulin through the interstitial space. In the present report, we examined whether insulin resistance induced by an HFD would simulate lipid infusion studies in which injected insulin was not detected in lymph. If so, this would implicate insulin access to muscle cells as an important factor in dietary fat-induced insulin resistance.

RESEARCH DESIGN AND METHODS

Animals. One-year-old male mongrel dogs were housed in individual cages in the University of Southern California Medical School Vivarium and were kept under controlled conditions (12-h light/12-h dark). Animals had free access to water, and were fed once per day from 9:00–10:00 A.M. with 825 g dry chow (60% ProLab Canine 2000, 40% Laboratory High Density Canine Diet 5LI8; PMI Nutrition International, Brentwood, MO) and one can of Purina Pro-Plan Puppy Classic (Nestle Purina PetCare, St Louis, MO). For fat feeding, animals were subjected to the basal diet, supplemented with 6 g/kg bacon grease, presented to the dogs for the remainder of the study (15.2 ± 0.8 weeks). We have previously published data from injecting insulin into normal-weight dogs (lean) fed ad lib dry chow (15); these previous results are reproduced with permission herein for comparison and were not different when performed before or after the HFD experiments. Dogs were used for experiments only if they were judged to be in good health as determined by visual observation, body weight, hematocrit, and body temperature. Protocols were approved by the University of Southern California Institutional Animal Care and Use Committee and were in accordance with the Principles of Laboratory Animal Care.

Study design. Weekly fasted (basal) plasma samples for measurement of FFAs were taken into tubes with 50 μ l EDTA and paraoxon to inhibit lipase activity and centrifuged, and the plasma was frozen before analysis using a colorimetric kit (NEFA-HR(2); Wako Diagnostics, Richmond, VA). Prior to beginning the HFD, each animal ($n = 6$) underwent magnetic resonance imaging (MRI) of the abdominal area as outlined below, and a hyperinsulinemic euglycemic clamp (EGC) for determination of insulin sensitivity (S_i) and glucose turnover. Once these baseline parameters were established, the HFD was administered, and the MRIs and EGCs were repeated (weeks 12–13). Finally, an acute injection procedure was performed prior to death to determine the local dynamics of injected insulin as detailed below. Lean animals received the acute injection procedure, but did not receive MRIs or EGCs.

MRI. On the morning of each MRI scan, a subcutaneous injection of acepromazine (0.04 mg/kg; Bioceutical, St Joseph, MO) and atropine sulfate (0.1 mg/kg, 1/120 grain; Western Medical, Arcadia, CA) was administered for preanesthesia; dogs then received intravenous anesthesia of ketamine HCl (10 mg/kg; Phoenix Pharmaceutical, St Joseph, MO) and diazepam (0.2 mg/kg; Abbott Laboratories, North Chicago, IL). Body composition was then assessed in 30 1-cm axial abdominal images using 1.5 Tesla Horizon Magnet (version 5.7 software). Each MRI slice was analyzed using Scion Image Software (Windows 2000 version Beta 4.0.2; Scion, Frederick, MD), which quantifies fat tissue (pixel values 121–254) and nonfat tissue (pixel values 20–120). The total pixels of fat and nonfat tissue were used to calculate the area of fat located within the peritoneal cavity in an 11-cm region of the thorax identified by the central landmark slice, where the left renal artery branches from the abdominal aorta. Subcutaneous fat volume was calculated as total fat in the assessment region minus the visceral fat volume.

Hyperinsulinemic EGC studies. A primed infusion of [3 -H $_2$]-D-glucose tracer was started at $t = -120$ min (25- μ Ci bolus + 0.25 μ Ci/min; DuPont-NEN, Boston, MA) into the saphenous vein in conscious animals. An infusion of insulin (0.75 mU \cdot min $^{-1}$ \cdot kg $^{-1}$; Lilly, Indianapolis, IN) and somatostatin (1.0 μ g \cdot min $^{-1}$ \cdot kg $^{-1}$; Bachem California, Torrance, CA) was initiated at time 0, and radiolabeled dextrose (2.0 μ Ci/g, [3 -H $_2$]-D-glucose, 50% dextrose) was variably infused to maintain euglycemia and specific activity. Blood samples were drawn from the cephalic vein every 10 min from $t = -30$ to 180 min. Euglycemia was defined as the average basal glucose and steady state as the final 30 min of the clamp. S_i was calculated using the equation $S_i = \Delta \text{Ginf}/(\Delta \text{I} \times \text{G})$, where ΔGinf = change in glucose infusion at steady state from basal, ΔI = change in plasma insulin at steady state from basal, and G = steady-state plasma glucose concentration.

Acute insulin injection studies: surgery. These experiments were terminal studies performed under anesthesia. Injection studies were performed on lean animals and on animals given 15.2 ± 0.8 weeks of HFD. Animals were fasted 15 h before the experiment, which began at 6:00 A.M. They were preanesthetized with acepromazine maleate (Prom-Ace, 0.22 mg/kg; Auco, Fort Dodge, IA) and atropine sulfate (0.11 ml/kg; Western Medical). Anesthesia was induced with sodium pentobarbital (0.44 ml/kg; Western Medical) and maintained with isoflurane (Western Medical). Dogs were placed on heating pads to maintain body temperature. Indwelling catheters were inserted into the right jugular vein for a continuous saline drip (~1 l for the first 60 min of surgery and a slow drip thereafter) and into 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 and somatostatin infusion. Indwelling catheters were also placed into both the right and left femoral arteries and veins for blood sampling. Two perivascular ultrasonic flowprobes

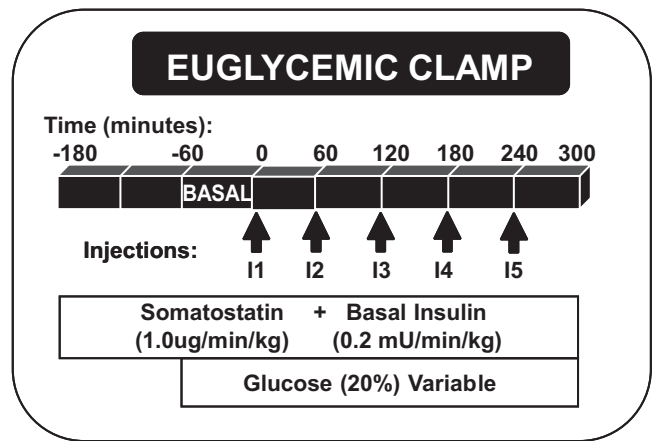


FIG. 1. Euglycemic basal insulin clamp protocol. Time in minutes is shown across the top. Somatostatin was infused with basal insulin replacement; glucose was infused at a variable rate to maintain euglycemia based on the INJ arterial glucose measurement. Samples were taken at 15-min intervals from -60 to 0 min, and at 10-min intervals from time 0 – 300 from the femoral artery, femoral vein, and lymph of INJ and NINJ legs. I1, I2, I3, I4, and I5 represent insulin injections of 0.3, 0.5, 0.7, 1.0, and 3.0 units, respectively, which occurred every 60 min from time 0.

(2-mm diameter; Transonic, Ithaca, NY) were placed around both right and left femoral arteries proximal to the femoral catheter. Left and right hind limb lymphatic vessels were cannulated by placing polyethylene catheters (PE10) into the afferent lymphatic vessels of the deep inguinal lymph node. Blood pressure, heart rate, O_2 saturation, and CO_2 were monitored continuously. At the conclusion of these experiments, animals were killed with an overdose of sodium pentobarbital (Eutha-6, 65 mg/kg; Western Medical).

Blood glucose control during insulin injection. Immediately after starting surgical procedures ($t = -180$ min), simultaneous systemic infusions of somatostatin (1 μ g \cdot min $^{-1}$ \cdot kg $^{-1}$) and basal insulin replacement (0.2 mU \cdot min $^{-1}$ \cdot kg $^{-1}$) were begun and continued for the duration of the study (Fig. 1). Exogenous 20% glucose was infused at variable rates to maintain euglycemia at the basal level based on glucose measurements from the injected leg's (INJ) femoral artery plasma glucose (measured prior to insulin infusion) throughout the entire experimental period ($t = -240$ to 300 min). Samples were taken simultaneously from the right and left femoral arteries and veins. Left and right hind limb lymph vessels were sampled by gently massaging the hind limb distal to the site of catheterization from 2 min prior to 2 min after each blood sample.

Unilateral intramuscular insulin injection. As previously described (15), at times 0, 60, 120, 180, and 240 min, porcine insulin (0.3, 0.5, 0.7, 1, and 3 U, represented as I1, I2, I3, I4, and I5, respectively) was injected directly into the vastus medialis of the quadriceps femoris of one leg. At each time point, two 30-gauge needles with a volume of 0.5 ml per syringe injected the appropriate concentration of insulin at ~0.6 ml/min. For individual experiments, either right or left hind limb was chosen at random for injections (INJ) and the 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. Arterial and venous tubes also contained 50 μ l EDTA (Sigma Chemicals). Blood samples were centrifuged immediately, and the supernatant was transferred and stored at -20°C until further assay. INJ femoral artery plasma samples were immediately assayed for glucose with a YSI 2700 auto analyzer (Yellow Springs Instruments, Yellow Springs, OH) before freezing at -20°C . Lymph samples were stored at -20°C after sampling. Samples were assayed for insulin by ELISA (Linco Research) adapted for canine plasma with a standard provided by Novo Nordisk, and for glucose with a YSI 2700 auto analyzer.

Calculations. Glucose uptake across each limb was calculated based on the Fick principle: $\text{LGU} = (\text{G}_A - \text{G}_V) \times \text{BF}$, where LGU = local glucose uptake; G_A = arterial glucose; G_V = venous glucose; and BF = femoral blood flow.

Statistical analyses. All experimental data are expressed as the means \pm SE. Statistical analyses were performed with unpaired Student t tests, or one- or two-way ANOVAs with Tukey pairwise comparisons as appropriate. The lean and HFD groups were compared using unpaired t tests and two-way ANOVAs.

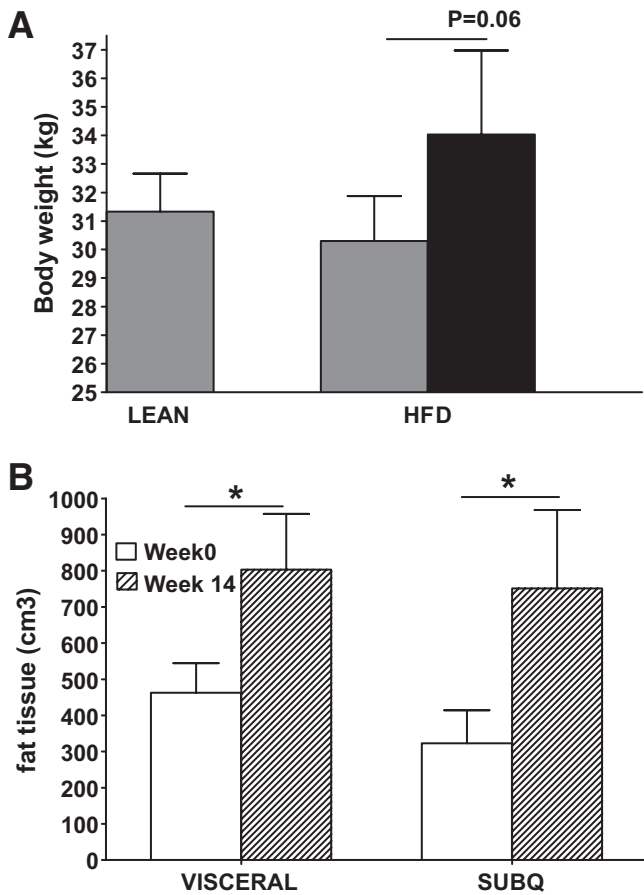


FIG. 2. A: Canine weights for lean animals and animals prior to (□) and after (■) 14 weeks of an HFD. There is no significant difference between lean animals and week-0 measurements for animals subjected to an HFD. B: MRIs were performed prior to (week 0, □) and after (week 14, ▨) an HFD. Fat feeding induced an accumulation of both visceral and subcutaneous adipose tissue. * $P < 0.05$ using paired t test.

RESULTS

Fourteen weeks of fat feeding caused an increase in body weight, as shown in Fig. 2A, and also resulted in an increase in both subcutaneous and abdominal fat, as measured by MRIs shown in Fig. 2B. On average, fat-fed dogs increased visceral fat by 79.4% (from $462.5 \pm 81.4 \text{ cm}^3$ to $803.2 \pm 154.3 \text{ cm}^3$, $P = 0.02$) and subcutaneous fat

by 108.9% (from $902.2 \pm 183.1 \text{ cm}^3$ to $1952.3 \pm 502.1 \text{ cm}^3$, $P = 0.03$).

Basal-fasted plasma FFAs decreased with fat feeding from $0.66 \pm 0.11 \text{ mmol/l}$ to $0.40 \pm 0.07 \text{ mmol/l}$ ($P = 0.02$). During the course of the experiment, the plasma FFA levels dropped to levels virtually undetectable by the FFA assay used (data not presented), as inhalant isoflurane anesthesia is known to suppress lipolysis (18).

Systemic insulin action. As expected, HFD induced significant peripheral insulin resistance determined by the hyperinsulinemic euglycemic clamp in conscious dogs (week 0: 8.72 ± 0.27 to week 12: $3.15 \pm 0.65 \text{ dl} \cdot \text{pM}^{-1} \cdot \text{mg}^{-1} \cdot \text{kg}^{-1}$, $P = 0.02$) (Fig. 3A). Glucose disposal (Rd) decreased from $11.49 \pm 1.53 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ prior to fat feeding to $7.26 \pm 1.55 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ after 12 weeks of HFD ($P = 0.03$) (Fig. 3B). Endogenous glucose production showed a trend to increase (week 0: 0.18 ± 0.27 to week 12: $0.69 \pm 0.41 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, $P = 0.36$) (Fig. 3C). HFD caused the dogs to become insulin resistant, and this was more evident in the periphery than in the liver.

Local insulin action: lean animals. As previously reported, insulin injections caused a small increase in both INJ and NINJ femoral artery insulin over the course of the experiment (INJ: 0 min, 11.9 ± 1.0 ; 300 min, $35.9 \pm 1.9 \text{ mU/l}$, $P < 0.001$. NINJ: 0 min, 12.4 ± 1.2 ; 300 min, $36.6 \pm 2.5 \text{ mU/l}$, $P < 0.001$) (Fig. 4A). Femoral vein insulin was significantly increased after the final injection in INJ compared with NINJ (300 min: NINJ, 35.8 ± 2.0 ; INJ, $64.0 \pm 10.5 \text{ mU/l}$, $P = 0.02$) (Fig. 4B), demonstrating a washout of insulin injected into the muscle that then entered the systemic circulation to cause the small elevations in arterial insulin. INJ lymph insulin concentrations increased after the second injection compared with NINJ and remained significantly elevated until the end of the experiment (300 min: NINJ, 13.2 ± 2.3 ; INJ, $73.8 \pm 7.1 \text{ mU/l}$, $P < 0.01$) (Fig. 4C). The concentration of insulin in the interstitial fluid of NINJ remained approximately half of that of the arterial insulin supply. Insulin injection into muscle induced an increase in glucose infusion required to maintain euglycemia in lean animals, as well as a significant increase in local glucose uptake in the injected limb (300 min: NINJ, 9.5 ± 4.5 ; INJ, $27.3 \pm 4.0 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$, $P = 0.03$) (Fig. 5A).

Local insulin action: fat-fed animals. In HFD-fed animals, in sharp contrast to lean animals, INJ lymph insulin

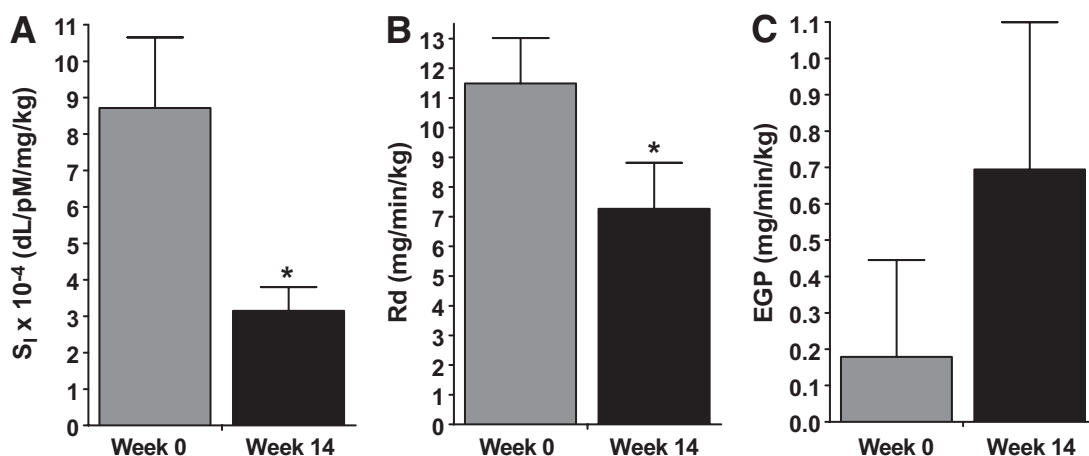


FIG. 3. Changes in (A) S_1 , (B) Rd, and (C) endogenous glucose production (EGP) as calculated from hyperinsulinemic EGCs prior to week 0 (□) and after 14 weeks (■) of HFD. *Significantly different from corresponding week 0, $P < 0.05$ (paired t test).

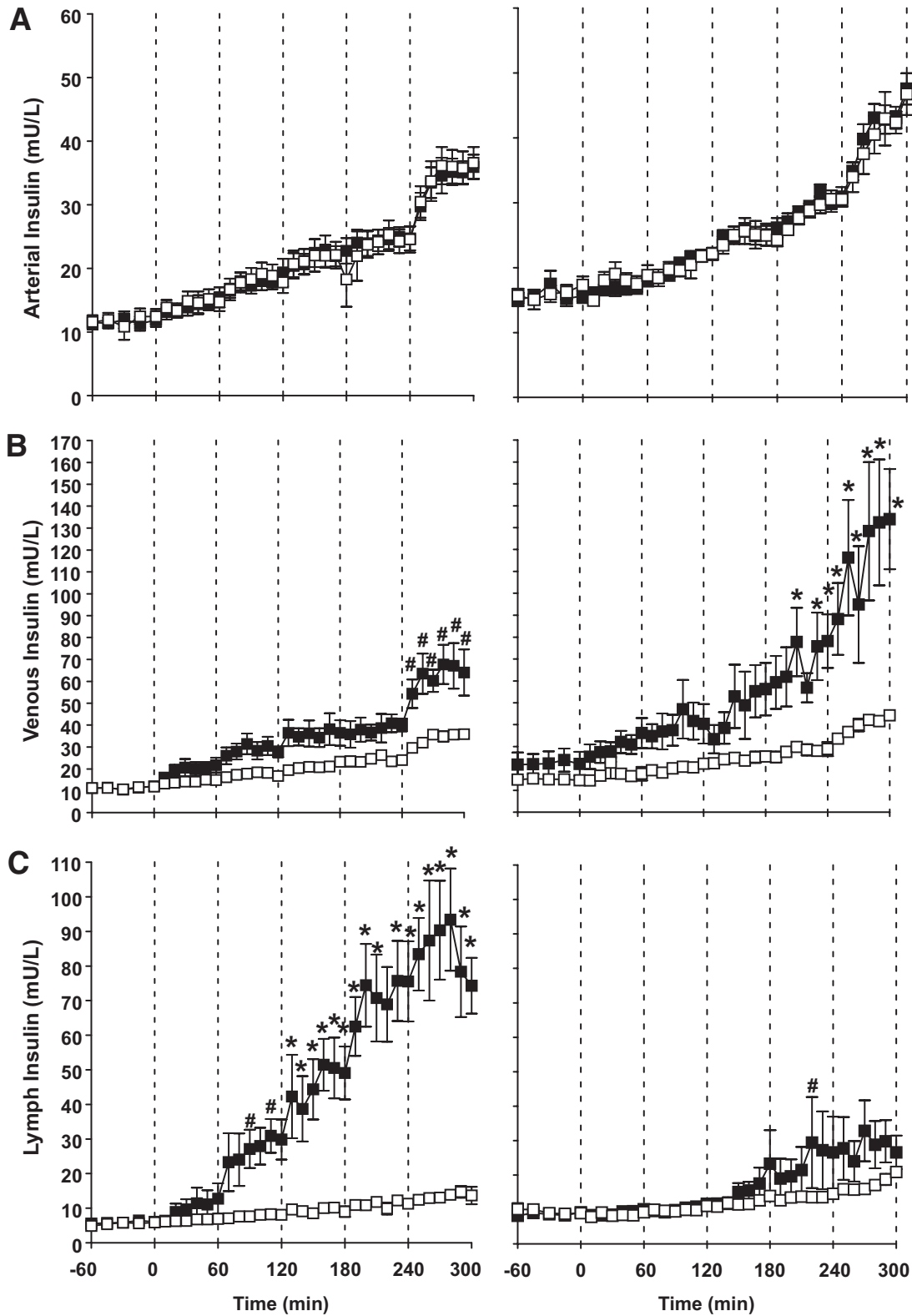


FIG. 4. Insulin concentration in femoral artery (A), femoral vein (B), and lymph (C). Lean animals are included (left column) compared with new data from animals fed an HFD (right column). In both columns, ■ represent data from INJ leg, □ represent data from NINJ leg, and : indicate the injection times. *Significantly different from all other groups (two-way ANOVA). #Significantly different from corresponding contralateral leg (paired *t* test).

concentrations showed no elevation in lymph insulin during injection into the muscle compared with the contralateral noninjected limb (300 min: NINJ, 20.9 ± 1.4 ; INJ, 26.6 ± 4.9 mU/L, $P = 0.232$) (Fig. 4C). The minor increase

in femoral artery insulin over time was observed similar to lean animals and not different between legs (INJ: 0 min, 15.7 ± 1.1 ; 300 min, 47.6 ± 2.4 mU/L, $P < 0.001$. NINJ: 0 min, 17.3 ± 1.4 ; 300 min, 46.8 ± 3.2 mU/L, $P < 0.001$) (Fig.

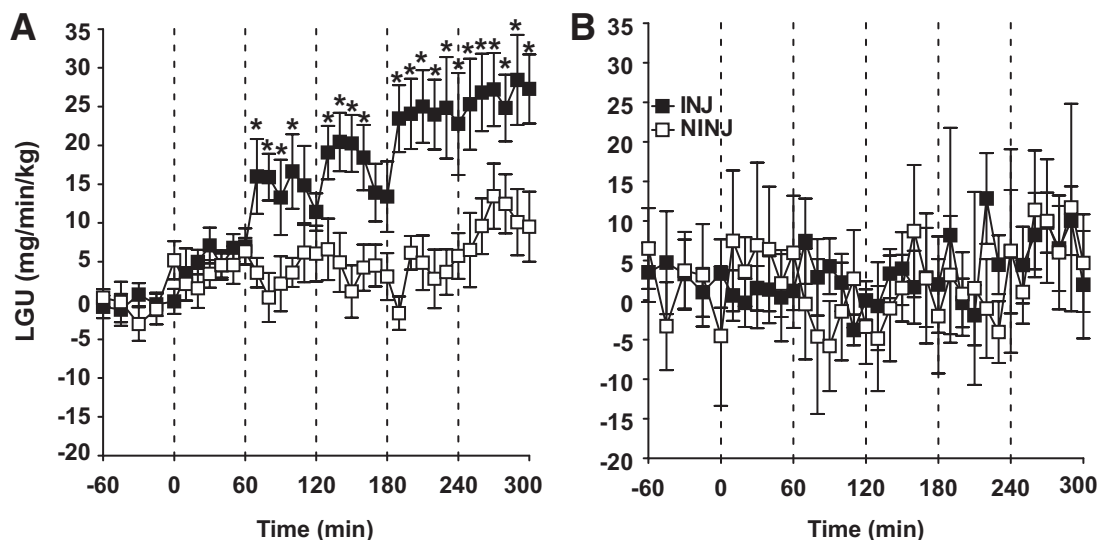


FIG. 5. Local glucose uptake (LGU) in lean (A) and HFD-fed (B) animals. INJ (■) LGU was significantly elevated above NINJ (□) in lean animals as marked. *Significantly different from NINJ (paired *t* test). There was no significant difference between INJ and NINJ in HFD-fed animals.

4A). INJ femoral vein insulin was significantly increased compared with NINJ (300 min: HFD NINJ, 49.6 ± 5.55 ; HFD INJ, 133.9 ± 22.9 mU/l, $P = 0.03$) and lean INJ (300 min: HFD INJ, 133.9 ± 22.9 ; lean INJ, 64.0 ± 10.5 mU/l, $P = 0.01$), reflecting a significantly greater washout of insulin from the injected area in HFD-fed compared with lean animals from 210 min to the end of the experiment (Fig. 4B). The interstitial fluid insulin concentration in NINJ was approximately half the arterial insulin concentration; this was not significantly different compared with lean animals and did not change throughout the experiment. The glucose infusion for fat-fed animals was not significantly different from lean animals, however the femoral artery glucose was significantly higher than in the lean animals due to a higher basal glucose measured prior to insulin and somatostatin infusion (lean: 96.0 ± 2.1 ; HFD: 11.6 ± 4.8 mg/dl, $P = 0.01$, unpaired *t* test). There was no significant glucose uptake due to insulin injection (300 min: INJ 5.9 ± 7.14 ; NINJ 4.68 ± 6.2 mg \cdot min $^{-1}$ \cdot leg $^{-1}$, $P = 0.328$), which was significantly lower than in lean animals ($P = 0.02$) (Fig. 5). There was no significant difference in blood flow (Fig. 6), mean arterial pressure (lean: 63.82 ± 3.01 ; HFD: 68.83 ± 3.56 mmHg), or heart

rate (lean: 110.09 ± 4.79 ; HFD: 111.67 ± 5.72 bpm) between groups.

DISCUSSION

Physiological insulin resistance induced by feeding an HFD for 14 weeks caused a failure of insulin injected into skeletal muscle to be detected in lymph and prevented glucose uptake in response to the injection compared with lean animals. This is reminiscent of previously published results that show experimentally elevated plasma lipids likewise inhibit the ability of injected insulin to diffuse through muscle and initiate glucose uptake (9). Interestingly, the ability of the HFD to impair insulin dispersion in muscle occurs in the absence of a rise in plasma FFAs, either under basal-fasting conditions or during the clamp.

There was no significant difference between the injected and noninjected arterial insulin in the fat-fed animals, as the arterial supply to both legs should be the same and the insulin injection occurs distal to the arterial sampling point. Also, there is no significant difference between the femoral artery and vein insulin concentrations of the noninjected leg, so the increase observed in the femoral vein insulin concentration of the injected leg was due to insulin overflow from the local area of the injection. Among the high-fat-fed animals, there was variation in the ability of lymph to reflect the more concentrated insulin injections. As the ratio of NINJ lymph to arterial insulin was maintained in the face of increasing systemic insulin, the ability of lymph to detect whole-body insulin changes was not altered.

Lymph measures of interstitial insulin have previously been used in this laboratory (9,15–17,19,20) and others (21–24), and agree with other measures of interstitial insulin such as microdialysis (25). Further, gold injected into the interstitium has been shown to concentrate in lymph vessels (26). Due to the low flow rate and apparent lack of size exclusion of lymph vessels, rapid equilibration of lymph with the intracellular fluid can occur similar to that used in microdialysis, and as such is an appropriate tool for measuring interstitial insulin concentrations, reflecting the makeup of the interstitial fluid to which myocytes are exposed. One limitation in our study is that

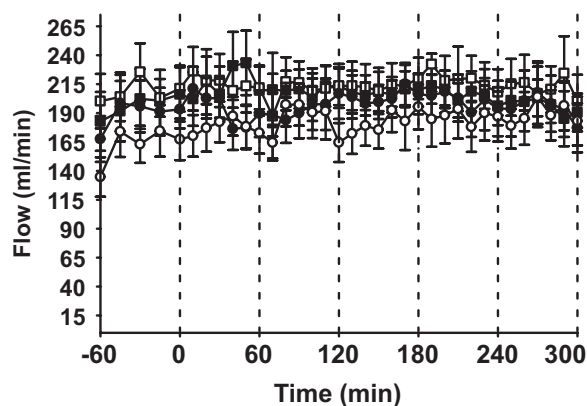


FIG. 6. Blood flow effects in lean (circles) and HFD-fed (squares) animals. INJ (black symbols) blood flow was not significantly different from NINJ (white symbols) at any time point, and did not change throughout the experiment (paired and unpaired *t* test as appropriate).

the lymph vessel insulin concentration is a mixture across the entire leg, whereas insulin is injected into a small area of one muscle; thus the interstitial concentration of insulin in the injected area may be higher than in lymph due to dilution of lymph from uninjected muscle. However, previous results have shown a significant increase in lymph insulin in lean dogs (15), thus HFD has impaired the appearance of insulin in lymph.

We show that the ability of insulin to access myocytes is impaired in physiological insulin resistance, and several proposed causes of insulin resistance are unable to describe the results we observe here. For example, mitochondrial dysfunction has been proposed as a critical component of insulin resistance (27), yet in our study, cells are not exposed to elevated insulin, and as such, mitochondrial dysfunction is unlikely to be responsible for the impaired dispersion of insulin. Studies in obese mice have shown that mitochondrial dysfunction is evident in adipose tissue, but not in skeletal muscle (28), and although stimulating FFA oxidation in skeletal muscle reduces insulin resistance (29), it does not necessarily follow that mitochondrial function is the primary cause of skeletal muscle insulin resistance. Similarly, it is unlikely that downstream signaling impairment in myocytes (30,31) or intracellular fat accumulation (6,32) impaired glucose uptake in our study, as there was apparently no measurable elevation of interstitial insulin to cause insulin's cellular effects. However, we cannot discount that myocytes would exhibit insulin resistance if the cells were actually exposed to injected insulin in our model.

The mechanism(s) by which HFD prevents injected insulin from appearing in the interstitial space cannot be clarified from the experiments presented here. As acute hyperlipidemia also resulted in reduced insulin appearance in the interstitial fluid (9), it is unlikely to be a structural change. One possibility is a lipid-induced alteration in permeability of the endothelial cell (33,34), which might allow a more rapid egress of insulin from the interstitial compartment into the bloodstream. However, this permeability theory does not identify the location of the injected insulin prior to appearance in the vein, which may occur as much as 1 h later. It was noted that, although insulin was not detected in lymph, venous insulin had not dropped to preinjection levels even 1 h after injection. As less than 30% of the injected insulin is removed by the vein during the course of the experiment, the remainder must in theory be contained within the muscle, although is not detected in the interstitial space. The excess may be taken up into some cells. Insulin is internalized by endothelial cells en route to the vein (35), and, as endothelial cells exhibit insulin resistance (36), this internalization may be increased in obesity to overcome the impaired insulin signaling, thus removing insulin from the interstitial space. As there is no insulin in the interstitial space, only myocytes immediately adjacent to the area of injection may internalize insulin. Alternatively, in lean animals, injected insulin could increase the perfusion of muscle and augment the blood flow to the area, therefore increasing the dispersion of insulin through the muscle (37). Obese rats (38) and those with pharmacologically elevated lipids (8) have impaired insulin-mediated capillary recruitment; insulin injected into the muscle may be trapped in the small area of injection, without the means to increase its dispersion area. It is also possible that the local high insulin concentration after injection is in the interstitial space, although not in an area sampled by the lymph. In lean

animals, injected insulin would cause an increase in the distribution volume to allow more complete dissemination of insulin through the muscle, and therefore detection in the lymph. Adiposity would prevent this increase in distribution volume (39) and reduce the appearance of insulin in the lymph, as less of the muscle is exposed to insulin.

The role of the endothelial cell in the transport of insulin is unclear—evidence exists for both paracellular and transcellular transport of insulin. A recent study has shown that in cell culture, endothelial cells concentrate insulin (38); whether this occurs *in vivo* has not been determined, and there is no indication of whether this is unidirectional or can move from plasma to interstitium and back. As lean animals show significant insulin appearance in the vein, insulin can move in both directions. Although endothelial cells exhibit insulin resistance (36), effects on insulin transport are not known; however, insulin resistance can impair the appearance of insulin in the interstitium (20,23), and transport is known to be rate limiting to insulin action (19,20,22).

Although we have shown that insulin resistance, either chronic (HFD) or acute (9), prevents dissemination of insulin through the muscle, the mechanism is not yet known. As the HFD does not increase, and in fact suppresses basal plasma FFAs, and isoflurane anesthesia reduces plasma FFAs by 70% (17), plasma FFAs are unlikely to be the primary cause of the impaired dissemination of injected insulin. However, as plasma lipids are elevated nocturnally by an HFD (13), it is possible that whereas plasma FFAs are not a direct cause of insulin resistance in our study, tissue FFA levels may be. Intramyocellular lipid content can be altered by diet (11) and acutely by lipid infusion (40); as many cells are not exposed to injected insulin, it is unlikely that the intracellular lipid would affect our results, although there is the possibility of extracellular fat accumulation. It is unclear whether lipids must be in the interstitial space to inhibit dispersion of intramuscularly injected insulin, or whether another factor may be inhibiting the dispersion of insulin.

The HFD dogs were subjected to a significantly higher glucose level than the lean animals throughout the injection procedure, as their plasma glucose level after anesthesia and prior to insulin and somatostatin infusion was higher. The arterial glucose of animals infused with lipid is not significantly different from either lean or HFD-fed animals, and values appear to be intermediate (9). Endothelial dysfunction may be caused by both acute hyperglycemia (41) and hyperlipidemia (42), which may prevent insulin-induced capillary recruitment. Thus, impaired insulin dispersion observed with lipid infusion (9) and fat feeding may result from different plasma factors that ultimately affect the capillary endothelium. Further studies are necessary to determine whether elevated glucose affects the dispersion of injected insulin.

In conclusion, in an HFD, which induces chronic insulin resistance, insulin injected directly into the muscle is not detectable in the interstitial space (lymph), but appears to wash out in the venous blood flow. This is similar to previous results (9) that demonstrate acute lipid-induced insulin resistance prevented injected insulin from diffusing through the interstitium. The apparent cellular insulin resistance occurs because myocytes are not exposed to insulin; potential downstream effectors such as mitochondrial dysfunction, intramyocellular fat accumulation, and signaling impairment are unlikely to be responsible for the inability of injected insulin to diffuse through the intersti-

tial space in this diet-induced insulin resistance. Although the injection study is not a physiological situation, there are clear differences in how insulin is dealt with in the interstitium, and its access to muscle. Therefore, studies on the insulin sensitivity of individual cells should consider whether insulin was able to access the tissue prior to any perceived defect in insulin signaling or response.

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