Early Microvascular Recruitment Modulates Subsequent Insulin-Mediated Skeletal Muscle Glucose Metabolism During Lipid Infusion

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OBJECTIVE—To test whether early, insulin-mediated microvascular recruitment in skeletal muscle predicts steady-state glucose metabolism in the setting of physiological elevation of free fatty acid concentrations.

RESEARCH DESIGN AND METHODS—We measured insulin’s microvascular and metabolic effects in 14 healthy young adults during a 2-h euglycemic insulin clamp. Plasma free fatty acid concentrations were raised (Intralipid and heparin infusion) for 3 h before the clamp and maintained at postprandial concentrations during the clamp. Microvascular blood volume (MBV) was measured by contrast-enhanced ultrasound (CEU) continuously from baseline through the first 30 min of the insulin clamp. Muscle glucose and insulin uptake were measured by the forearm balance method.

RESULTS—The glucose infusion rate (GIR) necessary to maintain euglycemia during the clamp varied by fivefold across subjects (2.5–12.5 mg/min/kg). The early MBV responses to insulin, as indicated by CEU video intensity, ranged widely, from a 39% decline to a 69% increase. During the clamp, steady state forearm muscle glucose uptake and GIR each correlated with the change in forearm MBV (P < 0.01). To explore the basis for the wide range of vascular and metabolic insulin sensitivity observed, we also measured VO2max in a subset of eight subjects. Fitness (VO2max) correlated significantly with the GIR, the forearm glucose uptake, and the percentage change in MBV during the insulin clamp (P < 0.05 for each).

CONCLUSIONS—Early microvascular responses to insulin strongly associate with steady state skeletal muscle insulin-mediated glucose uptake. Physical fitness predicts both metabolic and vascular insulin responsiveness.

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Insulin recruits underperfused capillaries to increase skeletal muscle microvascular blood volume (MBV), as measured by contrast-enhanced ultrasound (CEU), within 20 min in both rats (1) and humans (2,3). This effect occurs with physiological insulin concentrations (2,4) and precedes both changes in total limb blood flow (1,5,6) and insulin’s metabolic action (1). In rodents, microvascular recruitment enhances the rate at which insulin is delivered to muscle interstitium (7), thereby facilitating insulin’s metabolic action, and exercise training has been shown to enhance insulin-induced microvascular recruitment and muscle glucose disposal in rodents (8).

Raising plasma concentrations of free fatty acids (FFAs) induces insulin resistance within 2–4 h, can induce inflammation in muscle (9) and in circulating leukocytes (10), and produces endothelial dysfunction (10,11). Clinical studies have shown a marked impairment in insulin’s ability to recruit both muscle and skin microvasculature in chronically insulin-resistant obese subjects (12–14). FFA-induced insulin resistance impairs insulin-mediated microvascular recruitment in skin with elevation of FFA to physiological levels (~1 mmol/L) (15) and in muscle microvasculature with higher FFA levels ~3 mmol/L (16).

Both acute exercise and training can affect the metabolic response to raising plasma FFA. Raising plasma FFA acutely through lipid and heparin infusion has less effect on insulin sensitivity in individuals who exercised intensively the preceding day (17). Exercise training also prevents FFA-induced hepatic and peripheral insulin resistance (18). It is not known whether training affects insulin-induced microvascular recruitment or the ability of FFA to inhibit recruitment in humans.

Recently, we reported that human skeletal muscle insulin uptake (product of forearm blood flow and arteriovenous concentration) could be quantified and that it occurred through a saturable transport process at physiological concentrations of insulin (2). Whether FFA elevation would, by blocking insulin-induced increases in MBV, also limit muscle insulin uptake is not known.

In this study, CEU was used to measure muscle microvascular perfusion and paired arterial and venous sampling to measure muscle insulin and glucose uptake in response to a physiologic insulin infusion in 14 healthy volunteers whose plasma FFA levels were maintained in a range encountered in human insulin-resistant states (~1.0 mmol/L). To examine whether fitness was predictive of these responses, a subset of 8 volunteers underwent maximal exercise testing to quantifying the relationship between VO2max and muscle metabolic and microvascular insulin sensitivity.

RESEARCH DESIGN AND METHODS—Studies were performed in 14 lean (BMI 22 ± 1 kg/m^2), healthy
Euglycemic insulin clamp

At time 180 min, a primed 3 mU/min/kg insulin infusion was started in the arm contralateral to the arterial catheter. This infusion was decreased by 0.2 mU/min/kg each min during the next 10 min and then maintained at a rate of 1 mU/kg/min for the next 110 min. Arterial plasma glucose was maintained at basal levels with a variable rate 20% glucose infusion (euglycemic clamp) (20). Whole-body glucose disposal at steady state (80–120 min of the clamp) was estimated from the glucose infusion rate (GIR) required to keep arterial glucose constant. Forearm glucose and FFA balances (net uptake or release) were determined from the arteriovenous concentration difference obtained every 10 min from 150 to 300 min of lipid infusion. To avoid interference with the CEU images, no arterial or venous samples were collected from 180 to 210 min of lipid infusion.

MBV was measured with a SONOS 7500 ultrasound system (Philips Medical Systems, Bothell, WA) with harmonic imaging during the continuous infusion of perfluorocarbon gas–filled lipid microbubbles (Definity; Lantheus Medical Imaging Co., Billerica, MA), as described previously (2). CEU images were downloaded to an off-line image analysis system (Q-Laboratory; Philips Medical Systems, Andover, MA). Background-subtracted acoustic intensity was measured from a region of interest around the deep forearm flexor muscles, as described previously (12,21). Changes in MBV with time during insulin exposure were calculated from the acoustic intensity expressed as mean decibels.

Brachial artery blood flow was measured at baseline and every 20 min from 40 to 120 min of the insulin clamp with the SONOS 7500 ultrasound system with a linear-array transducer and a transmit frequency of 12 MHz. Two-dimensional imaging of the brachial artery was performed in the long axis approximately 10 cm proximal to the antecubital fossa. Images were triggered to the R wave of the cardiac cycle, and the brachial artery diameter was measured with online video calipers. At the same location, the time average mean blood velocity was measured with pulsed-wave Doppler ultrasound. Brachial artery mean blood flow was calculated according to the following equation: \( Q = v \cdot \pi (d/2)^2 \), where \( Q \) is brachial blood flow, \( v \) is mean brachial artery blood flow velocity, and \( d \) is brachial artery diameter.

Insulin was measured with a solid-phase two-site chemiluminescent assay (Diagnostic Products Corporation, Los Angeles, CA). The FFA level was measured with a colorimetric assay (Waco Diagnostics, Richmond, VA). Glucose

Table 1—The measured phenotypic characteristics of all subjects studied

|                      | Group 1 |                  | Group 2 |                  |
|----------------------|---------|-----------------|---------|-----------------|
|                      | Men (n = 1) | Women (n = 5) | Men (n = 6) | Women (n = 2) |
| Age (years)          | 28 ± 3  | 21 ± 0.6        | 20 ± 0.6 | 18 ± 1          |
| Height (cm)          | 193 ± 2 | 163 ± 6         | 181 ± 4 | 169 ± 6         |
| Weight (kg)          | 87 ± 5  | 57 ± 7          | 72 ± 4  | 56 ± 2          |
| BMI (kg/m²)          | 23 ± 3  | 21 ± 1          | 22 ± 1  | 20 ± 1          |
| Fat weight (kg)      | 9 ± 2   | 14 ± 3          | 12 ± 3  | 15 ± 0.5        |
| Lean weight (kg)     | 77 ± 5  | 43 ± 5          | 59 ± 5  | 41 ± 2          |
| Body fat (%)         | 11 ± 2  | 24 ± 4          | 17 ± 4  | 28 ± 1          |
| Waist circumference (cm) | 82 ± 3 | 70 ± 4         | 76 ± 2  | 71 ± 1          |
| Sagittal diameter (cm) | 20 ± 1 | 17 ± 1       | 19 ± 1  | 18 ± 0.5        |
| Total cholesterol (mg/dL) | 159 ± 6 | 137 ± 7      | 147 ± 11| 125 ± 12       |
| HDL cholesterol (mg/dL) | 100 ± 5 | 75 ± 5        | 88 ± 9  | 74 ± 5          |
| LDL cholesterol (mg/dL) | 53 ± 6 | 52 ± 6       | 48 ± 3  | 46 ± 5          |
| Triglycerides (mg/dL) | 39 ± 4 | 59 ± 19      | 63 ± 10 | 38 ± 15         |
| Systolic blood pressure (mmHg) | 114 ± 5 | 114 ± 5   | 120 ± 4 | 118 ± 6         |
| Diastolic blood pressure (mmHg) | 78 ± 5 | 67 ± 5    | 67 ± 2  | 69 ± 6          |

\( V_{O_2, max} \) (mL/kg/min)

Group 1 comprises the six subjects who did not undergo \( V_{O_2, max} \) testing; group 2 comprises the eight who did. Values are mean ± SEM.
and lactate were measured in duplicate with a YSI 2300 analyzer (Yellow Springs Instruments, Yellow Springs, OH). Baseline coagulation parameters, liver function tests, and fasting lipid profile were performed by standard assays in the University of Virginia Clinical Chemistries Laboratory.

Forearm balances for glucose, FFAs, and insulin were calculated as follows: balance = ([A] – [V]) · F, where [A] and [V] are arterial and venous concentrations and F is forearm blood flow in milliliters per minute per 100 mL forearm volume. A positive balance corresponded to a net uptake, whereas a negative balance signaled a net release of substrate. For calculation of glucose balance, blood flow was used; for FFA and insulin, we used forearm plasma flow, derived as blood flow · (1 – Hematocrit). The clearance of insulin was calculated as the product of the extraction fraction of insulin, derived as ([A] – [V])/[A], and forearm plasma flow per 100 mL forearm volume.

**Statistical analysis**
Data are presented as means ± SE. Comparisons were made by paired Student t test for the following: between mean baseline (−30 to −10 min) and mean steady state (80 to 120 min) values for forearm glucose uptake (FGU), forearm insulin uptake (FIU), FFA balance, insulin clearance and total forearm blood flow; between baseline and 25 min for CEU acoustic intensity; between 0 and 30 min for arterial FFA concentration; and between the highest and lowest tertile of percentage MBV change. Pearson product-moment correlation coefficient was computed to determine the relationship between specific variables. For all analyses, P < 0.05 was considered statistically significant. Statistics were calculated with Sigmastat 3.2 (Systat Co., Richmond, CA).

**RESULTS**—Table 1 gives the clinical characteristics of all 14 subjects studied broken down into the two groups who either did not (group 1) or did (group 2) have \( \dot{V}O_2 \text{max} \) measured. All were normotensive, were nonobese, and had normal values for serum lipids. Before beginning the insulin clamp, the 3-h Intralipid and heparin infusion had raised the arterial plasma FFA concentration to 2.0 ± 0.2 mmol/L. Plasma glucose averaged 5.1 ± 0.1 mmol/L, and forearm blood flow was 6.5 ± 0.4 mL/min/100 mL. The basal forearm glucose and FFA balances averaged 0.65 ± 0.1 and −0.1 ± 0.2 μmol/min/100 mL.

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**Figure 1**—A: The correlation between the GIR and FGU, each measured during the last 40 min of the euglycemic insulin clamp. B: The correlation between FGU measured during the last 40 min of the insulin clamp and the percentage change of MBV measured during the first 30 min of insulin infusion. C: The correlation of GIR measured during the last 40 min of the insulin clamp with the percentage change in MBV during the initial 30 min of insulin infusion. FAV, forearm volume.
respectively. The basal arterial insulin concentration was 37 ± 5 pmol/L, which significantly (P < 0.001) exceeded that in the forearm venous blood (32 ± 4 pmol/L), resulting in a significant F IU of 17.8 ± 3.0 fmol/min/100 mL. Basal forearm insulin clearance averaged 0.49 ± 0.07 mL/min/100 mL.

The intravenous insulin infusion raised arterial insulin concentrations from 36 to 231 ± 11 pmol/L during the 120 min of hyperinsulinemia. Arterial glucose averaged 5.1 ± 0.8 mmol/L during the baseline period and was maintained within 5% of baseline throughout. Arterial plasma FFA concentrations had declined sharply by 30 min of insulin infusion (18 ± 3.8 mmol/L in this subgroup the ranges of responses to insulin of GIR (2.0–12 mg/min/kg), FGU (−0.1 to +8.0 mmol/min/100 mL), and MBV percentage change (−40 to +40) were comparable to those of the group as a whole (Fig. 1A–C). In this subgroup there was again the expected correlation (r = 0.823; P < 0.02) between F GU and GIR (Fig. 3A). In these subjects there were significant correlations between VO2 max and F GU (Fig. 3B) and between VO2 max and percentage change in MBV (Fig. 3C). Finally, in this subgroup we again found a significant correlation (r = 0.743; P < 0.05) between the percentage change in MBV and F GU (Fig. 3D). In contrast, there was no correlation between changes in blood flow (either absolute or percentage

significant correlation between the GIR and percentage change in MBV (Fig. 1C). There was no significant correlation between forearm blood flow and GIR (r = 0.16; P = NS). Likewise, we found no correlation between percentage change in MBV and the plasma FFA concentration measured during the first hour of the insulin clamp (r = 0.09; P = NS).

This study was not powered to address whether there was an effect of sex on this response. We did, however, observe a significant correlation between percentage change in MBV and FGU in both women (r = 0.77; P < 0.05) and men (r = 0.88; P < 0.01) and a correlation between percentage change in MBV and whole-body GIR that was not significant in women (r = 0.60; P = NS) although it was nearly significant in men (r = 0.74; P = 0.06). This suggests that the relationship between MBV and muscle glucose uptake holds for both sexes.

Comparing the five subjects in the highest tertile with the five in the lowest tertile of MBV percentage change, we found that F GU was markedly higher (6.5 ± 0.6 pmol/L during the clamp) in the group that had the more responsive microvasculature (Fig. 2). F IU averaged nearly threefold greater in that group (92 ± 29 vs. 32 ± 7 fmol/min/100 mL; P = 0.08), but this difference was of borderline significance (Fig. 2).

Of the 14 subjects, 8 agreed to have VO2 max measured on a separate day from the clamp study. The mean VO2 max was 43 ± 4 mL/min/kg and ranged from 29 to 63 mL/min/kg. Compared with the other 6 subjects there were no differences in these 8 in BMI, age, fasting insulin or glucose, or the plasma concentrations of FFA (1.05 ± 0.16 vs. 1.14 ± 0.13 mmol/L), insulin (250 ± 15 vs. 252 ± 18 pmol/L), or glucose (4.9 ± 0.1 vs. 5.1 ± 0.1 mmol/L) during the last 40 min of the clamp. We observed that in this subgroup the ranges of responses to insulin of GIR (2.0–12 mg/min/kg), FGU (−0.1 to +8.0 mmol/min/100 mL), and MBV percentage change (−40 to +40) were comparable to those of the group as a whole (Fig. 1A–C). In this subgroup there was again the expected correlation (r = 0.823; P < 0.02) between F GU and GIR (Fig. 3A). In these subjects there were significant correlations between VO2 max and F GU (Fig. 3B) and between VO2 max and percentage change in MBV (Fig. 3C). Finally, in this subgroup we again found a significant correlation (r = 0.743; P < 0.05) between the percentage change in MBV and F GU (Fig. 3D). In contrast, there was no correlation between changes in blood flow (either absolute or percentage

![Figure 2](image-url)
change from basal) and GIR or $V_{O2\text{max}}$, suggesting that under these experimental conditions regulation of MBV is more closely linked than is total blood flow to insulin’s metabolic effect.

**CONCLUSIONS**—Previously, we reported that euglycemic hyperinsulinemia significantly enhanced forearm MBV in healthy humans (3) and that metabolic insulin resistance, such as occurs with obesity (12) and with lipid infusion (16), blunts insulin’s action to increase MBV. In those studies we did not directly measure muscle glucose uptake, however, and MBV was measured at baseline and after 2 h of hyperinsulinemia. Because insulin’s microvascular action in muscle occurs within 15–30 min (2) of infusion and because we (22) and others (23,24) have hypothesized that insulin’s access to muscle interstitium is rate limiting for insulin’s metabolic action in muscle, we wanted to compare early insulin-induced changes in MBV with subsequent muscle glucose metabolism and to do so in the setting of physiological FFA elevation to levels observed in the postprandial state in insulin-resistant individuals. In this study, changes in MBV were measured during the first 30 min of hyperinsulinemia and forearm glucose metabolism between 80 and 120 min. In the current study, by maintaining postprandial plasma FFA concentrations (~1.1 mmol/L), we found that both muscle MBV and FGU varied over a wide range in healthy young adults. Most intriguingly, there was a strong correlation between insulin’s early microvascular action and subsequent metabolic action in muscle, underscoring the physiological importance of microvascular insulin sensitivity to muscle glucose metabolism. Beyond that, we noted that the level of fitness appeared to impact both microvascular and metabolic responses to insulin during the lipid infusion. This is of particular interest in light of recent reports that both an acute bout of endurance exercise (17) and overall fitness (18,25) interfere with the ability of lipid infusions to diminish insulin sensitivity. This suggests that muscle microvasculature, like muscle itself, responds to exercise and training to preserve insulin responsiveness.

FFAs are thought to induce muscle insulin resistance at least in part through the activation of an inflammatory response (9), which itself may result from increased oxidative stress (26). In humans, acutely raising plasma FFA level (as was done here) has been observed to

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**Figure 3**—A: The correlation between whole-body GIR and FGU, each measured during the last 40 min of the insulin clamp, in eight individuals in whom fitness was assessed by $V_{O2\text{max}}$. B: The correlation between the $V_{O2\text{max}}$ and FGU in the same individuals. C: The correlation between $V_{O2\text{max}}$ and the percentage change in MBV seen during the first 30 min of insulin infusion in the same individuals. D: The correlation between FGU measured during the last 40 min of the insulin clamp and the percentage change of MBV measured during the first 30 min of the insulin infusion in the same individuals. FAV, forearm volume.
enhance nuclear factor-κB activity in circulating mononuclear cells and plasma concentrations of macrophage migration inhibition factor, consistent with an acute inflammatory response. This was accompanied by a decrease in brachial artery flow-mediated dilation consistent with an impact of raised FFA level, with or without inflammation, on endothelial function (10). Exercise has repeatedly been shown to increase production of reactive oxygen species; however this reactive oxygen species production appears to play a synergistic role in activating and regulating antioxidant pathways (27), including manganese superoxide dismutase (28), glutathione peroxidase (29), and heme oxygenase-1 (30). This tightly regulated bidirectional redox signaling appears to occur in part through the NF-κB and mitogen-activated protein kinase (27) signal transduction pathways. The observation that fitness mitigates the inhibitory effect of FFAs on muscle’s microvascular response to insulin suggests that the muscle vascularization of fit volunteers has developed a capacity to protect against oxidative stress induced by FFA infusion. Insulin’s action to enhance microvascular perfusion is dependent on nitric oxide production. FFAs have been shown in rats to impair endothelial cell nitric oxide production acting through the inhibitor of κB kinase Β pathway (31) and to impair insulin-induced nitric oxide production and leg blood flow changes in humans (32). We have shown that insulin’s effect to increase MBV is blocked by inhibition of nitric oxide synthase. The greater response of MBV to insulin in fit individuals seen here suggests that fitness may abrogate the effect of FFAs to inhibit vascular nitric oxide production.

In the current study, we observed a significant uptake of insulin by forearm muscle under both basal and hyperinsulinemic conditions. The basal FIU and clearance of insulin observed here were not different than we reported previously in healthy controls not receiving lipid (2). Likewise, insulin uptake by muscle during the clamp was comparable to that which we reported earlier (2). We noted however that there was a wide range of FIU among subjects. As was seen with glucose, there appeared to be greater uptake among persons who responded to insulin by increasing MBV (Fig. 1). Among the 8 subjects who had VO₂$_{2\text{max}}$ measured, the mean rate of FIU during the last 40 min of the insulin clamp ranged from 22 to 112 fmol/min/100 mL. We divided these 8 subjects into two groups, four with high VO₂$_{2\text{max}}$ and four with low VO₂$_{2\text{max}}$ (average 50 ± 4 vs. 36 ± 3 mL/min/kg) and compared FIU rates. FIU during the clamp was nearly twofold greater in the 4 physically fit individuals (82 ± 16 vs. 46 ± 9; P = 0.06). This suggests that enhanced insulin delivery in physically fit individuals may contribute to the increased skeletal muscle insulin sensitivity seen with increasing fitness.

As noted in RESULTS, FGU was much greater in subjects with good microvascular responses to insulin, as reflected by increases in MBV. In four subjects the MBV actually declined below basal level during insulin infusion. We had previously observed this behavior during Intralipid infusion in rat studies and found that the decline could be prevented by infusion of BQ123, an endothelin A receptor blocker (33). This led us to suggest it may be due to selective inhibition by FFAs of endothelial nitric oxide synthase activation with preservation of insulin’s action to increase endothelin 1 production in the microvasculature, as has been observed in the Zucker (fa/fa) rat (34) and in several in vitro studies (35,36). A similar decrease in microvascular perfusion was reported for human cardiac muscle in response to meal ingestion in diabetic patients but was not seen in healthy volunteers (37).

A limitation of the current study is that we do not have measures of MBV before beginning the lipid infusion. This is due to the limitation of the amount of Definity that can be infused in humans during a single study. In rats, Intralipid with heparin infusion alone did not increase MBV (33). Another limitation relates to whether fitness per se or some other lifestyle difference associated with fitness explains the correlation between VO₂$_{2\text{max}}$ and insulin-induced changes in MBV.

In summary, we have observed that during mild, physiological increases in plasma FFA concentrations, both metabolic and vascular insulin sensitivities vary widely in otherwise healthy humans. Early microvasculature recruitment correlates strongly with subsequent muscle glucose uptake. This is consistent with a role for insulin’s microvascular action in modulating insulin’s metabolic action in muscle. Impaired microvascular responses may also diminish muscle insulin uptake, perhaps accounting in part for the muscle insulin resistance seen. Finally, physical fitness appears to blunt the inhibitory effect of raising plasma FFA on insulin-induced muscle microvascular recruitment and glucose.

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No potential conflicts of interest relevant to this article were reported.

E.M.E. and L.A.J. participated equally in the design and conduct of the studies and data analysis. E.J.B. participated in the design, conduct, data analysis, and manuscript preparation and provided grant support for the study. E.J.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References
1. Vincent MA, Clerk LH, Lindner JR, et al. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. Diabetes 2004;53:1418–1423
2. Eggleston EM, Jahn LA, Barrett EJ. Hyperinsulinemia rapidly increases human muscle microvascular perfusion but fails to increase muscle insulin clearance: evidence that a saturable process mediates muscle insulin uptake. Diabetes 2007;56:2958–2963
3. Coggins MP, Lindner J, Rattigan S, et al. Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. Diabetes 2001;50:2682–2690
4. Zhang L, Vincent MA, Richards SM, et al. Insulin sensitivity of muscle capillary recruitment in vivo. Diabetes 2004;53:447–453
5. Yki-Järvinen H, Utriainen T. Insulin-induced vasodilatation: physiology or pharmacology? Diabetologia 1998;41:369–379
6. Baron AD, Brechtel-Hook G, Johnson A, Cronin J, Leaming R, Steinberg HO. Effect of perfusion rate on the time course of insulin-mediated skeletal muscle glucose uptake. Am J Physiol 1996;271:E1067–E1072
7. Inyard AC, Clerk LH, Vincent MA, Barrett EJ. Contraction stimulates nitric oxide independent microvascular recruitment and increases muscle insulin uptake. Diabetes 2007;56:2194–2200
8. Rattigan S, Wallis MG, Youl JM, Clark MG. Exercise training improves insulin-mediated capillary recruitment in association with glucose uptake in rat hindlimb. Diabetes 2001;50:2659–2665

9. Kim JK, Kim YJ, Fillmore JJ, et al. Prevention of fat-induced insulin resistance by salicylate. J Clin Invest 2001;108:437–446

10. Tripathy D, Mohanty P, Dhindsa S, et al. Free fatty acids induce inflammation and impair vascular reactivity in healthy subjects. Diabetes 2003;52:2882–2887

11. Steinberg HO, Tarshoby M, Monestel R, et al. Elevated circulating free fatty acids levels impair endothelium-dependent vasodilation. J Clin Invest 1997;100:1230–1239

12. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. Diabetes 2006;55:1436–1442

13. Keske MA, Clerk LH, Price WJ, Jahn LA, Barrett EJ. Obesity blunts microvascular recruitment in human forearm muscle after a mixed meal. Diabetes Care 2009;32:1672–1677

14. de Jongh RT, Ijzerman RG, Serné EH, et al. Visceral and truncal subcutaneous adipose tissue are associated with impaired capillary recruitment in healthy individuals. J Clin Endocrinol Metab 2006;91:5100–5106

15. de Jongh RT, Serné EH, Ijzerman RG, de Vries G, Stehouwer CD. Free fatty acid levels modulate microvascular function: relevance for obesity-associated insulin resistance, hypertension, and microangiopathy. Diabetes 2004;53:2873–2882

16. Liu Z, Liu J, Jahn LA, Fowler DE, Barrett EJ. Infusing lipid raises plasma free fatty acids and induces insulin resistance in muscle microvasculature. J Clin Endocrinol Metab 2009;94:3543–3549

17. Schenk S, Horowitz JF. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. J Clin Invest 2007;117:1690–1698

18. Haus JM, Solomon TPJ, Marchetti CM, Edmison JM, González F, Kirwan JP. Free fatty acid-induced hepatic insulin resistance is attenuated following lifestyle intervention in obese individuals with impaired glucose tolerance. J Clin Endocrinol Metab 2010;95:323–327

19. Yki-Jarvinen H. Insulin sensitivity during the menstrual cycle. J Clin Endocrinol Metab 1984;59:350–353

20. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223

21. Vincent MA, Clerk LH, Lindner JR, et al. Mixed meal and light exercise each recruit muscle capillaries in healthy humans. Am J Physiol Endocrinol Metab 2006;290:E1191–E1197

22. Barrett EJ, Eggleston EM, Inyard AC, et al. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. Diabetologia 2009;52:752–764

23. Yang YJ, Hope ID, Ader M, Bergman RN. Insulin transport across capillaries is rate limiting for insulin action in dogs. J Clin Invest 1989;84:1620–1628

24. Prager R, Wallace P, Olefsky JM. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. J Clin Invest 1986;78:472–481

25. Solomon TPJ, Haus JM, Marchetti CM, Stanley WC, Kirwan JP. Effects of exercise training and diet on lipid kinetics during free fatty acid-induced insulin resistance in older obese humans with impaired glucose tolerance. Am J Physiol Endocrinol Metab 2009;297:E532–E539

26. Anderson EJ, Lustig ME, Boyle KE, et al. Mitochondrial H2O2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. J Clin Invest 2009;119:573–581

27. Ji LL. Modulation of skeletal muscle antioxidant defense by exercise: Role of redox signaling. Free Radic Biol Med 2008;44:142–152

28. Fukai T, Siegfried MR, Ushio-Fukai M, Cheng Y, Kojda G, Harrison DG. Regulation of the vascular extracellular superoxide dismutase by nitric oxide and exercise training. J Clin Invest 2000;105:1631–1639

29. Leeuwenburgh C, Fiebig R, Chandwaney R, Ji LL. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. Am J Physiol 1994;267:R430–R445

30. Niess AM, Sommer M, Schneider M, et al. Physical exercise-induced expression of inducible nitric oxide synthase and heme oxygenase-1 in human leukocytes: effects of RRR-alpha-tocopherol supplementation. Antioxid Redox Signal 2000;2:113–126

31. Kim F, Tyselling KA, Rice J, et al. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. Arterioscler Thromb Vasc Biol 2005;25:989–994

32. Steinberg HO, Paradisi G, Hook G, Crowder K, Cronin J, Baron AD. Free fatty acid elevation impairs insulin-mediated vasodilation and nitric oxide production. Diabetes 2000;49:1231–1238

33. Inyard AC, Chong DG, Klibanov AL, Barrett EJ. Muscle contraction, but not insulin, increases microvascular blood volume in the presence of free fatty acid-induced insulin resistance. Diabetes 2009;58:2457–2463

34. Jiang ZY, Lin YW, Clement A, et al. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. J Clin Invest 1999;104:447–457

35. Eringa EC, Stehouwer CD, Merlijn T, Westerhof N, Sipkema P. Physiological concentrations of insulin induce endothelium-mediated vasoconstriction during inhibition of NOS or PI3-kinase in skeletal muscle arterioles. Cardiovasc Res 2002;56:464–471

36. Bakker W, Sipkema P, Stehouwer CD, et al. Protein kinase C theta activation induces insulin-mediated constriction of muscle resistance arteries. Diabetes 2008;57:706–713

37. Scognamiglio R, Negut C, De Kreutzenberg SV, Tiengo A, Avogaro A. Postprandial myocardial perfusion in healthy subjects and in type 2 diabetic patients. Circulation 2005;112:179–184