Lower capillary density but no difference in VEGF expression in obese vs.lean young skeletal muscle in humans

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Abstract

Obesity is associated with lower skeletal muscle capillarization and lower insulin sensitivity. Vascular endothelial growth factor (VEGF) is important for the maintenance of the skeletal muscle capillaries. To investigate whether VEGF and VEGF receptor [kinase insert domain- containing receptor (KDR) and Flt-1] expression are lower with obesity, vastus lateralis muscle biopsies were obtained from eight obese and eight lean young sedentary men before and 2 h after a 1-h submaximal aerobic exercise bout for the measurement of VEGF, KDR, Flt-1, and skeletal muscle fiber and capillary characteristics. There were no differences in VEGF or VEGF receptor mRNA at rest between lean and obese muscle. Exercise increased VEGF (10-fold), KDR (3-fold), and Flt-1 (5-fold) mRNA independent of group. There were no differences in VEGF, KDR, or Flt-1 protein between groups. Compared with lean skeletal muscle, the number of capillary contacts per fiber was the same, but lower capillary density (CD), greater muscle cross sectional area, and lower capillary-to-fiber area ratio were observed in both type I and II fibers in obese muscle. Multiple linear regression revealed that 49% of the variance in insulin sensitivity (homeostasis model assessment) could be explained by percent- age of body fat (35%) and maximal oxygen uptake per kilogram of fat-free mass (14%). Linear regression revealed significant relation- ships between maximal oxygen uptake and both CD and capillary-to- fiber perimeter exchange. Although differences may exist in CD and capillary-to-fiber area ratio between lean and obese skeletal muscle, the present results provide evidence that VEGF and VEGF receptor expression are not different between lean and obese muscle.

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Lower capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in humans

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Obesity is associated with lower skeletal muscle capillarization and lower insulin sensitivity. Vascular endothelial growth factor (VEGF) is important for the maintenance of the skeletal muscle capillaries. To investigate whether VEGF and VEGF receptor [kinase insert domain- containing receptor (KDR) and Flt-1] expression are lower with obesity, vastus lateralis muscle biopsies were obtained from eight obese and eight lean young sedentary men before and 2 h after a 1-h submaximal aerobic exercise bout for the measurement of VEGF, KDR, Flt-1, and skeletal muscle fiber and capillary characteristics. There were no differences in VEGF or VEGF receptor mRNA at rest between lean and obese muscle. Exercise increased VEGF (10-fold), KDR (3-fold), and Flt-1 (5-fold) mRNA independent of group. There were no differences in VEGF, KDR, or Flt-1 protein between groups. Compared with lean skeletal muscle, the number of capillary contacts per fiber was the same, but lower capillary density (CD), greater muscle cross sectional area, and lower capillary-to-fiber area ratio were observed in both type I and II fibers in obese muscle. Multiple linear regression revealed that 49% of the variance in insulin sensi- tivity (homeostasis model assessment) could be explained by percent- age of body fat (35%) and maximal oxygen uptake per kilogram of fat-free mass (14%). Linear regression revealed significant relation- ships between maximal oxygen uptake and both CD and capillary-to- fiber perimeter exchange. Although differences may exist in CD and capillary-to-fiber area ratio between lean and obese skeletal muscle, the present results provide evidence that VEGF and VEGF receptor expression are not different between lean and obese muscle.

kinase insert domain-containing receptor; Flt-1; exercise; homeostasis model assessment; maximal oxygen uptake; vascular endothelial growth factor

OBESITY IS A MAJOR HEALTH PROBLEM in the United States and many other industrialized countries. Increasing adiposity is associated with lower skeletal muscle oxidative capacity and capillarization (22). Skeletal muscle capillaries are a fundamental component in the diffusion of various substances including oxygen, glucose, insulin, and fatty acids from the circulation to skeletal muscle (18).

The transcapillary delivery of insulin and glucose is mediated through passive diffusion. Fick's law of diffusion states that the amount of substance transferable via diffusion is directly proportional to the surface area available for diffusion and the concentration gradient of substance of interest, and is inversely proportional to diffusional distance. Therefore, an increase in the concentration gradient should increase the diffusion of substance across the capillary membrane, unless a limit exists as a result of the surface area or diffusional distance. Transcapillary transport of insulin is an important contributor to insulin sensitivity (2). In muscle, an increase in plasma insulin does not increase the concentration of insulin in the interstial space between the capillary and muscle fi (11), suggesting that a limit may exist in the diffusional conductance of insulin in skeletal muscle. In obese muscle, a lower muscle capillary-to-fi area ratio (CFA) is associated with lower insulin action (21). Compared with lean, obese individuals demonstrate a delayed transport of insulin over the capillary wall (31), which may be attributed to lower skeletal muscle capillary density (CD) in obese muscle.

Present data suggest that vascular endothelial growth factor (VEGF) is an important regulator of skeletal muscle capillaries. Inhibition of endogenous VEGF production reduces skeletal muscle capillarization by 64% (32). Blockade of VEGF during chronic electrical stimulation or exercise training inhibits skeletal muscle angiogenesis (3, 4). There are no differences in circulating VEGF between lean and obese individuals (27); however, myocardial VEGF expression and VEGF receptor 2 [kinase insert domain-containing receptor (KDR) human/Flk-1 murine analog], but not VEGF receptor 1 (Flt-1) are lower in obese compared with lean Zucker rats (7, 33). Whether VEGF or VEGF receptor expression is lower in obese human muscle at rest or whether the VEGF or VEGF receptor responses to acute aerobic exercise are lower in obese human vastus lateralis muscle is unknown. Accordingly, the present study was designed to investigate the hypothesis that obesity alters resting and exercise-induced expression of VEGF and VEGF receptors in human muscle.

METHODS

Subjects. Eight sedentary lean (LM; range 19.0–25.0 body mass index) and eight sedentary obese men (OM; range 31.8–47.4 body mass index) volunteered to participate in the study after written and verbal explanations of the content and intent of the study were given in accordance with the University & Medical Center Institutional Review Board. All subjects were healthy, nonsmokers, with no history of cardiopulmonary disease or diabetes, and completed a physical activity readiness questionnaire before commencement of the study. Subject characteristics are listed in Table 1.

Maximal oxygen consumption. Maximal oxygen consumption ($\dot{V}O_{2\ max}$) was measured on an electronically braked cycle ergometer (Lode, Excaliber Sport, Groningen, The Netherlands) by open-circuit

Table 1. *Subject characteristics*

	Lean	Obese	*P* Value
Age, yr	23±1	22±1	0.223
Height, cm	175.5±2.0	179.6±2.8	0.250
Mass, kg	68.8±3.0	117.0±7.6	0.000
Body fat, %	17.3±1.5	31.1±2.9	0.001
BMI	22.4±1.1	36.2±1.9	0.000
Rest systolic blood pressure, mmHg	113.0±3.2	126.3±3.9	0.080
Rest diastolic blood pressure, mmHg	78.0±2.4	83.7±2.1	0.191
$\dot{V}_{O_2max}$, ml $O_2 \cdot kg^{-1} \cdot min^{-1}$	40.2±1.5	29.7±1.5	0.000
$\dot{V}_{O_2max}$, ml $O_2 \cdot kg\ FFM^{-1} \cdot min^{-1}$	48.6±1.2	43.5±2.2	0.055
Maximum PO, W	231±10	256±12	0.141
Fasting glucose, mM	4.58±0.16	5.16±0.23	0.057
Fasting insulin, μU/ml	6.57±2.44	14.62±2.23	0.029
Fasting HOMA	1.24±0.37	3.49±0.63	0.009

Values are means ± SE. $N = 8$ men/group. BMI, body mass index. $\dot{V}_{O_2max}$, maximal oxygen consumption; Maximum PO, power output at $\dot{V}_{O_2max}$; HOMA, homeostasis model assessment.

spirometry (True Max 2400, Parvo Medics, Salt Lake City, UT). The test began with a 5-min warm-up at 75 W. After the warm-up, the workload was increased 25 W every 2 min until volitional fatigue.

Body composition. Body density was determined via hydrostatic weighing. Residual volume was measured by oxygen dilution (36). Body fat percentage was determined from body density based on the two-compartment model of Siri et al. (30).

Submaximal exercise and muscle biopsies. At least 1 wk after the $\dot{V}_{O_2\ max}$ test, subjects completed 1 h of exercise at 50% $\dot{V}_{O_2\ max}$. Before the commencement of exercise and at 2 h postexercise, a muscle biopsy was obtained from the vastus lateralis. The resting and postexercise muscle biopsy samples were obtained from alternate legs. Samples were stored at -80°C until analysis. A section of the resting sample was oriented in an optimal cutting temperature (OCT) compound (Sakura Finetek, Torrance, CA) and tragacanth (Sigma, St. Louis, MO) mixture, frozen in liquid nitrogen-cooled isopentane, and stored at -80°C until processing for the measurement of muscle morphometry and capillarization.

Insulin and glucose. Insulin and glucose were determined from a 12-h fasting plasma sample by an immobilized enzyme biosensor for glucose (YSI 2300 STAT plus glucose and lactate analyzer, Yellow Spring Instruments, Yellow Springs, OH) and by chemiluminescent enzyme immunoassay for insulin (Access, Beckman Coulter, Brea, CA) according to the manufacturer's instructions. The homeostasis model assessment (HOMA) allows for the quantitative assessment of insulin action using fasting concentrations of glucose and insulin. The formula is based on an array of predicted glucose and insulin values that would be expected for many potential combinations of insulin action (23).

RNA isolation and Northern analysis. Total cellular RNA was isolated from each biopsy sample, and standard Northern blot analysis was performed for VEGF, KDR, and Flt-1 mRNA as previously described (10). Blots were exposed to XAR-5 X-ray film (Eastman Kodak, New Haven, CT) by use of a Cronex Lightning Plus screen at -80°C. Autoradiographs were quantitated by densitometry within the linear range of signals and normalized to r3-actin mRNA levels.

Protein isolation and analysis. A portion of the muscle biopsy sample was homogenized in RIPA (1X PBS, 1% Igepal, 0.5% sodium deoxycholate, 0.1% SDS with protease inhibitors) as previously described (10). Total protein was measured by bicinchoninic acid (Bio-Rad Laboratories, Hercules, CA). For each sample, VEGF (from 50 μg of total protein), KDR (from 125 μg of total protein), and Flt-1 (from 50 μg of total protein) were measured in duplicate. Commercial VEGF, KDR, and Flt-1 ELISA kits were used according to the manufacturer's instructions and within the linear range of the kits (R&D Systems, Minneapolis, MN).

Morphometric and morphological analysis. Muscle tissue mounted in OCT tragacanth was sectioned to a thickness of 10 μm on a cryostat, mounted on slides, and kept at -20°C until fixation. Sections were stained for capillaries using a modified ATPase method (Rosenblatt technique; Ref. 29), which simultaneously provides fiber typing (type I and II) and capillary visualization. There is no difference in the number of capillaries visualized with frozen biopsy samples using the Rosenblatt technique and the number visualized in tissue sections prepared from perfusion-fixed muscle (15).

Muscle sections were viewed under a light microscope (Nikon 400) at a magnifi of X200, and a digital image was taken of the section (Nikon Coolpix 990). Capillaries were quantifi manually from the digital image on individual fi The following indexes were measured (13): *1*) the number of capillaries around a fi [capillary contacts (CC)], *2*) the capillary-to-fi ratio on an individual-fi basis (C/F_i), *3*) the number of fi sharing each capillary (sharing factor), and *4*) CD. Capillary-to-fi perimeter exchange index (CFPE) was calculated as an estimate of the capillary-to-fi surface area (13), whereas the capillary-to-fi area ratio (CFA) was calculated as an estimate of the muscle fi area supplied by a capillary (21). The sampling strategy employed for image capture involved randomly selecting two to three fi from the muscle biopsy sample as previously described by Hepple et al. (16). For each image collected, a sampling frame was created within the image, such that all fi intersecting the edges of the sampling frame were contained completely within the image. By allowing the complete area of all fi wholly or partially contained within the sampling frame to be measured, this technique prevents bias of the data toward smaller fi that would otherwise occur due to the fact that smaller fi have a greater probability than larger fi of being completely contained within the image (16). Quantifi of skeletal muscle capillarization was

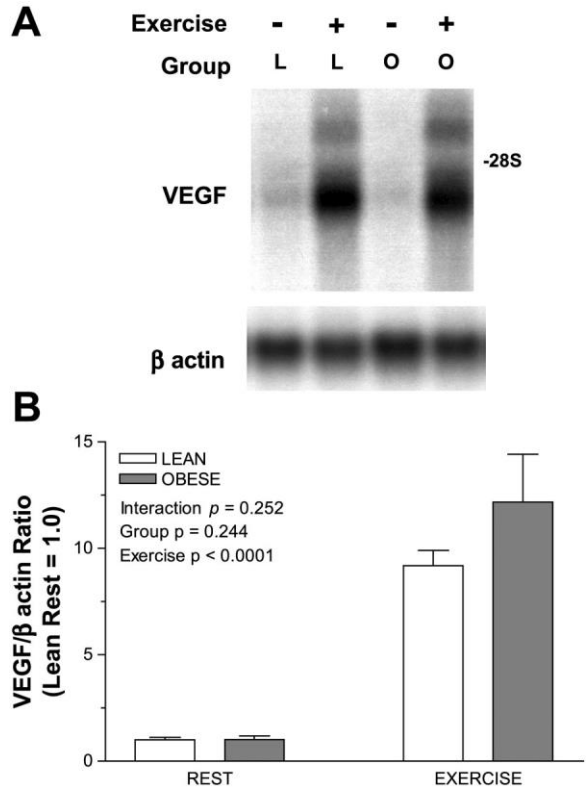

Fig. 1. Representative Northern blot (*A*) and quantitative densitometry for the ratio of vascular endothelial growth factor (VEGF) mRNA to r3-actin mRNA (*B*) in skeletal muscle of lean (L) and obese (O) men at rest and 2 h after the completion of 1 h of cycle ergometer exercise. Lean rest data were normalized to 1.0. All other data were normalized to lean + rest to allow for comparisons. VEGF was significantly increased by exercise. There was no difference in VEGF mRNA between lean and obese men. Error bars represent standard errors. $N = 8$ men/group.

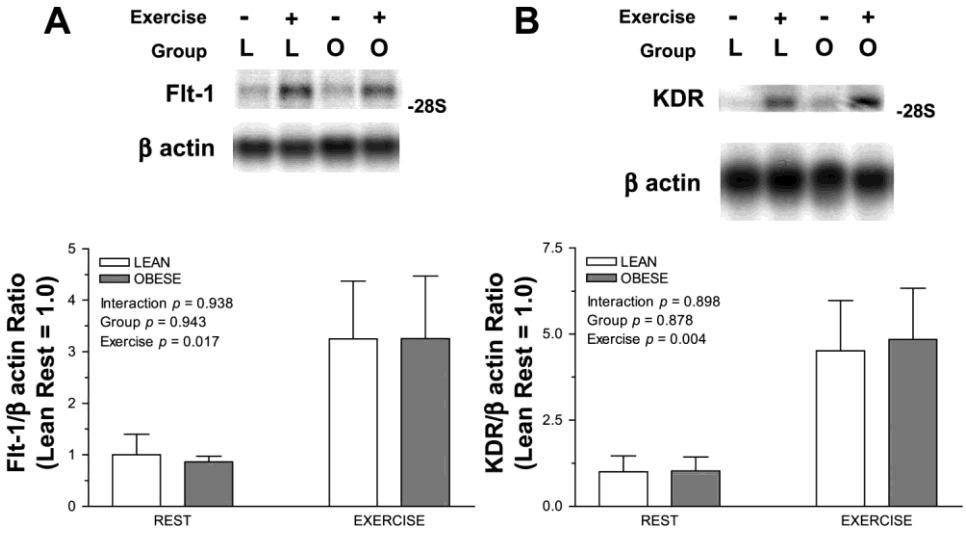

Fig. 2. Representative Northern blot and quantitative densitometry for the ratio of kinase insert domain-containing receptor (KDR; *A*) and Flt-1 (*B*) mRNA to r3-actin mRNA in skeletal muscle of lean (L) and obese (O) men at rest and 2 h after the completion of 1 h of cycle ergometer exercise. Lean rest data were normalized to 1.0. All other data were normalized to lean + rest to allow for comparisons. KDR and Flt-1 mRNA were significantly increased by exercise. There were no differences in KDR or Flt-1 mRNA between lean and obese men. Error bars represent standard errors. $N = 8$ men/group.

performed on at least 50 fi (mean 56.0 ± 2.0 fi since a greater sampling size does not improve the estimation of capillarization in human skeletal muscle (26). Fiber cross-sectional area (FCSA) and perimeter were measured with the image-analysis system and commercial software (SigmaScan, Jandel Scientifi calibrated to transform the number of pixels (viewed on a computer monitor) into micrometers.

Statistical treatment. For both mRNA and protein, a two-way analysis of variance (group X exercise level) was used. After a significant *F* ratio, a Bonferroni post hoc analysis was used. Unpaired Student's *t*-tests were used to compare differences in all other variables. Multiple linear regression was performed to identify relationships between variables. One resting mRNA sample was lost for LM and one from OM, and these samples were not replaced. Significance was established at $P < 0.05$ for all statistical sets, and data are reported as means ± SE.

RESULTS

Subject characteristics. OM demonstrated significantly lower $\dot{V}O_{2\,max}$ than LM (LM: 40.2 ± 1.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$; OM: 29.7 ± 1.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$); however, when corrected for fat-free mass (FFM), the difference in $\dot{V}O_{2\,max}$ was no longer statistically significant ($P = 0.055$) (Table 1). OM demonstrated greater fasting insulin than LM (LM: 6.57 ± 2.44 μU/ml; OM: 14.62 ± 2.23 μU/ml) and HOMA (LM: 1.24 ± 0.37; OM: 3.49 ± 0.63) (Table 1).

VEGF and VEGF receptor expression. In Figs. 1 and 2, mRNA at rest and in response to acute systemic exercise for VEGF (Fig. 1), KDR (Fig. 2*A*), and Flt-1 (Fig. 2*B*) are shown with representative Northern blots and quantitative densitom-

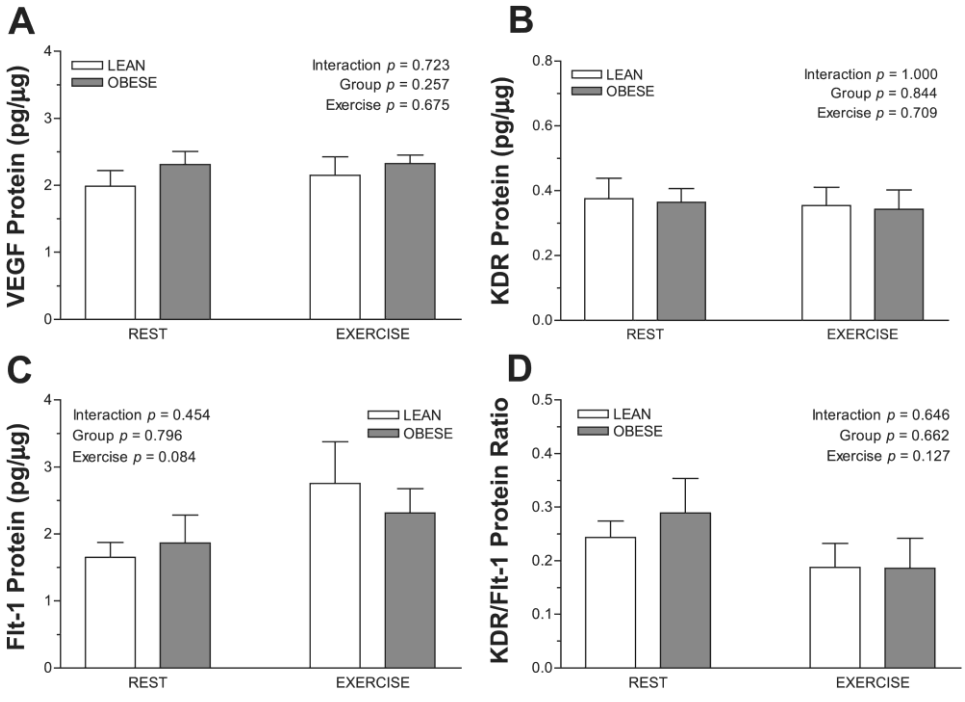

Fig. 3. Skeletal muscle protein for VEGF (*A*), KDR (*B*), Flt-1 (*C*), and KDR-to-Flt-1 ratio (*D*) at rest and 2 h after the completion of 1 h of cycle ergometry exercise. There were no differences in VEGF, KDR, Flt-1, or KDR/Flt-1 between lean and obese men. Error bars represent standard errors. $N = 8$ men/group.

Table 2. *Skeletal muscle fiber type characteristics*

	Lean	Obese	*P* Value
Overall fiber area, μm²	4,618±220	6,694±426	0.001
Type I fiber area, μm²	4,273±311	5,936±498	0.013
Type II fiber area, μm²	4,924±256	7,182±399	0.000
Overall fiber perimeter, μm	286.6±7.6	345.7±12.5	0.001
Type I fiber perimeter, μm	275.8±11.5	324.8±15.0	0.021
Type II fiber perimeter, μm	295.8±7.2	359.2±11.5	0.000
Type I fibers, %	38.8±4.7	37.7±2.8	0.842
Area of type I fibers, %	35.5±4.2	33.1±2.4	0.626

Values are means ± SE. $N = 8$ men/group.

etry normalized to r3-actin mRNA. The expression of mRNA was analyzed at rest and 2 h after a single, 1-h submaximal exercise bout in the vastus lateralis. Systemic exercise increased VEGF mRNA, KDR mRNA, and Flt-1 mRNA independent of group.

VEGF, KDR, Flt-1, and the KDR/Flt-1 protein at rest and in response to acute systemic exercise are shown in Fig. 3. There were no differences in VEGF, KDR, Flt-1, or KDR/Flt-1 ratio between groups.

Muscle morphology. In type I fibers, OM demonstrated larger muscle FCSA (LM: 4,273 ± 311 μm²; OM: 5,936 ± 498 μm²) and perimeter (LM: 275.8 ± 11.5 μm; OM: 324.8 ± 15.0 μm) than LM (Table 2). Despite the larger fibers, there were no differences in CC, C/F_i, sharing factor, or CFPE between groups (Table 3). Both CD (LM: 277.4 ± 13.0 capillaries/mm²; OM: 217.0 ± 17.8 capillaries/mm²) and CFA (LM: 2.67 ± 0.12 capillaries/10,000 μm²; OM: 2.10 ± 0.18 capillaries/10,000 μm²) were lower in OM.

In type II fibers, FCSA (LM: 4,924 ± 256 μm²; OM: 7,182 ± 399 μm²) and perimeter (LM: 295.8 ± 7.2 μm; OM: 359.2 ± 11.5 μm) were larger in OM. As in type I fibers, there were no differences in CC, C/F_i, sharing factor, or CFPE between groups, but CD (LM: 252.3 ± 11.5 capillaries/mm²; OM: 198.8 ± 17.7 capillaries/mm²) and CFA (LM: 2.47 ± 0.12 capillaries/10,000 μm²; OM: 1.93 ± 0.18 capillaries/10,000 μm²) were lower in OM. Representative photomicrographs from lean and obese muscle are in Fig. 4.

Insulin action, $\dot{V}_{O_2\,max}$, and skeletal muscle capillaries. Multiple linear regression revealed that body composition (body fat %) ($P = 0.008$ to enter) can account for 35% of the variance in HOMA, whereas $\dot{V}_{O_2\,max}$ as expressed per kilogram FFM ($P = 0.049$ to enter) can account for an additional 14% of the variance in HOMA [HOMA = (0.110 × body fat %) − (0.136 × $\dot{V}_{O_2\,max}$ kg FFM) + 5.97; overall adjusted $R^2 = 0.49$]. There were no significant relationships identified between any measure of skeletal muscle capillarization and HOMA.

Regression revealed a significant relationship between $\dot{V}_{O_2\,max}$ and CD (Fig. 5*A*). When $\dot{V}_{O_2\,max}$ was expressed per kilogram FFM, regression revealed significant relationships between $\dot{V}_{O_2\,max}$ and both CD (Fig. 5*B*) and CFPE (Fig. 5*C*).

DISCUSSION

The principal fi of the present study is that VEGF, KDR, and Flt-1 expression are similar in young LM and OM at rest and in acute aerobic exercise. In addition, we have also shown that measures of capillarization independent of FCSA (CC, C/F_i, and CFPE) are not different between lean and obese muscle, whereas measures accounting for FCSA (CD and CFA) are lower in obese muscle. The present result of lower CD in obese muscle is consistent with the relationship previously observed between CD and body fat % (r =-0.59) by Lillioja et al. (22). We are unaware of any reports that have compared measures of capillarization that do and do not account for FCSA in lean and obese muscle.

Obesity and basal VEGF and VEGF receptor expression. In Zucker rats, myocardium from obese animals demonstrates lower VEGF and VEGF receptor 2 mRNA expression compared with lean animals (7). There is a positive correlation between myocardial VEGF expression and CD in obese Zucker rats (33), suggesting that lower VEGF is responsible for the lower capillary supply observed in obese myocardium. We did not observe any differences in VEGF or VEGF receptor mRNA or protein expression between lean or obese skeletal muscle, which is consistent with the similar values for CC and C/F_i observed between lean and obese muscle but inconsistent with the lower CD and CFA.

Table 3. *Skeletal muscle capillary supply characteristics*

	Lean	Obese	*P* Value
Overall capillary contacts	3.12±0.14	3.43±0.30	0.363
Type I capillary contacts	3.01±0.21	3.18±0.26	0.608
Type II capillary contacts	3.16±0.13	3.59±0.34	0.260
Overall individual capillary-to-fiber ratio	1.19±0.06	1.31±0.12	0.358
Type I individual capillary-to-fiber ratio	1.14±0.09	1.21±0.09	0.586
Type II individual capillary-to-fiber ratio	1.20±0.05	1.38±0.14	0.269
Overall sharing factor	2.76±0.06	2.76±0.04	0.937
Type I sharing factor	2.78±0.07	2.77±0.04	0.934
Type II sharing factor	2.76±0.06	2.75±0.05	0.932
Overall capillary density, capillaries/mm²	266.9±10.5	206.8±18.0	0.012
Type I capillary density, capillaries/mm²	277.4±13.0	217.0±17.8	0.016
Type II capillary density, capillaries/mm²	252.3±11.5	198.8±17.7	0.024
Overall CFPE, capillaries/1,000 μm	4.12±0.15	3.80±0.32	0.382
Type I CFPE, capillaries/1,000 μm	4.08±0.20	3.75±0.27	0.332
Type II CFPE, capillaries/1,000 μm	4.06±0.15	3.82±0.36	0.543
Overall CFA, capillaries/10,000 μm²	2.58±0.10	1.99±0.18	0.013
Type I CFA, capillaries/10,000 μm²	2.67±0.12	2.10±0.18	0.021
Type II CFA, capillaries/10,000 μm²	2.47±0.12	1.93±0.18	0.026

Values are means ± SE. CFPE, capillary-to-fiber perimeter exchange index; CFA, capillary-to-fiber area. $N = 8$ men/group.

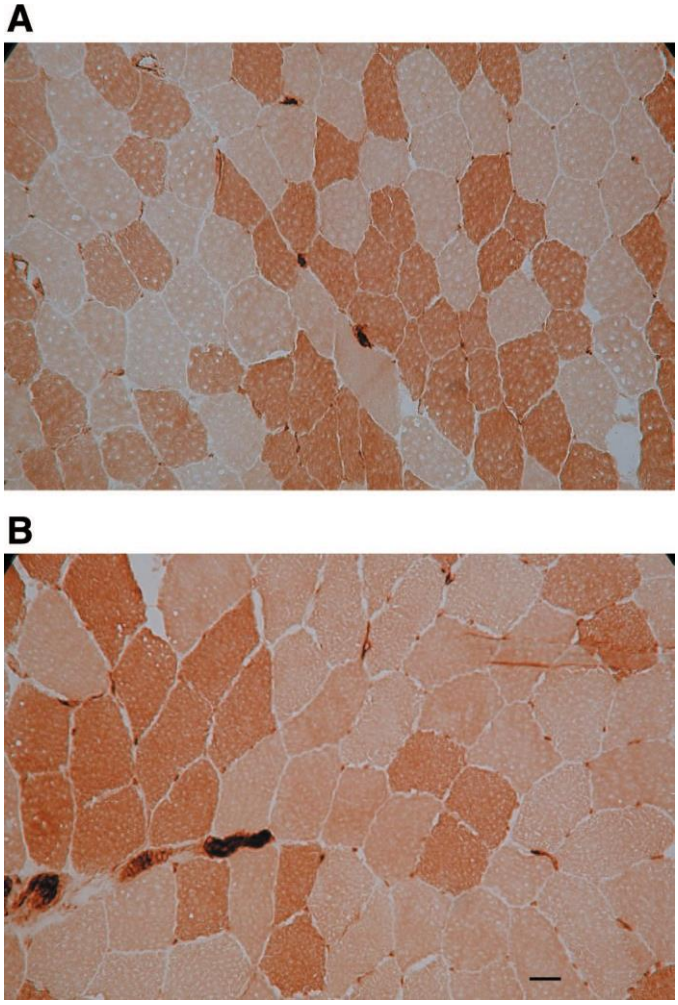

Fig. 4. Photomicrographs of muscle stained for capillaries in young lean (*A*) and obese (*B*) men. Capillaries appear as dark-stained regions between fibers. Type I fibers stain as dark and type II fibers stain as light. Bar = 50 μm.

Obesity, exercise, and VEGF and VEGF receptor expression. Exercise is a stress to muscle that produces an increase in the capillary supply (5, 6, 28). Consistent with this, acute aerobic exercise increases VEGF mRNA in human muscle (10, 12, 17, 19, 28, 35). In the present study, exercise increased VEGF, KDR, and Flt-1 mRNA independent of group, suggesting that the regulation of VEGF and VEGF receptor expression in obese muscle in response to aerobic exercise is not different than in lean muscle. This is consistent with findings that the capillary supply is increased in response to aerobic exercise training in obese muscle (21).

Muscle fiber size and capillarization. It has been proposed that the size of the capillary supply is loosely related to muscle fiber size (8). In the present study, OM demonstrated larger FCSA indicative of muscle fiber hypertrophy in both type I and type II fibers compared with lean muscle. Despite these larger fibers, the number of capillaries expressed as either CC or C/F_i was not different between groups, and as a result CFA was lower in OM. This is in contrast to the results obtained in young men in response to resistance exercise-induced muscle hypertrophy where CFA is well maintained (24). Thus obese muscle may demonstrate a reduced angiogenic response to muscle fiber hypertrophy.

Recent work suggests that small increases in muscle VEGF can promote physiological angiogenesis (25). Therefore, it might be hypothesized that greater VEGF protein expression in obese muscle could lead to an increase in capillarization and maintain the capillary-to-fiber size relationship observed in lean muscle and in response to resistance exercise-induced muscle hypertrophy (24). To our knowledge, there are no studies investigating the effects of resistance exercise on angiogenesis in obese muscle.

In the present study, individuals performed aerobic exercise to investigate whether VEGF and VEGF receptor responses to exercise are lower in obese muscle. It is possible that, although the VEGF and VEGF receptor responses to aerobic exercise are similar between lean and obese muscle, the response to resistance exercise could be different. Acute resistance exer-

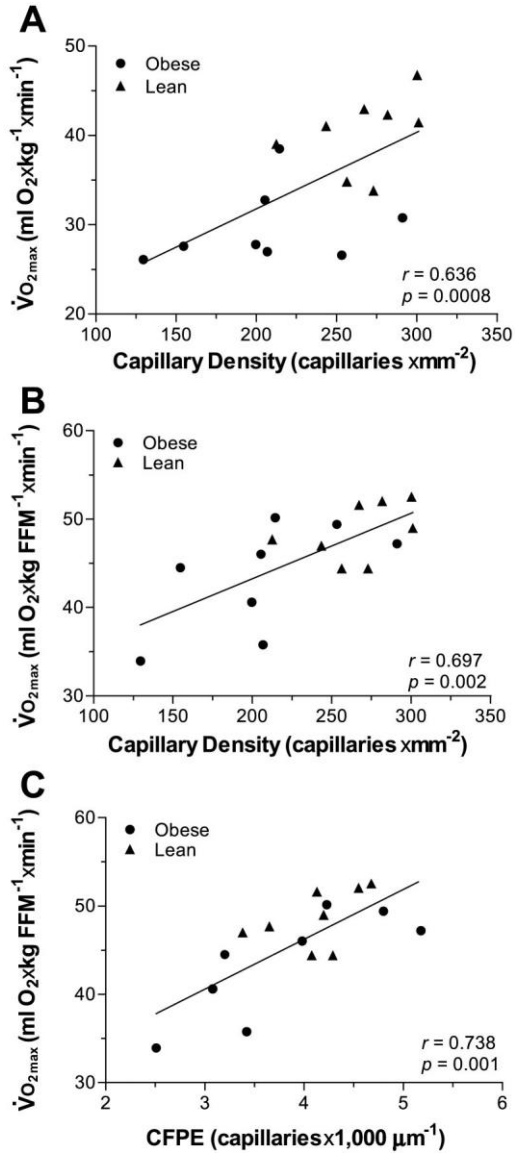

Fig. 5. Linear regression between relative maximal oxygen consumption ($\dot{V}O_{2\,max}$) per kilogram and capillary density (*A*) and between relative $\dot{V}O_{2\,max}$ per kilogram FFM and capillary density (*B*) and capillary-to-fiber perimeter exchange index (CFPE; *C*), demonstrating that capillary supply is an important determinant of aerobic capacity in young sedentary men. N = 16 men.

cise does promote increases in VEGF mRNA in human muscle (20). In rats, VEGF mRNA and protein are increased during compensatory muscle hypertrophy (9). Whether obese muscle demonstrates a reduced VEGF response to acute resistance exercise is unknown.

Regulation of $\dot{V}_{O_2\,max}$. The most prominent theory on the regulation of the capillary supply suggests that the size of the capillary supply is proportional to the metabolic demand of the muscle fiber (1). According to Fick's law of diffusion, an increase in the capillary-to-fiber surface area (CFPE estimates the capillary-to-fiber surface area) or a reduction in diffusional distance (CD estimates diffusional distance) will improve diffusional conductance from the capillary lumen to the muscle cell membrane for substances such as oxygen and insulin (18, 34). In support of the metabolic regulation of the capillary supply, significant relationships were identified between $\dot{V}_{O_2\,max}$, CD, and CFPE, suggesting that the capillary supply is appropriately regulated in lean and obese muscle to match metabolic demand. Although it has been suggested that capillary-to-fiber surface area is more tightly regulated than diffusional distance (14), evaluations such as this are not possible from the present data.

Regulation of insulin action. It is well known that both aerobic capacity and body composition are positively correlated with insulin action (18). Consistent with this, multiple linear regression did reveal relationships between insulin action, body composition, and aerobic capacity in the present report. In contrast, there were no relationships identified between any measure of the capillary supply and insulin action, which is in contrast to Lillioja et al. (22). Differences between our data and that of Lillioja et al. may arise due to differences in the measurement technique used to assess insulin sensitivity (HOMA vs. euglycemic clamp, respectively) or the size and the composition of the sampled populations (two distinct populations of lean/obese and a cross-sectional design, respectively).

In summary, we have demonstrated that basal and acute aerobic exercise-induced VEGF and VEGF receptor expression are not altered in obese skeletal muscle. We have also demonstrated a lower CD and CFA in obese muscle but no difference in absolute capillary numbers or CFPE (an estimate of the capillary-to-fiber interface), raising questions as to the variables regulating the capillary supply in obese muscle.

GRANTS

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