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# Muscle Microvascular Dysfunction in Central Obesity Is Related to Muscle Insulin Insensitivity but Is Not Reversed by High-Dose Statin Treatment

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**OBJECTIVE**—To test the hypotheses that decreased insulin-mediated glucose disposal in muscle is associated with a reduced muscle microvascular exchange capacity ( $K_f$ ) and that 6 months of high-dose statin therapy would improve microvascular function in people with central obesity.

**RESEARCH DESIGN AND METHODS**—We assessed skeletal muscle microvascular function, visceral fat mass, physical activity levels, fitness, and insulin sensitivity in skeletal muscle in 22 female and 17 male volunteers with central obesity whose age (mean  $\pm$  SD) was  $51 \pm 9$  years. We tested the effect of atorvastatin (40 mg daily) on muscle microvascular function in a randomized, double-blind, placebo-controlled trial lasting 6 months.

**RESULTS**— $K_f$  was negatively associated with a measure of glycemia (A1C;  $r = -0.44$ ,  $P = 0.006$ ) and positively associated with insulin sensitivity (the ratio of insulin-stimulated glucose effectiveness, or  $M$  value, to the mean insulin concentration, or  $I$  value;  $r = 0.39$ ,  $P = 0.02$ ). In regression modeling, A1C, visceral fat mass, and  $M:I$  explained 38% of the variance in  $K_f$  (in a linear regression model with  $K_f$  as the outcome [ $R^2 = 0.38$ ,  $P = 0.005$ ]).  $M:I$  was associated with  $K_f$  independently of visceral fat mass (B coefficient 3.13 [95% CI 0.22–6.02],  $P = 0.036$ ). Although 6 months' treatment with atorvastatin decreased LDL cholesterol by 51% ( $P < 0.001$ ) and plasma high-sensitivity C-reactive protein by 75% ( $P = 0.02$ ), microvascular function was unchanged.

**CONCLUSIONS**—Decreased insulin-mediated glucose uptake in skeletal muscle is associated with impaired muscle microvascular exchange capacity ( $K_f$ ), independently of visceral fat mass. Muscle microvascular function is not improved by 6 months of high-dose statin treatment, despite marked statin-mediated improvements in lipid metabolism and decreased inflammation. *Diabetes* 58:1185–1191, 2009

**M**icrovascular dysfunction is a cardinal long-term complication of type 2 diabetes. Reported microvascular defects in type 2 diabetes include impaired endothelium-dependent vasodilatation, reduced substrate delivery, and lower capillary density in insulin-sensitive tissues (1). Increased glycation of erythrocyte membrane proteins causing rigidity may result in an increased resistance to travel through the microcirculation (2), while concomitant alterations of the endothelial cell surface glycocalyx may modulate vascular permeability and exchange surface area (3). Obesity is the most important modifiable risk factor for the development of type 2 diabetes. Microvascular dysfunction has also been reported in obese subjects in the absence of diabetes, but it remains unclear which component(s) of obesity-linked pathophysiology contributes to microvascular dysfunction (1,4–6). Increased body fat mass is associated with molecular changes that contribute to altered vasodilatory responses, oxidative stress, abnormalities of vasoconstriction, and altered platelet adhesion; all of these defects could potentially influence solute delivery via the microvasculature (5). Insulin increases blood flow and microvascular perfusion in skin (7,8) and skeletal muscle (9,10), and impairment of insulin-induced microvascular dilator responses in skeletal muscle in animal models of insulin resistance, even at basal insulin concentrations, is believed to be a key factor in reduced glucose uptake (4,11). Thus, microvascular dysfunction might contribute to obesity-associated insulin resistance. Studies in insulin-resistant states in humans, such as obesity with or without the presence of type 2 diabetes (12), have shown impaired microvascular function where both insulin-mediated muscle microvascular perfusion and glucose uptake are reduced (5,13). The attenuation of insulin-stimulated muscle microvascular perfusion recruitment in obese humans is reminiscent of that reported in obese Zucker rats (6), being suggestive of common mechanistic pathways, including increased production of reactive oxygen species and reduced nitric oxide (NO) availability.

Based on results in animal models, it has been proposed that insulin acts to dilate the arterioles governing flow through capillary beds (14), thereby increasing substrate delivery (15). This occurs independently of, and appears to precede, increases in total blood flow and glucose disposal (14) resulting from dilatation of upstream arteriolar vessels (16). Entangled with this hypothesis is the concept of insulin-mediated redistribution of blood flow through the preferential perfusion of so-called nutritive vessels at the expense of nonnutritive routes (17). In healthy humans,

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many, but not all, experimental studies have shown insulin to have dose- and time-dependent effects, increasing blood flow in a manner that parallels glucose disposal (18). However, whether the capacity of insulin to increase the number of patent capillaries (capillary recruitment) is impaired in insulin-resistant states, such as obesity, remains uncertain (19–21).

It is well accepted that treatment with statins decreases risk of macrovascular disease and that statins have pleiotropic actions. Statins are also effective treatments for targeting vascular risk in people with features of the metabolic syndrome (22). However, the extent to which statin therapy has beneficial effects on the microvasculature has been little studied and remains unclear. Within the macrovasculature, statins have been shown to improve endothelial function and attenuate endothelial dysfunction in the presence of atherosclerotic risk factors through upregulation of endothelial NO synthase and the increased production of NO, and such an effect has been shown to occur after 6 months of therapy (rev. in 23). However, the potential for statins to modulate endothelial function in smaller vessels has yet to be elucidated.

Although it is known that microvascular dysfunction occurs in obesity, the nature of the relationship between microvascular function in an insulin-sensitive tissue such as skeletal muscle and insulin-mediated glucose disposal in skeletal muscle is uncertain. The role of potential confounders, such as fitness and physical activity levels, in the relationship between skeletal muscle microvascular function and insulin-mediated glucose disposal in skeletal muscle have not been fully clarified. Moreover, it is not certain whether statin therapy confers any benefit on muscle microvascular function via hypothesized pleiotropic actions independently of an effect on circulating LDL cholesterol concentrations.

The aims of our study were to assess the relationship between insulin-mediated glucose disposal and measures of skeletal muscle microvascular function, including microvascular filtration capacity ( $K_f$ ), a measure of microvascular integrity (isovolumetric pressure [ $P_{vi}$ ]), and resting limb blood flow ( $Q_a$ ), and to test the effect of statins on these factors in individuals with central obesity. We hypothesized 1) that decreased insulin-mediated glucose disposal in muscle is associated with a reduced muscle  $K_f$  and 2) that 6 months' intensive high-dose statin treatment would reverse this microvascular dysfunction (potentially via the pleiotropic actions of statins on NO production). We took care to assess potential confounders, such as physical inactivity and cardiorespiratory fitness, that are known to influence  $K_f$  (24).

## RESEARCH DESIGN AND METHODS

The study was approved by the Southampton General Hospital research ethics committee (LREC05/Q1704/38) and conducted in accordance with the Declaration of Helsinki. All participants were unpaid volunteers and gave informed written consent. White European subjects aged 18–75 years were invited to participate in the study. Volunteers were eligible for the main study if they had central obesity and at least one other feature of the metabolic syndrome as assessed by International Diabetes Federation criteria (25). For ethical reasons, subjects were only included in the study if estimated cardiovascular risk was <20% over 10 years based on the equation derived from the Framingham Heart Study because national guidelines indicate that people at higher cardiovascular risk should receive statin treatment for primary prevention of cardiovascular disease. Exclusion criteria were known diabetes; renal, liver, or uncontrolled thyroid disease; uncontrolled hypertension (blood pressure >160/100 mmHg); treatment with lipid-modifying drugs; antihypertensive medication; corticosteroid therapy; or hormone replacement therapy.

For more information on the subjects and methods, please refer to the supplemental appendix, available online at <http://dx.doi.org/10.2337/db08-1688>.

After completing the baseline tests, subjects were randomized in a double-blind placebo-controlled trial study design by an independent pharmacist to either 40 mg atorvastatin daily or to matched placebo for 26 weeks. The primary end point of the trial was a change in microvascular function. Previously, Charles et al. (26) studied 12 individuals in a 14-week training program during which lower limbs were trained for endurance exercise, and these authors showed a 79% improvement in  $K_f$  (from  $2.4 \pm 0.8$  to  $4.3 \pm 0.9$ ,  $P < 0.05$ ). Brown et al. (24), using electrical stimulation for 4 weeks in five sedentary individuals (8 Hz,  $3 \times 20$  min/day for 5 days per week), showed that  $K_f$  increased ~200%, from  $3.38 \pm 0.38$  to  $6.68 \pm 0.62$  ( $P < 0.05$ ). We estimated that a sample size of  $n = 40$  subjects would give us 99% power at the 5% significance level to detect a 1-SD increase in  $K_f$  and that, based on the changes shown with exercise and electrical stimulation studies, such a functional change would be physiologically relevant. Data are presented on 39 subjects because one person was unable to complete the study after suffering side effects of the prescribed trial medication.

Body composition, fat mass, and lean body mass were measured using a dual X-ray absorptiometry Delfia W 4500 instrument (coefficient of variation = 0.68%; Hologic, Bedford, MA) using a standard visual method to divide images into trunk, limb, and head. An abdominal magnetic resonance imaging scan was undertaken to assess visceral fat (27–29). An oral glucose tolerance test was performed with a 75-g glucose load with samples collected after 2 h.

The following tests were undertaken at baseline and at 26 weeks: microvascular function was assessed using a Filtrass venous occlusion plethysmographic system using a passive inductive transducer with an accuracy of  $\pm 5 \mu\text{m}$  (Compumedics DWL, Singen, Germany). Filtration ( $J_v$  in  $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ ) was measured from the slope of volume change in response to each pressure step over the last 2 min of its application, to allow for completion of vascular filling, and plotted against cuff pressure ( $P_{\text{cuff}}$ ). The slope of this relationship, at pressures above those giving rise to net filtration, is a measure of microvascular filtration capacity ( $K_f$ ) (30). Extrapolation of the relationship to its intercept on the  $P_{\text{cuff}}$  axis gives the isovolumetric venous pressure ( $P_{vi}$ ) at which there is neither net filtration nor absorption (30,31). Muscle strength was assessed by measurement of handgrip strength using a Jamar dynamometer (Promedics, Blackburn, U.K.).

**Hyperinsulinemic-euglycemic clamp.** A hyperinsulinemic-euglycemic clamp was undertaken to assess whole-body glucose uptake (insulin-stimulated glucose effectiveness, or  $M$  value) during the steady state of the clamp (final 30 min of the clamp), both at baseline and after intervention while subjects were taking their trial medication (32).

**Measurement of insulin sensitivity.** Whole-body insulin sensitivity was measured as glucose uptake during the steady state of the clamp with an insulin infusion rate of  $1.5 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ . All individuals achieved euglycemia during the clamp with glucose concentrations clamped at 5.0 mmol/L. Whole-body glucose uptake ( $M$  value) was defined as the glucose infusion rate during the final 30 min of the test (in  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) when steady-state insulin concentrations had been achieved. The ratio of  $M$  to the mean insulin concentration ( $I$  value) was used as an index of insulin sensitivity.  $M:I$  values were estimated by dividing the  $M$  value by the  $I$  value during the last 30 min of the clamp.

**Cardiorespiratory fitness and physical activity energy expenditure.** Cardiorespiratory fitness measured in terms of maximal oxygen uptake ( $\dot{V}O_{2\text{max}}$ ) was determined using a treadmill test and Cortex Metalyzer, and physical activity (physical activity energy expenditure and metabolic equivalents) was assessed using an activity monitor (Armband Sensewear Pro2) (33).

**Statistical analyses.** All statistical analyses were performed using SPSS for Windows version 16.0 (SPSS, Chicago, IL). Student's  $t$  test comparisons were undertaken to compare mean values of normally distributed data. Pearson correlation coefficients are presented for univariate analyses of normally distributed data. Where variables were not normally distributed, log transformation was undertaken to normalize the distribution. Multivariate linear regression models were used to describe factors that were independently associated with  $K_f$  as the dependent (outcome) variable. A  $P$  value of <0.05 was considered to be statistically significant for all analyses. Data are expressed as the mean  $\pm$  SD and range unless otherwise stated. To test the effect of statin on measures of microvascular function, we analyzed microvascular function at the end of the trial, adjusting for randomization and baseline microvascular measures by factorial ANOVA.

## RESULTS

Table 1 shows the baseline characteristics of subjects recruited to the study. The 39 healthy volunteers included 17 men and were aged  $51 \pm 9$  years. Of the study subjects,

TABLE 1  
Baseline characteristics of participants

	Mean $\pm$ SD	Range
Age (years)	51.4 $\pm$ 9.0	29.0–69.6
BMI (kg/m <sup>2</sup> )	32.1 $\pm$ 4.6	26.0–47.9
Waist (cm)	105.3 $\pm$ 12.9	86.5–151.0
Total fat (%)	35.6 $\pm$ 7.4	21–48
Truncal fat (% of total)	52.2 $\pm$ 5.6	43–64
Systolic blood pressure (mmHg)	133 $\pm$ 14	93–155
Diastolic blood pressure (mmHg)	85 $\pm$ 9	64–104
Cardiovascular disease risk (% per 10 years)*	7.3 $\pm$ 5.1	0–17.3
Total cholesterol (mmol/l)	5.7 $\pm$ 1.1	3.2–9.3
LDL cholesterol (mmol/l)	3.7 $\pm$ 0.9	1.7–7.0
HDL cholesterol (mmol/l)	1.45 $\pm$ 0.36	0.92–2.45
Triglyceride (mmol/l)	1.4 $\pm$ 0.6	0.4–2.7
Glucose (mmol/l)	5.2 $\pm$ 0.7	4.0–7.4
A1C (%)	5.5 $\pm$ 0.3	4.9–6.3

% fat estimated by DEXA. \*Estimated using Framingham risk score.

11 had two features, 18 had three features, 9 had four features, and 1 had all five features of the metabolic syndrome. Subjects were excluded if they had known diabetes at recruitment. On baseline testing one subject was found to have a fasting glucose of 7.4 mmol/l and therefore analyses were undertaken both including and excluding this person. Inclusion of data from this individual (who received no glucose-lowering medication during the study) did not change or affect the results, and the data are therefore presented for all 39 subjects who completed the 6-month trial. For more information on the results of this study, please refer to the supplementary material in the online appendix.

Figure 1 shows baseline  $K_f$ ,  $Q_a$ , and  $P_{vi}$  measurements. Mean values for  $K_f$ ,  $Q_a$ , and  $P_{vi}$  were  $3.91 \pm 0.18 \times 10^{-3} \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \cdot \text{mmHg}^{-1}$ ,  $4.01 \pm 0.48 \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ , and  $20.5 \pm 1.1 \text{ mmHg}$ , respectively. There was considerable variability in all measures within the cohort with an approximately threefold difference in  $K_f$  levels between subjects.

We investigated the relationships between measures of microvascular function ( $K_f$ ,  $Q_a$ , and  $P_{vi}$ ), measures of obesity and features of the metabolic syndrome, together with measures of physical activity and fitness. Table 2 shows correlation coefficients describing the relationships between  $K_f$ , age, features of the metabolic syndrome,  $VO_{2\text{max}}$ , physical activity energy expenditure, and metabolic equivalents. Of the readily measurable features of the

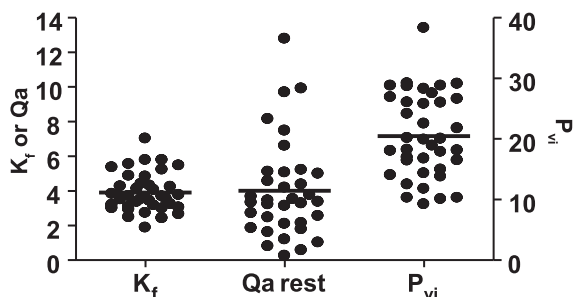


FIG. 1. Baseline measurements of filtration capacity ( $K_f$ ), resting limb blood flow ( $Q_a$ ), and endothelial integrity ( $P_{vi}$ ) from 39 individuals. All values were derived using venous congestion plethysmography from the raw data (see supplemental information in the online appendix).  $Q_a$  = resting ( $\text{ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$ );  $K_f$  =  $\times 10^{-3} \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \cdot \text{mmHg}^{-1}$ ;  $P_{vi}$  = mmHg.

TABLE 2  
Correlations between filtration capacity ( $K_f$ ) and features of the metabolic syndrome, abdominal obesity, fitness, and physical activity

	<i>r</i>	<i>P</i>
Age (years)	0.02	0.89
Waist (cm)	-0.36	0.025
Visceral fat (kg)	-0.43	0.015
Subcutaneous fat (kg)	-0.28	0.12
Systolic blood pressure (mmHg)	-0.15	0.36
Diastolic blood pressure (mmHg)	0.08	0.63
Cardiovascular disease risk (% per 10 years)	-0.15	0.38
HDL cholesterol (mmol/l)	0.13	0.43
Triglyceride (mmol/l)	-0.30	0.07
Glucose (mmol/l)	-0.05	0.77
A1C (%)	-0.44	0.006
Steps ( <i>n</i> )	0.35	0.04
Physical activity energy expenditure (metabolic equivalents)	0.20	0.24
$VO_{2\text{max}}$ ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )	0.21	0.20
Glucose disposal ( $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{mIU}^{-1} \cdot \text{l}^{-1}$ )	0.39	0.021

metabolic syndrome (waist circumference, blood pressure, glucose, HDL cholesterol, and triglyceride concentrations),  $K_f$  was statistically significantly associated with waist circumference ( $r = -0.36$ ,  $P = 0.025$ ). There was a borderline significant association with triglycerides ( $r = -0.30$ ,  $P = 0.07$ ), and there were no significant associations between  $K_f$  and age, blood pressure, glucose, or HDL cholesterol concentrations. Figure 2 shows the scatter plots for the relationships between  $K_f$  and visceral fat, A1C, and  $M:I$ .  $K_f$  was negatively associated with visceral fat ( $r = -0.43$ ,  $P = 0.015$ ) and A1C ( $r = -0.44$ ,  $P = 0.006$ ).  $K_f$  was also negatively associated with plasma high-sensitivity C-reactive protein (hsCRP) and soluble intracellular adhesion molecule (ICAM)-1 (both  $P < 0.05$ ).  $K_f$  was positively associated with  $M:I$  ( $r = 0.39$ ,  $P = 0.02$ ). There were no significant associations of note with  $P_{vi}$ . Resting  $Q_a$  was associated with respiratory exchange ratio ( $r = 0.52$ ,  $P = 0.002$ ). Neither  $P_{vi}$  nor  $Q_a$  were associated with age ( $r = -0.20$ ,  $P = 0.24$ , and  $r = -0.12$ ,  $P = 0.94$ , respectively).

Multiple regression modeling was undertaken to further explore factors that were associated with  $K_f$ . In a regression model that included  $K_f$  as the outcome, 38% of the variance in  $K_f$  was explained by A1C,  $M:I$ , and visceral fat mass as the explanatory variables in the model ( $R^2 = 0.38$ ,  $P = 0.005$ ). To determine whether the association between  $M:I$  and  $K_f$  (observed in univariate analysis) (Table 2) was independent of visceral fat, we undertook regression modeling with  $K_f$  as the outcome variable and included  $M:I$  and visceral fat as explanatory variables. In this model,  $M:I$  and visceral fat explained 30% of the variance in  $K_f$  ( $R^2 = 0.30$ ,  $P = 0.008$ ).  $M:I$  was associated with  $K_f$  independently of visceral fat (B coefficient = 3.13, 95% CI 0.22–6.02,  $P = 0.036$ ), whereas visceral fat was not associated with  $K_f$  independently of  $M:I$  (B coefficient = -0.09, 95% CI -0.40 to 0.22,  $P = 0.55$ ). There was no effect of sex in our model.

Having observed associations with measures of insulin sensitivity and microvascular function in muscle, we investigated whether a functional measure of muscle performance (grip strength) was associated with measures of microvascular function. Only  $P_{vi}$  was associated with grip

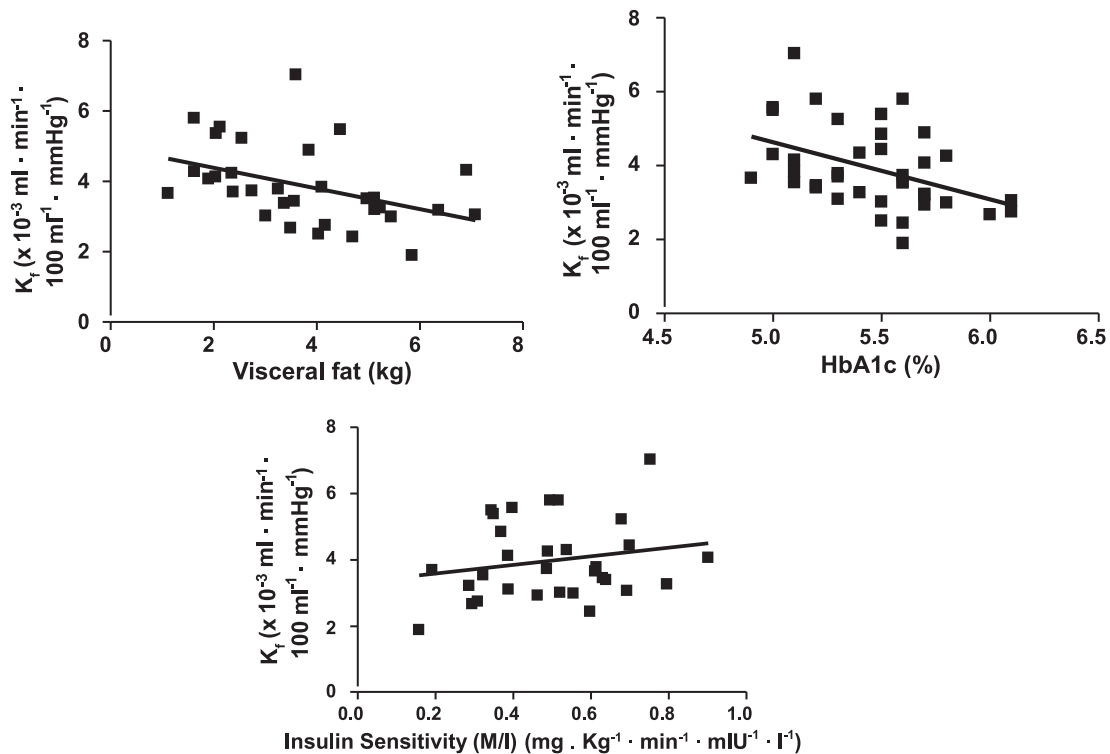


FIG. 2. Scatter plots for the relationships between  $K_f$  and visceral fat, A1C, and  $M:I$  from 39 individuals at baseline.  $K_f$  was negatively associated with visceral fat ( $r = -0.43$ ,  $P = 0.015$ ) and A1C ( $r = -0.44$ ,  $P = 0.006$ ) and positively associated with  $M:I$  ( $r = 0.39$ ,  $P = 0.02$ ).

strength {mean handgrip strength [(right + left)/2]} and  $P_{vi}$  ( $r = -0.55$ ,  $P < 0.001$ ). We observed similar results for the relationship between  $P_{vi}$  and left handgrip strength ( $r = -0.59$ ,  $P < 0.001$ ) and right handgrip strength ( $r = -0.51$ ,  $P = 0.001$ ).

We investigated the effect of 6 months of 40 mg/day of atorvastatin treatment in the randomized placebo controlled trial. In subjects in the placebo arm of the study ( $n = 20$ ), mean baseline LDL cholesterol was  $3.78 \pm 1.05$  mmol/l, and it was  $3.70 \pm 0.90$  mmol/l at follow-up ( $P = 0.51$ ). In contrast, in the treatment arm of the study ( $n = 19$ ), LDL cholesterol fell from  $3.53 \pm 0.81$  mmol/l at baseline to  $1.73 \pm 0.71$  mmol/l at follow-up ( $P < 0.001$ ). Subjects in the placebo arm of the study had a mean baseline triglyceride of  $1.31 \pm 0.68$  mmol/l, and triglyceride was  $1.19 \pm 0.61$  mmol/l at follow-up ( $P = 0.34$ ). Mean baseline fasting triglyceride concentration in the treatment arm of the study was  $1.41 \pm 0.62$  mmol/l, compared with  $1.00 \pm 0.58$  mmol/l at follow-up ( $P = 0.001$ ). Median baseline hsCRP in subjects in the placebo arm of the study was 2.0 mg/l (95% CI 1.09–10.47) compared with 3.0 mg/l (1.62–6.35) at follow-up ( $P = 0.73$ ). Statins reduced hsCRP from a baseline value of 2.0 mg/l (95% CI 1.31–5.59) to 0.5 mg/l (0.35–4.65) at follow-up ( $P = 0.02$ ). The change in LDL cholesterol concentration was positively correlated with the change in hsCRP ( $r = 0.27$ ,  $P < 0.002$ ). In both the placebo and treatment arms of the study, there was no change in body fat,  $M:I$ , A1C, or ICAM-1 measurements after 6 months of treatment (data not shown). Figure 3 shows  $K_f$ ,  $P_{vi}$ , and  $Q_a$  measurements before and after 6 months' treatment with atorvastatin. There was no change in  $K_f$ ,  $P_{vi}$ , and  $Q_a$  measurements with statin treatment, adjusting for baseline measures, age, and sex ( $K_f$ :  $P = 0.99$ ;  $P_{vi}$ :  $P = 0.28$ ;  $Q_a$ :  $P = 0.29$ ).

## DISCUSSION

Our results show that in adults with central obesity, decreased insulin-mediated glucose uptake in skeletal muscle is associated with impaired muscle  $K_f$  independently of visceral fat mass. Our data demonstrate that the association between  $K_f$  and insulin-mediated glucose disposal is independent of visceral fat mass, with no confounding by physical inactivity or low fitness levels. Despite a wealth of evidence showing that statins confer a benefit in the macrovasculature, and despite a marked statin effect on LDL cholesterol levels (lowered by ~50%) and hsCRP (decreased by 75%), our results clearly show no effect of statin on measures of muscle microvascular function.

In our study, insulin-mediated glucose disposal ( $M:I$ ) was positively associated with exchange capacity ( $K_f$ ), suggesting that the more insulin-sensitive individuals have a greater exchange capacity, thereby facilitating muscle nutrient delivery. Although the range of values of  $K_f$  measured in our study group was considerable, they were similar to those reported previously for similarly aged individuals (26,34). The values of  $K_f$  are also within the range reported for individuals with pre-diabetes or diabetes without microvascular complications (35–38). We failed to show any association between age and any of the measures of microvascular function in our study. However, the mean age of our volunteers was 51.4 years with an SD of only 9 years. Thus, it is plausible that in our relatively small sample size of predominantly middle-aged subjects, we have failed to detect true associations between aging and measures of microvascular function. It is also possible that because we have not studied very aged individuals, we have not detected any obesity-independent, age-related change in microvascular function.

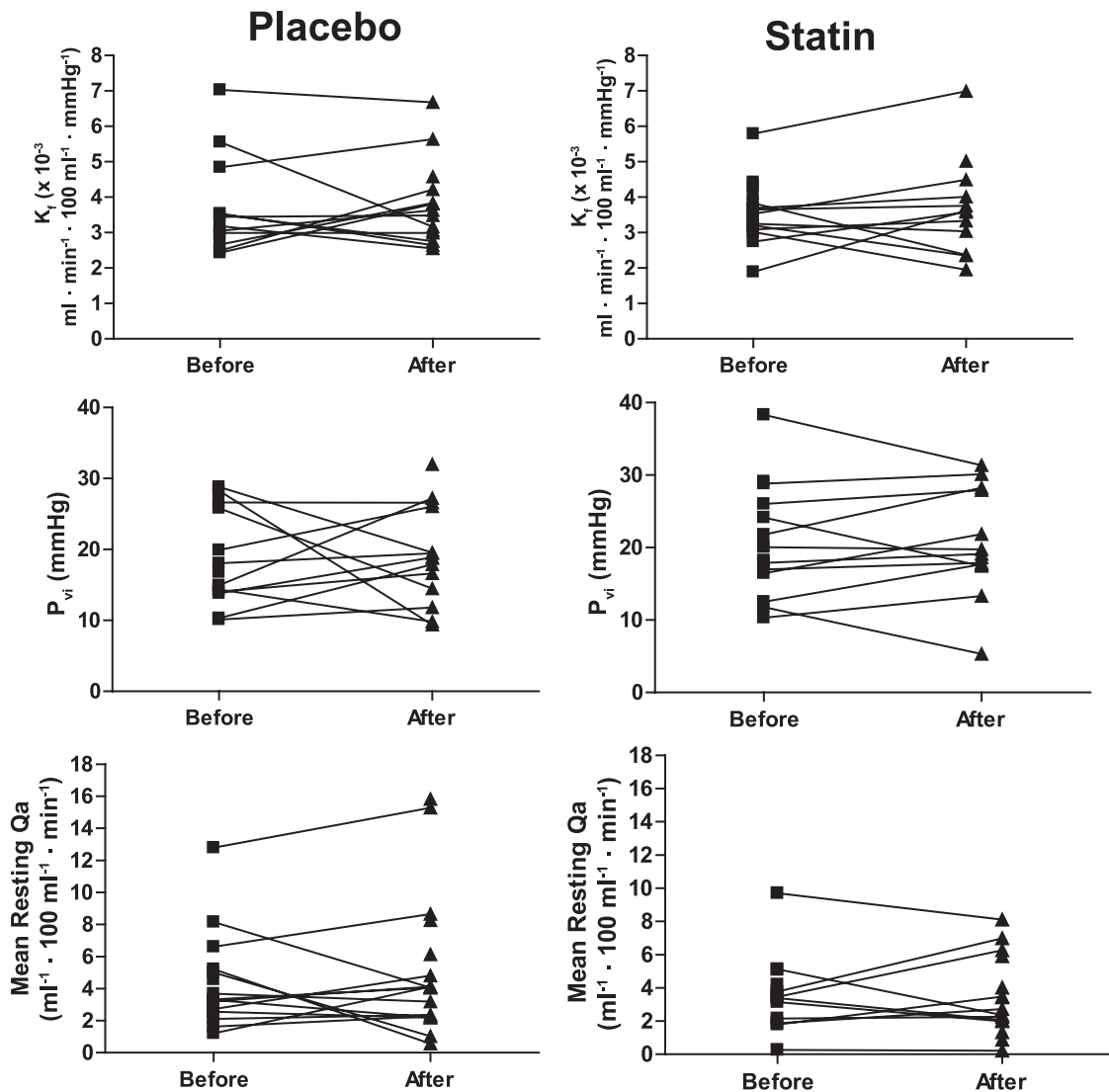


FIG. 3.  $K_p$ , isovolumetric pressure ( $P_{vi}$ ), and resting limb blood flow ( $Q_a$ ) measured before and after 26 weeks' treatment with atorvastatin (40 mg once daily) or matched placebo.

Having shown associations between insulin-mediated glucose uptake in skeletal muscle and  $K_p$ , we also explored whether a functional measure of skeletal muscle performance (grip strength) was associated with measures of muscle microvascular function, since we have recently shown in a large cohort study that decreased grip strength was associated with metabolic syndrome (39) and type 2 diabetes (40). The mechanism underlying decreased hand-grip strength in type 2 diabetes and metabolic syndrome is uncertain, but, interestingly, our results show that one measure of microvascular integrity ( $P_{vi}$ ) was associated with decreased grip strength. In other studies, increases in  $P_{vi}$  have been associated with inflammatory disease (41), but whether vascular inflammation within skeletal muscle may contribute to loss of muscle strength in people with type 2 diabetes has yet to be explored.

Our results showed a marked effect of atorvastatin to decrease LDL cholesterol and CRP, but, in keeping with previous work (42) testing the effects of 12 weeks' treatment with atorvastatin, we showed no effect of atorvastatin on levels of inflammatory markers (e.g., ICAM-1, tumor necrosis factor- $\alpha$ , interleukin-6, endothelin 1, retinol binding protein 4, leptin, adiponectin, and resistin) (data not

shown). Whereas statins have been shown to have a beneficial effect on endothelial function and blood flow within the macrovasculature (e.g., flow-mediated dilation), our data with high-dose atorvastatin for 6 months showed no effect of statin on any of the measured aspects of muscle microvascular function. Fegan et al. (43) showed, in individuals with type 2 diabetes, no improvement in cutaneous vascular response after 3 months' treatment with single or combined lipid-lowering therapy. Although no effect of 4 weeks' treatment with 20 mg/day atorvastatin was observed on vasomotor function by high-resolution ultrasound examination of the brachial artery (flow-mediated dilation and sublingual nitrate) (44), a beneficial effect of statins on aspects of endothelial function has been noted over 6 months (45). It is plausible that turnover of endothelial cells or neovascularization is needed to improve aspects of microvascular function as measured in our study. Six months' treatment with statins may be insufficient time for this to occur. It is possible that our failure to detect a difference in microvascular function with statin treatment represents a type 2 statistical error. However, our randomized placebo-controlled trial sample size gave us 97% power to detect a 1.0-SD change in  $K_p$  at

the 5% significance level, and a 1.0-SD change in  $K_f$  represents a  $\sim 1.1$ -unit change in  $K_p$ , or an increase in mean  $K_f$  from  $3.9$  to  $5.0 \times 10^{-3} \text{ ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \cdot \text{mmHg}^{-1}$ . Our study was therefore powered to detect relatively modest changes in  $K_f$ , and more marked changes in  $K_f$  have been observed with electrical stimulation and with exercise. Electrical stimulation for 4 weeks increased  $K_f$  from  $3.38 \pm 0.38$  to  $6.68 \pm 0.62$  ( $P < 0.05$ ) (24), and a 14-week training program during which lower limbs were trained for endurance caused a 79% improvement in  $K_f$  (from  $2.4 \pm 0.8$  to  $4.3 \pm 0.9$ ,  $P < 0.05$ ) (26,35–38). Thus, these data suggest that our study was powered to detect physiologically relevant changes in  $K_f$ .

The methods available to investigate muscle microvasculature are limited, and those used to measure the whole tissue or insulin-mediated capillary perfusion and flow are very invasive, for example, radiolabeled imaging techniques (46), contrast enhanced ultrasound using albumin microbubbles (9), needle-inserted laser Doppler probes (8), or measurements of the distribution of blood flow by [ $^{15}\text{O}$ ]H $_2$ O as an index of flow heterogeneity (47). One way of assessing blood/muscle exchange, and hence to assess impaired muscle microvascular function, is to quantify the capacity of the microvascular bed to filter fluid. Plethysmography is a well-validated, noninvasive technique that uses small-step increases in venous occlusion pressure and measurement of the resultant changes in limb volume to provide a measure of microvascular filtration capacity ( $K_f$ ) (48).  $K_f$ , which is measured predominantly in the muscle of the lower limb, has been shown to be differentially sensitive to increases in capillary surface area, as found in training schedules (26), as well as sensitive enough to detect increases in capillary perfusion, as in studies involving chronic electrical stimulation (24).  $K_f$  thus appears to be an important and sensitive measure to detect impairment of microvascular function, and the methodology used (plethysmography) does not affect the function of the vasculature under study (17). In the current study, a noninvasive measure was used that was acceptable to subjects returning for testing at the end of the study. We reasoned that the greater technical ease of the Filtrass system and its good reproducibility, together with its noninvasive nature, would result in better compliance in our nonpaid volunteers who were required to return for follow-up measurements at the end of the intensive 6-month clinical trial. Many of the techniques mentioned above used to assess changes in blood flow in muscle rely on visualization of erythrocyte movement or their particulate surrogates. Moreover, the movement of plasma, which determines bulk transfer and microvascular surface interchange of small solutes to sustain the optimal diffusion gradient, cannot be readily visualized. Plethysmographic assessment of  $K_f$  goes some way toward addressing this matter by measuring the rate of fluid exchange across the whole muscle microvascular bed. This enables evaluation of microvascular filtration parameters, by which significant differences due to pathophysiology and/or therapeutic interventions can be studied. Other more invasive techniques provide evidence of an insulin-mediated microvascular “recruitment” in human muscle through a selective action on precapillary arterioles (9–11,13,49) to redirect blood to “nutritive” vascular beds. The measurement of  $K_f$  or  $Q_a$  by plethysmography does not allow us to distinguish between variations in muscle blood flow due to shifts, or redistribution, within microvascular networks and those in total blood flow, as

determined by the resistance vessels supplying the muscle. It is possible that variation in muscle blood flow due to shifts, or redistribution, within microvascular networks could explain some of the wide variance in microvascular measurements (Fig. 1) that we observed across our cohort.

In summary, we have shown a strong association between skeletal muscle  $K_f$  and decreased insulin sensitivity in skeletal muscle in men and women with central obesity. Despite marked decreases in LDL cholesterol and hsCRP concentrations caused by 6 months’ high-dose statin treatment, there was no improvement in any measure of skeletal muscle microvascular function, suggesting that these factors do not make an important contribution to control of microvascular function. Our data emphasize that further studies are now required to investigate the effects of insulin-sensitizing agents on microvascular function both in individuals at risk of type 2 diabetes and in those who have type 2 diabetes.

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