Muscle Microvascular Dysfunction in Central Obesity is Related to Muscle Insulin Insensitivity but is not Reversed by High-Dose Statin Treatment

Clough GF¹, Turzyniecka M¹, Walter L¹, Krentz AJ¹, Wild SH², Chipperfield AJ³, Gamble J⁴, Byrne CD¹

¹School of Medicine, University of Southampton and ²Public Health Sciences, University of Edinburgh, ³School of Engineering, University of Southampton, ⁴University of Birmingham, UK

First author: Geraldine Clough

Corresponding author: Christopher D Byrne
The Institute of Developmental Sciences (IDS Building) (University of Southampton), MP 887 Southampton General Hospital Tremona Rd, Southampton SO16 6YD Email: C.D.Byrne@soton.ac.uk

Clinical Trial Registration Information: EUDRACT 2005000512-28

Submitted 5 December 2008 and accepted 2 February 2009.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org
ABSTRACT

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

Methods & Results. 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 mean (SD) age was 51 (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. Microvascular exchange capacity (Kf) was negatively associated with a measure of glycaemia (HbA1c) (r=−0.44, p=0.006); and positively associated with insulin sensitivity (M/I) (r=0.39, p=0.02). In regression modelling, HbA1c, visceral fat mass and M/I explained 38% of the variance in Kf [in a linear regression model with Kf as the outcome (R^2 =0.38, p=0.005)]. M/I was associated with Kf 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 LDLc by 51%, p<0.001 and plasma C reactive protein (hsCRP) 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, 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.
Microvascular 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 glyocalyx 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 contribute 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 an insulin-stimulated muscle microvascular perfusion recruitment in obese humans is reminiscent of that reported in the obese Zucker rat (6), being suggestive of common mechanistic pathways including increased production of reactive oxygen species (ROS) 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 non-nutritive routes (17). In healthy humans, 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 to attenuate endothelial dysfunction in the presence of atherosclerotic risk factors through upregulation of endothelial nitric oxide synthase (eNOS) and the increased production of nitric oxide (NO) and such an effect has been shown to occur after
months of therapy (for review see (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, whether statin therapy confers any benefit on muscle microvascular function via hypothesised pleiotropic actions, independently of an effect on circulating LDL cholesterol concentrations is uncertain.

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 (Kf), a measure of microvascular integrity (isovolumetric pressure, (Pvi) and resting limb blood flow (Qa) and to test the effect of statins on these factors in individuals with central obesity. We hypothesised (a) that decreased insulin mediated glucose disposal in muscle is associated with a reduced muscle microvascular exchange capacity and (b) that 6 months intensive high dose statin treatment would reverse this microvascular dysfunction (potentially via the pleiotrophic actions of statins on nitric oxide production). We took care to assess potential confounders such as physical inactivity and cardiorespiratory fitness that are known to influence microvascular exchange capacity (24).

**RESEARCH DESIGN AND METHODS**

*Subjects and methods (see supplemental file)*

**Study design.** 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 (IDF) 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, as 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, anti-hypertensive medication, corticosteroid therapy, hormone replacement therapy.

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 weeks training programme during which lower-limbs were trained for endurance exercise and these authors showed a 79% improvement in Kf (from 2.4 ± 0.8 to 4.3 ± 0.9 p<0.05). Brown et al (24) showed using electrical stimulation for 4 weeks in 5 sedentary individuals (8 Hz, 3 x 20 min day⁻¹, for 5 days week⁻¹), that Kf increased ~200% from, 3.38 ± 0.38 to 6.68 ± 0.62 (p<0.05).
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 Kf 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 as 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 dual X-Ray absorptiometry (DEXA) Hologic Delfia W 4500 instrument (Hologic, Bedford, MA, USA) (CV=0.68%) using a standard visual method to divide images into trunk, limb and head. Abdominal MRI was undertaken to assess visceral fat (27-29). An oral glucose tolerance test (OGTT) was performed with a 75 g glucose load with samples collected after 2 hours.

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 +/−5 μm (Compumedics.dwl, Singen, Germany). Filtration, Jv (mL min±1 100 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 (Pcuff). The slope of this relationship, at pressures above those giving rise to net filtration, is a measure of Kf, microvascular filtration capacity (30). Extrapolation of the relationship to its intercept on the Pcuff axis gives the isovolumetric venous pressure, Pvi, 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 UK).

**Hyperinsulinaemic euglycaemic clamp.** A hyperinsulinemic euglycaemic clamp was undertaken to assess whole body glucose uptake (M value) during the steady state of the clamp (final 30 minutes 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 the insulin infusion rate of 1.5 mU kg⁻¹ min⁻¹. All individuals achieved euglycaemia 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 mg kg⁻¹ min⁻¹, when steady state insulin concentrations had been achieved. The ratio M/I was used as an index of insulin sensitivity. M/I values were estimated by dividing the M value by the mean insulin concentration (I value) during the last 30 minutes of the clamp.

**Cardiorespiratory fitness and physical activity energy expenditure.** Cardiorespiratory fitness measured in terms of maximal oxygen uptake (VO₂ max) was determined using treadmill test and Cortex Metalyzer, and physical activity (Physical activity energy expenditure (PAEE) and Metabolic equivalents (METS)) were 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, USA). 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 normalise the distribution. Multivariate linear regression models were used to describe factors that were independently associated with Kf as the dependent (outcome) variable. A p value of <0.05 was considered to be statistically significant for all analyses. Data are expressed as mean ± SD and range unless otherwise stated. To test the effect of statin on measures of microvascular function we analysed microvascular function at the end of the trial, adjusting for randomization and baseline microvascular measures by factorial ANOVA.

RESULTS

(See supplemental file)

Table 1 shows the baseline characteristics of subjects recruited to the study. The 39 healthy volunteers included 17 men and had a mean (SD) age of 51 (9) years. 11 subjects had 2 features, 18 had 3 features, 9 had 4 features and 1 had all 5 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 person (who received no glucose lowering medication during the study), did not change or affect the results, and the data is therefore presented for all 39 people who completed the 6 month trial.

Fig 1 shows baseline Kf, Qa and Pvi measurements. Mean values for Kf, Qa and Pvi were 3.91± 0.18x10-3 ml/min/100ml/mmHg, 4.01 ± 0.48 ml/min/100ml and 20.5 ± 1.1 mmHg, respectively. There was considerable variability in all measures within the cohort with an approximately 3-fold difference in Kf levels between subjects.

We investigated the relationships between measures of microvascular function (Kf, Qa and Pvi), 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 Kf, age, features of the metabolic syndrome, VO2 max, PAEE and METS. Of the readily measureable features of the metabolic syndrome (waist circumference, blood pressure, glucose, HDLc and triglyceride concentrations), Kf was statistically significantly associated with waist circumference (r=-0.36, p=0.025). There was a borderline significant association with triglyceride (r=-0.30, p=0.07) and there were no significant associations between Kf and age, blood pressure, glucose or HDLc concentrations. Fig 2 shows the scatter plots for the relationships between Kf and visceral fat, HbA1c and M/I. Kf was negatively associated with visceral fat (r=-0.43, p=0.015) and HbA1c (r=-0.44, p=0.006). Kf was also negatively associated with plasma hsCRP and soluble ICAM-1 (both p<0.05). Kf was positively associated with M/I (r=0.39, p=0.02). There were no significant associations of note with Pvi. Resting Qa was associated with respiratory exchange ratio (r=0.52, p=0.002). Neither Pvi nor Qa were associated with age (r=-0.20, p=0.24 and r=-0.12, p=0.94 respectively).

Multiple regression modelling was undertaken to explore further factors that were associated with Kf. In a regression model that included Kf as the outcome, 38% of the variance in Kf was explained by HbA1c, M/I and visceral fat mass as the explanatory variables in the model (R2=0.38, p=0.005). In order to determine whether the association between M/I and Kf (observed in univariate analysis see table 2) was independent of visceral fat, we undertook regression modelling with Kf 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 Kf ($R^2=0.30$, $p=0.008$). M/I was associated with Kf independently of visceral fat (B coefficient 3.13, (95% CI 0.22, 6.02), $p=0.036$), whereas visceral fat was not associated with Kf independently of M/I (B coefficient= -0.09 (95% CI -0.40, 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 Pvi was associated with grip strength (mean hand grip strength ([(right + left) / 2]) and Pvi (r=−0.55, $p<0.001$). We observed similar results for the relationship between Pvi and left hand grip strength ($r=−0.59$, $p<0.001$) and right hand grip 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 LDLc was 3.78 ± 1.05 mmol/l and 3.70 ± 0.90 mmol/l at follow up, $p=0.51$. In contrast in the treatment arm of the study (n=19) LDLc fell from 3.53 ± 0.81 mmol/l at baseline to 1.73 ± 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 ± 0.68 mmol/l and 1.19 ± 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 ± 0.62 mmol/l compared with 1.00 ± 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 (95%CI 1.09, 10.47) mg/l compared with 3.0 (95% CI 1.62, 6.35) mg/l at follow up ($p=0.73$). Statins reduced hsCRP from a baseline value of 2.0 (95%CI 1.31, 5.59) mg/l to 0.5 (95%CI 0.35, 4.65) mg/l at follow up ($p=0.02$). The change in LDLc 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, HbA1c or ICAM-1 measurements after 6 months treatment (data not shown). Fig 3 shows Kf, Pvi and Qa measurements before and after 6 months treatment with atorvastatin. There was no change in Kf, Pvi and Qa measurements with statin treatment, adjusting for baseline measures, age and sex (Kf $p=0.99$, Pvi $p=0.28$, Qa $p=0.29$).

**DISCUSSION**

Our results show that in adults with central obesity, decreased insulin mediated glucose uptake in skeletal muscle is associated with impaired muscle microvascular exchange capacity (Kf), independently of visceral fat mass. Our data demonstrate that the association between microvascular exchange capacity 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 LDLc 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 (Kf), suggesting that the more insulin-sensitive individuals have a greater exchange capacity, thereby facilitating muscle nutrient delivery. Although the range of values of Kf measured in our study group was considerable, they were similar to those reported previously for similarly aged individuals (26;34). The values of Kf are also within the range reported for individuals with prediabetes, 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 a standard deviation 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 ageing and measures of microvascular function. It is also possible that as we have not studied very aged individuals, we have not detected any obesity-independent, age-related change in microvascular function.

Having shown associations between insulin mediated glucose uptake in skeletal muscle and Kf, 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, type 2 diabetes and metabolic syndrome, is uncertain but interestingly, our results show that one measure of microvascular integrity (Pvi) was associated with decreased grip strength. In other studies, increases in Pvi 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 LDLc 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 inflammatory markers e.g. ICAM-1, TNFα, IL-6, endothelin 1, and retinol binding protein 4, leptin, adiponectin and resistin levels (data not shown). Whilst 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 following 3 months treatment with single or combined lipid-lowering therapy. Although, no effect of 4 weeks treatment with atorvastatin 20 mg/day 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 (see (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 Kf at the 5% significance level and a 1.0 SD change in Kf represents a ~ 1.1 unit change in Kf, or an increase in mean Kf from 3.9 to 5.0 x10⁻³ ml/min/100ml/mmHg. Our study was therefore powered to detect relatively modest changes in Kf and more marked changes in Kf have been observed with electrical stimulation and with exercise. Electrical stimulation for 4 weeks increased Kf from, 3.38 ± 0.38 to 6.68 ± 0.62 (p<0.05) (24), and a 14-week training programme during which lower-limbs were trained for endurance, caused a 79% improvement in Kf (from 2.4 ± 0.8 to 4.3 ± 0.9 p<0.05) (26).

(35-38). Thus these data suggest that our study was powered to detect physiologically relevant changes in Kf.
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 radiolabelled 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 [15O]H2O 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, non-invasive 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 (Kf) (48). Kf, 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). Kf thus appears to be an important and sensitive measure to detect impairment of microvascular function and the methodology used (plethysmography) does not impact on the function of the vasculature under study (17). In the present study, a non-invasive 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, good reproducibility, together with its non-invasive nature would result in better compliance in our non-paid volunteers who were required to return for follow up measurements at the end of the intensive 6 month clinical trial. Many of the techniques used to assess changes in blood flow in muscle, mentioned above, rely upon visualization of erythrocyte movement or their particulate surrogates. Moreover, the movement of plasma that determines bulk transfer and microvascular surface interchange of small solutes, to sustain the optimal diffusion gradient cannot be readily visualized. Plethysmographic assessment of Kf goes some way towards 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 Kf or Qa 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 microvascular exchange capacity (Kf) and decreased insulin sensitivity in skeletal muscle in men and women with central obesity. Despite marked decreases in LDLc 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 emphasise that further studies are now required to investigate the effects of insulin-sensitising agents on
Microvascular function both in people at risk of type 2 diabetes and who have type 2 diabetes.

ACKNOWLEDGEMENTS

The authors would like to thank Debbie Smith and Gina Schreiber, School of Medicine, University of Southampton for their technical help in data collection and analysis, the nurses of Wellcome Trust Clinical Research facility, especially Clare Grocott, for their help with the physiological measurements, and Lucinda England for help with recruitment. Dr Margaret Brown, University of Birmingham is acknowledged for her help in protocol development.

Disclosures

The study was supported by an Independent Research Grant from Pfizer UK to CDB and AJK.
Fig 1 Baseline measurements of filtration capacity ($K_f$), resting limb blood flow ($Q_a$) and isovolumetric pressure (endothelial integrity, $P_{vi}$) from 39 individuals. All values were derived using venous congestion plethysmography from the raw data (see supplemental information).

$Q_a = \text{resting (ml/100ml/min)}$

$K_f = 10^{-3}\text{ml/min/100ml/mmHg}$

$P_{vi} = \text{mmHg}$
Fig 2 Scatter plots for the relationships between Kf and visceral fat, HbA1c and M/I from 39 individuals as baseline. Kf was negatively associated with visceral fat (r=-0.43, p=0.015) and HbA1c (r=-0.44, p=0.006) and positively associated with M/I (r=0.39, p=0.02).
**Fig 3** Microvascular exchange capacity (Kf), isovolumetric pressure (Pvi) and resting limb blood flow (Qa) measured before and after 26 weeks treatment with atorvastatin (40 mg o.d.) or matched placebo.
Table 1. Baseline characteristics of participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ±SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>51.4 ± 9.0</td>
<td>29.0 – 69.6</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>32.1 ± 4.6</td>
<td>26.0 – 47.9</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>105.3 ± 12.9</td>
<td>86.5 – 151.0</td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>35.6 ± 7.4</td>
<td>21 – 48</td>
</tr>
<tr>
<td>Truncal fat (% of total)</td>
<td>52.2 ± 5.6</td>
<td>43 – 64</td>
</tr>
<tr>
<td>Systolic Bp (mmHg)</td>
<td>133 ± 14</td>
<td>93 – 155</td>
</tr>
<tr>
<td>Diastolic Bp (mmHg)</td>
<td>85 ± 9</td>
<td>64 – 104</td>
</tr>
<tr>
<td>CVD risk* (%.10 y⁻¹)</td>
<td>7.3 ± 5.1</td>
<td>0 – 17.3</td>
</tr>
<tr>
<td>TC (mmol.l⁻¹)</td>
<td>5.7 ± 1.1</td>
<td>3.2 – 9.3</td>
</tr>
<tr>
<td>LDLc (mmol.l⁻¹)</td>
<td>3.7 ± 0.9</td>
<td>1.7 – 7.0</td>
</tr>
<tr>
<td>HDLc (mmol.l⁻¹)</td>
<td>1.45 ± 0.36</td>
<td>0.92 – 2.45</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.4 ± 0.6</td>
<td>0.4 – 2.7</td>
</tr>
<tr>
<td>Glucose (mmol.l⁻¹)</td>
<td>5.2 ± 0.7</td>
<td>4.0 – 7.4</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5 ± 0.3</td>
<td>4.9 – 6.3</td>
</tr>
</tbody>
</table>

* estimated using Framingham risk score. % fat estimated by DEXA.
<table>
<thead>
<tr>
<th>Feature</th>
<th>r value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>-0.36</td>
<td>0.025</td>
</tr>
<tr>
<td>Visceral fat (kg)</td>
<td>-0.43</td>
<td>0.015</td>
</tr>
<tr>
<td>Subcutaneous fat (kg)</td>
<td>-0.28</td>
<td>0.12</td>
</tr>
<tr>
<td>Systolic Bp (mmHg)</td>
<td>-0.15</td>
<td>0.36</td>
</tr>
<tr>
<td>Diastolic Bp (mmHg)</td>
<td>0.08</td>
<td>0.63</td>
</tr>
<tr>
<td>CVD risk (%/10 y)</td>
<td>-0.15</td>
<td>0.38</td>
</tr>
<tr>
<td>HDLc (mmol.l⁻¹)</td>
<td>0.13</td>
<td>0.43</td>
</tr>
<tr>
<td>Triglyceride (mmol.l⁻¹)</td>
<td>-0.30</td>
<td>0.07</td>
</tr>
<tr>
<td>Glucose (mmol.l⁻¹)</td>
<td>-0.05</td>
<td>0.77</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>-0.44</td>
<td>0.006</td>
</tr>
<tr>
<td>Steps (n)</td>
<td>0.35</td>
<td>0.04</td>
</tr>
<tr>
<td>PAEE (METS)</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>V02 max (ml.min⁻¹.kg⁻¹)</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>Glucose disposal mg.kg⁻¹.min⁻¹.mIU⁻¹.L⁻¹</td>
<td>0.39</td>
<td>0.021</td>
</tr>
</tbody>
</table>
REFERENCES


20. Poole, D, Brown, M, Hudlicka, O: Last word on Point: Counterpoint: There is/is not capillary recruitment in active skeletal muscle during exercise. J Appl Physiol 104:901, 2008


40. Sayer, AA, Dennison, EM, Syddall, HE, Gilbody, HJ, Phillips, DI, Cooper, C: Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg? Diabetes Care 28:2541-2542, 2005