Subcutaneous abdominal adipose tissue blood flow: variation within and between subjects and relationship to obesity

Lucinda K. M. SUMMERS, Jaswinder S. SAMRA, Sandy M. HUMPHREYS, Richard J. MORRIS* and Keith N. FRAYN

Oxford Lipid Metabolism Group and *Diabetes Research Laboratories, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Oxford, U.K.

(Received 28 March/19 July 1996; accepted 27 August 1996)

1. We assessed the variation in subcutaneous abdominal adipose tissue blood flow within and between subjects and investigated whether it is correlated with body mass index.

2. We measured body mass index and subcutaneous abdominal adipose tissue blood flow in 38 fasting subjects on the same day and on different days and, in a subgroup of 16 subjects, after a mixed meal.

3. In 190 measurements in the fasted state, subcutaneous abdominal adipose tissue blood flow was significantly more variable between subjects than could be accounted for by the within-subject variation alone. Subcutaneous abdominal adipose tissue blood flow was also significantly more variable between days within subjects than could be accounted for by within-day variation alone. Fasting and post-prandial subcutaneous abdominal adipose tissue blood flow were negatively correlated with body mass index, as was the post-prandial rise in subcutaneous abdominal adipose tissue blood flow. Multiple regression analysis showed that fasting blood flow was not dependent on insulin concentration after allowing for body mass index. There was no correlation between post-prandial subcutaneous abdominal adipose tissue blood flow and insulin concentration.

4. Insulin does not appear to have a direct vasodilatory effect in subcutaneous adipose tissue. Obese subjects have lower fasting and post-prandial subcutaneous abdominal adipose tissue blood flow. This may be because of a blunted response to sympathetic stimulation, or it may be another aspect of the insulin-resistant state.

INTRODUCTION

It has been suggested that insulin has a physiological role as a vasodilator of skeletal muscle vasculature [1] and that one of the phenomena associated with insulin resistance is a reduced skeletal muscle blood flow in response to insulin [2, 3]. This failure to vasodilate in response to insulin results in a decreased insulin-mediated glucose uptake in skeletal muscle [4]. After an oral glucose load, fasting and post-prandial insulin concentrations were two- to threefold higher in obese than in lean subjects [5]. However, leg blood flow increased post-prandially only in lean subjects, not in the obese. The increase in leg blood flow seen in lean subjects may be due to an insulin-mediated increase in cardiac output, and it has been suggested that this is the result of activation of the sympathetic nervous system by insulin [6]. It is also possible that lowering of leg vascular resistance by a nitric oxide-dependent mechanism partially accounts for the increase in leg blood flow [1]. In obese, insulin-resistant subjects there is a specific impairment of sympathetic neural responsiveness to physiological hyperinsulinaemia in skeletal muscle [7].

Blood flow is an important regulator of metabolism in white adipose tissue [8–10], the site of most of the initial hydrolysis of chylomicron triacylglycerol in the post-prandial period. It is not known whether insulin stimulates adipose tissue blood flow or whether there is decreased insulin-mediated glucose uptake into adipose tissue cells in vivo in the insulin-resistant state. It has previously been observed that obese subjects have lower subcutaneous abdominal adipose tissue blood flow (sATBF) than non-obese subjects and that the β-adrenergically mediated sATBF response is diminished in obesity [11, 12]. In obese subjects sATBF has been found to be similar to femoral ATBF, whereas in non-obese individuals it is nearly twice as high [13]. This may be due to differences in lipolytic activity or responses to vasoactive substances and could be linked to the increased cardiovascular morbidity associated with abdominal obesity [13].

Factors that are known to influence ATBF include exercise, skin temperature and oxygen partial pressure [14, 15]. Intravenous infusion of adrenaline or isoprenaline increases ATBF through β-adrenergic stimulation [11, 16]. ATBF has been
noted to rise post-prandially [17, 18], and this might be due to regional sympathetic nervous activation [19]. Physiological concentrations of insulin decrease the catecholamine-induced production of vasodilating prostaglandins in adipose tissue. It has been suggested that in hyperinsulinaemia the stimulatory effect of adrenergic agonists on the production of these prostaglandins is inhibited [20].

A significant day-to-day variation in sATBF within individuals has previously been described in three subjects [21]. We have now compared the variation in sATBF among different subjects and the variation within the same subject on different occasions. We have also investigated whether the variation in sATBF in one subject on different days was greater than the variation in repeated measurements of sATBF of the same subject on the same day. In considering the possible effects of insulin resistance on sATBF we have hypothesized that sATBF is negatively correlated with body mass index (BMI) and that the rise in this blood flow seen post-prandially is also negatively correlated with BMI.

METHODS

Subjects

Studies were conducted on 38 healthy subjects (21 men) aged 18–68 years (median 30.5 years), BMI 19–53 kg/m² (median 24 kg/m²) who had volunteered for other research projects involving measurement of sATBF. Subjects were studied on 1–4 different days. For 24 h before the study they were asked to avoid smoking cigarettes, drinking alcohol or taking ‘unaccustomed exercise’. The evening before the study subjects were asked to eat a low fat meal and then to fast from 20.00 hours onwards. During this period subjects were also asked to avoid caffeine-containing fluids. The studies were approved by the Central Oxford Research Ethics Committee and all subjects gave informed consent.

Experimental design

Each study began at 08.00 hours with subjects resting in a supine position for at least an hour. A dose of 3 MBq of ¹³³Xe (XE-133-S-2B2, CIS UK, High Wycombe, U.K.) dissolved in 0.9% saline was injected paraumbilically using an extrafine needle with an external diameter of 0.36 mm (MICROFINE 111 syringe, B-D LO-DOSE, Cowley, U.K.). The injected volume (0.02–0.5 ml) has been shown not to influence blood flow values [21]. The first sATBF measurement was not taken until at least 30 min later to allow recovery from the hyperaemic phase caused by the injection. sATBF was then measured on two occasions 20 min to 1 h apart, except in three subjects in whom, on one of the days they attended, only one measurement was made.

sATBF was measured by collecting 10-s counts over a 10-min period with a caesium iodide detector (Oakfield Instruments, Eynsham, U.K.) placed immediately over the injection site [18]. A subgroup of 24 subjects had been treated overnight with various pharmaceutical agents that did not affect blood flow (shown by a paired t-test). In another subgroup of 16 subjects (four men), six obese (BMI>30 kg/m²) and 10 non-obese (BMI<25 kg/m²), sATBF was measured on two different days before and after a mixed meal containing 85 g carbohydrate, 60 g fat and 13 g protein. On each day measurements were taken on two occasions before the meal and 30, 60, 90, 120, 180, 240, 300 and 360 min after the meal. ‘Resting’ blood flow was taken as the mean of the basal readings from both days and the ‘post-prandial’ value was taken as the mean of the 30- and 60-min readings from both days. The post-prandial rise was taken as the difference between these two values.

Calculations

A semilog plot of disappearance of counts versus time was produced using Mediscint software (Oakfield Instruments, Eynsham, U.K.). ATBF was then calculated using the following equation [22]:

\[ \text{ATBF} = \text{slope of semilog plot} \times \text{partition coefficient.} \]

The partition coefficient for human adipose tissue has been shown that a tissue-blood partition coefficient of 10.0 ml/g may be used with reasonable accuracy in both lean and obese patients [25]. Possible errors arising from this source are discussed below.

Statistical analysis

Blood flow in adipose tissue was measured in 38 fasting subjects on up to 4 days each, with one or two measurements taken each day. A total of 190 measurements were analysed over 98 subject-days. Data were analysed by hierarchical analysis of variance (ANOVA) using a random effects model with blood flow as the dependent variable and fitting terms for subject and day (within subject). The model residuals were normally distributed and showed no systematic relationship with the fitted values. The effect of the increased duration of fasting between the first and second sATBF measurement on each study day was analysed by ANOVA. An independent samples t-test was used to analyse areas under the curve for both the fasting and post-prandial sATBFs of the subgroup that was given a mixed meal (a Shapiro–Wilks test was used to demonstrate that the values were normally distributed). The Spearman rank correlation test was used to analyse the relationship between sATBF, BMI and insulin concentrations. Multiple regression
Adipose tissue blood flow

RESULTS

Overall, the mean basal sATBF was 3.65 ml min\(^{-1}\) 100 g\(^{-1}\), with a standard deviation of 1.93 ml min\(^{-1}\) 100 g\(^{-1}\) and a range of 0.56–10.41 ml min\(^{-1}\) 100 g\(^{-1}\) (Fig. 1). There was no significant difference between the two measurements taken on the same day. ANOVA showed that there was greater variation of sATBF between subjects than could be accounted for by the within-subject variation alone \((P<0.001)\). Within each subject there was considerable variation, but there was more variation in sATBF measured on different days than could be accounted for by the within-day variation alone \((P<0.001)\). The SD of the subject means for the 38 subjects was 1.56 ml min\(^{-1}\) 100 g\(^{-1}\). The mean of the between-day SDs for each subject was 1.0-9 ml min\(^{-1}\) 100 g\(^{-1}\) and the mean of the within-day SDs for each subject and day was 0.9 ml min\(^{-1}\) 100 g\(^{-1}\). The estimates of the three SDs in the random effects model were 1.21 ml min\(^{-1}\) 100 g\(^{-1}\) between subject, 1.05 ml min\(^{-1}\) 100 g\(^{-1}\) between day (within subject) and 1.13 ml min\(^{-1}\) 100 g\(^{-1}\) within day.

While considering the possible causes of between-subject variability, we noted that obese subjects tended to have lower sATBF values. The mean fasting sATBF for the non-obese subjects in the subgroup that was given a meal was 4.38 ml min\(^{-1}\) 100 g\(^{-1}\) (SD 2.03 ml min\(^{-1}\) 100 g\(^{-1}\), range 0.98–7.69 ml min\(^{-1}\) 100 g\(^{-1}\)), whereas that for the obese subjects in the same subgroup was significantly different \((P<0.005)\) at 1.75 ml min\(^{-1}\) 100 g\(^{-1}\) (SD 0.87 ml min\(^{-1}\) 100 g\(^{-1}\), range 0.60–3.55 ml min\(^{-1}\) 100 g\(^{-1}\)). The sATBF of these subjects was also significantly different during the post-prandial period \((P<0.005)\) (Fig. 2). Basal sATBF was negatively correlated with BMI \((P<0.005, r_s = -0.72)\), as was post-prandial blood flow \((P<0.005, r_s = -0.77)\). The post-prandial rise in sATBF was also negatively correlated with BMI \((P<0.05, r_s = -0.60)\). Basal sATBF was negatively correlated with basal insulin concentrations \((P<0.0005, r = -0.84)\). BMI and basal insulin concentrations were themselves strongly positively correlated \((P<0.05, r = 0.72)\). Using multiple regression analysis it was shown that basal blood flow was not dependent on the basal insulin concentration after allowing for BMI. Post-prandial sATBF and insulin concentrations were not correlated (Fig. 2).

DISCUSSION

These results indicate that within-person variability in sATBF on different days is greater than would be expected from method variability alone, based on the variability observed within a day. This within-person variability may reflect fluctuations in regional sympathetic activity: adipose tissue vasodilatation is known to be mediated by sympathetic nervous system activity, probably via \(\beta_1\)-adrenoceptors, with \(\beta_2\)-adrenoceptors playing only a
minor role, in contrast to skeletal muscle, in which the opposite is true [26]. α2-Adrenoceptor activation has been shown to cause vasoconstriction in adipose tissue [10] and inhibit the mobilization of non-esterified fatty acids from adipose tissue [10] and inhibit the mobilization of non-esterified fatty acids from adipose tissue after sympathetic nervous system activation [27]. There is some evidence that in dogs vascular β3-adrenoceptors may play a role in adipose tissue vessel vasodilation [28], but as yet there is no evidence for this mechanism in humans, although there is evidence of a functional β3-adrenoceptor in adipose tissue [29].

It has been suggested that vascular control in skeletal muscle may involve site-specific vasomodulators, direct responses to changes in the rate of supply of nutrients or removal of products, or the release of a signal substance by vascular tissue [30]. It is possible that similar mechanisms may be involved in the control of adipose tissue vasculature, but this has not yet been investigated. In particular, it is not known whether nitric oxide plays a role in adipose tissue vessel vasodilation, as it is thought to do in muscle [1].

Our initial hypotheses were correct: basal abdominal subcutaneous adipose tissue blood flow was negatively correlated with BMI. The tissue/blood partition coefficient, λ, used in this experiment was 10.0 ml/g. It has previously been shown that the subcutaneous λ on the abdomen is linearly correlated with the local skinfold thickness (SFT) with the equation $\lambda = 0.22 \times \text{SFT} + 2.99$ [31]. That study concluded that a $\lambda$ value of 10 ml/g is generally too high. However, comparative studies in lean and obese subjects have shown that a partition coefficient for adipose tissue of 10.0 ml/g may be used as an estimate in both groups [25]. The obese subjects (BMI = 39.9 ± 2.1 kg/m²) used in that study were similar to the obese group in this study (BMI = 38.7 ± 3.8 kg/m²). It is unlikely that error in estimation of the partition coefficient is solely responsible for our findings because there is a twofold difference in the λ values for our subjects (5.5–11.0 ml/g) using the equation suggested by Bülow et al. [31], whereas there is a 13-fold difference in their sATBF (0.60–7.69 ml min⁻¹ 100 g⁻¹).

Also, it could not explain the large difference in the post-prandial pattern of sATBF between obese and non-obese subjects (Fig. 2). The effect of BMI on sATBF may not be directly mediated by insulin, but may instead be due to blunting of the β3-mediated increase in blood flow [11].

Teleologically, ATBF should increase after a meal in order to deliver triacylglycerol-rich chylomicrons to the lipoprotein lipase enzyme on the adipose tissue capillary endothelial wall. The triacylglycerol molecules can then be hydrolysed to produce glycerol and fatty acids. The latter can either be taken up and stored in the adipocytes or allowed to escape into the venous plasma as non-esterified fatty acids. The mechanism of the post-prandial ATBF increase appears to be via stimulation of vascular β-adrenoceptors in the subcutaneous adipose tissue as continuous intravenous propranolol infusion abolishes the rise in post-prandial sATBF but does not affect the overall metabolic and hormonal response to the meal [17]. The post-prandial rise in blood flow in our experiments was seen as early as 30 min after the meal. This corresponds with sympathetic nervous system stimulation: after a large meal an increase in total body spillover of noradrenaline to plasma is seen, peaking at 30 min, with a return towards fasting levels by 90 min [19]. The sympathetic nervous system appears to be activated by food intake, possibly as a reflex response to vasodilatation occurring in the gut. The mediators of this are not known, but it has been suggested that (Gln4)-neurotensin acts as a hormone affecting the regional deposition of fat post-prandially by regulating ATBF [32]. The post-prandial rise in sATBF is diminished in obese subjects, again probably as a result of blunting of the sympathetic nervous system response after a meal. We believe that this change in sATBF is a reflection of the obese state rather than a cause of it, but this requires further investigation. The diminution in sATBF in obesity obviously has important implications for post-prandial fat metabolism as adipose tissue is a major site of chylomicron triacylglycerol hydrolysis, directing non-esterified fatty acids either into storage or into the circulation.
ACKNOWLEDGMENTS

This research was funded by a grant from the Ministry of Agriculture, Fisheries and Food, and partly by the Wellcome Trust.

REFERENCES