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► **To cite this version:**

Jun-Ichi Funada, a Louise Dennis, Rachel Roberts, Fredrik Karpe, Keith Frayn. Regulation of subcutaneous adipose tissue blood flow is related to measures of vascular and autonomic function. *Clinical Science*, 2010, 119 (8), pp.313-322. 10.1042/CS20100066 . hal-00606283

**HAL Id: hal-00606283**

**<https://hal.science/hal-00606283>**

Submitted on 6 Jul 2011

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## Regulation of subcutaneous adipose tissue blood flow is related to measures of vascular and autonomic function

Jun-ichi Funada\*<sup>†</sup>, A Louise Dennis\*<sup>‡</sup>, Rachel Roberts\*, Fredrik Karpe\*<sup>‡</sup>, Keith N Frayn\*

\* Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford OX3 7LJ, UK

<sup>†</sup> Present address: The Department of Cardiology, NHO Ehime National Hospital, Toon, Ehime, 791-0281, Japan

<sup>‡</sup> NIHR Oxford Biomedical Research Centre, Oxford Radcliffe Hospitals NHS Trust, Churchill Hospital, Oxford OX3 7LJ, UK

Key words: Adipose tissue blood flow, body mass index, endothelial function, flow-mediated vasodilatation, heart rate variability, insulin resistance.

Short title: Adipose tissue and cardiovascular function

Abbreviations: ATBF, adipose tissue blood flow; FMD, flow-mediated vasodilatation; HOMA-IR, homeostatic model assessment of insulin resistance; HRV, heart rate variability; IMT, intima-media thickness of the common carotid artery; NEFA, non-esterified fatty acids; SDnn, standard deviation of the R-R intervals; TG, triacylglycerols

Correspondence:

Keith N Frayn

OCDEM

Churchill Hospital

Oxford OX3 7LJ

Tel: 01865 857220

Fax: 01865 857213

keith.frayn@oxlip.ox.ac.uk

**ABSTRACT**

Appropriate blood vessel function is important to cardiovascular health. Adipose tissue plays an important role in metabolic homeostasis. Subcutaneous abdominal ATBF (adipose tissue blood flow) is responsive to nutritional stimuli. This response is impaired in obesity, suggesting parallels with endothelial function. We assessed whether regulation of ATBF is related to the regulation of endothelial function, assessed by FMD (flow-mediated vasodilatation) of the brachial artery. Impaired FMD is a marker of atherosclerotic risk so we also assessed relationships between ATBF and a marker of atherosclerosis, common carotid artery IMT (intima-media thickness). Since ATBF is responsive to sympatho-adrenal stimuli we also investigated relationships to HRV (heart rate variability). Seventy-nine healthy volunteers (44 female) were studied fasting and after ingestion of 75 g glucose. FMD, fasting ATBF and the responsiveness of ATBF to glucose were all negatively related to BMI, confirming the adverse cardiovascular effects of adiposity. FMD was related to fasting ATBF ( $r_s = 0.32$ ,  $P = 0.008$ ) and, at least in males, this relationship was independent of BMI ( $P = 0.02$ ). Common carotid artery IMT, measured in a subset of participants, was negatively related to fasting ATBF ( $r_s = -0.51$ ,  $P = 0.02$ ,  $n = 20$ ). On the other hand, ATBF responsiveness to glucose showed no relation with either FMD or IMT. In multiple regression models, both fasting and stimulated ATBF showed relationships with HRV. Our data show that the regulation of ATBF has features in common with endothelial function, but also relationships with autonomic cardiovascular control as reflected in HRV.

## INTRODUCTION

Over the past few years, it has been appreciated that blood flow through metabolically-active tissues is regulated, and that this may relate to cardiovascular and metabolic health [1, 2]. One such tissue is adipose tissue. Subcutaneous adipose tissue blood flow (ATBF) is highly regulated by nutritional state, increasing markedly after meals in lean, healthy subjects [3, 4]. However, regulation of ATBF is impaired in obese or otherwise insulin-resistant people [4-6]. This observation suggests a parallel with endothelial function, which is an important component of cardiovascular health, and is known to be impaired in the early stages of atherosclerosis [7].

Endothelium-derived NO is one of main regulators of endothelial function in the human vasculature [8]. ATBF is also highly dependent upon NO in the fasting state: local micro-infusion of NG-monomethyl-L-arginine (L-NMMA), an inhibitor of endothelial NO synthase, reduces subcutaneous abdominal ATBF by 40% [9]. But L-NMMA does not affect the ATBF response to glucose ingestion [9], which appears instead to be driven by sympatho-adrenal activation and is largely blocked by local or systemic infusion of the beta-adrenergic antagonist, propranolol [9, 10]. It might therefore be speculated that endothelial function, as measured by the index of flow-mediated vasodilatation (FMD) in the brachial artery, would relate more to fasting ATBF than to its regulation in the postprandial state. Measurements relating to the activity of the autonomic nervous system, such as heart rate and heart rate variability (HRV), might, in contrast, be expected to relate to postprandial activation of ATBF.

Impaired endothelial function is associated with increased atherosclerotic risk [11, 12]. Increased intima-media thickness of the common carotid artery (IMT) is a surrogate marker of early atherosclerosis [13], although not always closely associated with impairment of FMD [14]. As noted earlier, ATBF responsiveness to nutrients is impaired in obesity or insulin resistance, but it is not known whether it is also associated with direct markers of atherosclerotic risk.

We have therefore investigated whether there are relationships between measurements of endothelial function and HRV, and ATBF and its responsiveness to glucose ingestion. Our working hypothesis was that, because of the common involvement of endothelial NO generation, there would be stronger relationships in the fasting state than with postprandial stimulation of ATBF. On the other hand, we hypothesised that HRV would relate specifically to the postprandial regulation of ATBF. We also took the opportunity to assess relationships between these measures of vascular function and measurements of early atherosclerosis, as reflected by IMT, in a pilot study in a sub-group of these volunteers.

## MATERIALS AND METHODS

### Subjects

We recruited 79 healthy volunteers (44 female) by advertisement and from the Oxford BioBank (OBB), a collection of samples and clinical data from the local Oxfordshire healthy population [15]. The OBB is set up such that the database can be screened, and suitable participants invited to attend for further studies. The studies were approved by the Oxfordshire Clinical Research Ethics Committee, and all subjects gave their informed consent.

The study was conducted in two phases. In both phases, ATBF and FMD were measured in the fasting state, together with standard biochemical and cardiovascular measurements (heart rate, blood pressure). In one phase, measurements of ATBF and FMD were combined with measurements of HRV. The participants in this phase were also given an oral glucose tolerance test (oGTT) to assess metabolic and cardiovascular responses to a challenge. In the other phase, ATBF and FMD measurements were combined with ultrasound measurements

of carotid intima-media thickness (IMT). Some of these participants were given the oGTT. The disposition of the participants amongst the various procedures is outlined in Fig. 1.

Those who were to have measurements of HRV attended for a screening visit to assess suitability. Some of the participants in this phase went on to take part in a dietary intervention; full details have been published separately [16]. In addition, some aspects relating to adipose tissue fatty acid composition, adipocyte size and insulin sensitivity in a sub-set of the volunteers have been published separately [17].

All subjects (both phases) were asked to refrain from strenuous exercise or alcohol intake for 24 hours before the experimental day. Investigations started around 8 am following an overnight fast. We did not standardize the stage of the menstrual cycle for pre-menopausal female participants. Details of the procedures are given below.

### Measurement of adipose tissue blood flow

ATBF was measured by the  $^{133}\text{Xe}$  washout technique [18, 19]. In brief, 1 MBq  $^{133}\text{Xe}$  was injected into the para-umbilical area of the subcutaneous adipose tissue to a depth of approximately 10 mm. After an equilibration period of 30 min, ATBF was monitored by collecting continuous 20 s readings from a  $\gamma$ -counter probe placed over the site of injection [20]. In 74 of the volunteers, as noted above, following a period of measurement of fasting blood flow, an oral glucose load (75 g) was given, and readings were continued for the next 2 h. Blood flow was calculated from a semilog plot of the disappearance of  $^{133}\text{Xe}$  counts versus time in 10 min intervals. ATBF was then calculated according to the following equation:  $\text{ATBF} = \text{slope of semilog plot of (counts per second)} \times \text{partition coefficient} \times 100$  [18]. The partition coefficient between adipose tissue and blood was taken as  $10 \text{ ml}\cdot\text{g}^{-1}$ . This value was based on a review of appropriate literature and represents a typical value [21]. Peak ATBF was calculated as previously [4], and the ATBF response to glucose as peak minus baseline.

### Heart rate variability measurement

An electrocardiograph (ECG) was recorded during the screening visit to assess suitability for inclusion, and for acclimatisation of the volunteers. Subjects with  $>3\%$  ectopic beats were excluded at the screening visit. HRV was then assessed on the study day, when two fasting baseline measurements of HRV were recorded for 10 min following the same procedure. Recordings were also made following consumption of glucose at 20, 70 and 130 min.

Subjects were semi-supine in a quiet room. Respiration was controlled using an electronic metronome at a rate comfortable for the subject at 9-11 breaths per minute. A chest strain gauge was worn to assess respiratory compliance. A 3-lead continuous ECG signal was recorded using a Powerlab/8Sp, MLS310 HRV Module (AD Instruments Pty Ltd, Castle Hill, NSW, Australia). The R-R intervals for the last consecutive 5 min for each 10 min recording were manually selected and analyzed (minimum of 250 cycles). Time and frequency-domain analyses of HRV and Poincaré plot were performed. All R-R intervals were edited by visual inspection to exclude ectopic beats or artefacts.

The various parameters of HRV tended to be highly-intercorrelated, and for illustration the key parameter of standard deviation of R-R intervals (SDnn) is presented in Results. There was no consistent pattern of change in any of the HRV parameters following glucose ingestion, so average values for SDnn over the whole experimental day are presented.

### Ultrasound study

FMD measurements were made in a dark, quiet, temperature-controlled laboratory using a high-resolution ultrasound machine (ATL 5000, Philips Ltd., Andover, MA, USA) equipped with a 14 MHz linear artery transducer. The limit of axial resolution was 0.1mm and all subjects rested in the supine position for at least 10 min before the investigation.

FMD, the index of endothelium-dependent vasodilatation, was determined by the maximal change in the diameter of the brachial artery during hyperaemia [7, 22], which was created by

placing a cuff on the forearm and inflating it to 200 mmHg for 5 min, thereby occluding blood flow to the forearm. Longitudinal scans of the right brachial artery were obtained proximal to the antecubital fossa with the probe positioned so that the optimum images could be obtained. The peak diameter change during reactive hyperaemia occurred approximately 1 min after cuff deflation. Three consecutive frames at rest and at peak reactive hyperaemia were stored in order to determine FMD. The brachial artery diameter, between media-adventitia interface on the anterior wall and intima-lumen interface on the posterior wall, was measured manually four times in each frame. A total of 12 measurements in each condition were taken to determine FMD. Arterial flow velocity was measured using a pulsed Doppler signal in the centre of the vessel at baseline and during the first 15 s of reactive hyperaemia after cuff deflation. The degree of hyperaemic response was assessed as the brachial blood flow at peak hyperaemia as a percentage of the resting flow. In 20 of the volunteers, additional ultrasound measurements were made of the common carotid artery. A region of interest for each common carotid artery was selected at least 1 cm proximal to the carotid bulb. The region of interest was manually moved to a more proximal position to avoid any plaque formation. Carotid artery intima-media thickness (IMT) was evaluated as the distance between the intima-lumen interface on both anterior and posterior walls of the bilateral common carotid artery [13, 23]. The average IMT of the bilateral common carotid arteries was employed in this investigation.

Ultrasound measurements were made by one of two observers (ALD, JF) who were responsible for the two phases of the study. One of them (ALD) performed HRV measurements. The other (JF) performed common carotid artery IMT measurements. Hence, IMT and HRV measurements were not made in the same individuals, as described earlier and shown on Fig. 1. Intra-observer variability was assessed by making repeated measurements on volunteers on separate occasions. For FMD, the correlation coefficient between first and second measurements was 0.81 ( $n = 28$ ), coefficient of variation of repeated measurements (c.v.) 13%. For IMT ( $n = 19$ ), the correlation coefficient was 0.90, and the c.v. 5.7%. For SDnn ( $n = 25$ ), the correlation coefficient was 0.92 and the c.v. 15%.

### Analytical methods

Samples of blood were taken in the fasting state and at intervals throughout the glucose tolerance test into heparinised syringes. Plasma concentrations of glucose, insulin, non-esterified fatty acids (NEFA) and triacylglycerols (TG) were measured as described previously [16].

### Calculations and statistical analysis

Glucoregulatory insulin resistance in the fasting state (HOMA-IR) was assessed from fasting glucose and insulin concentrations [24]. In those participants who underwent the oGTT, indices of insulin sensitivity were calculated from responses of plasma glucose, insulin and NEFA concentrations as described by Belfiore *et al.* [25]. These refer to post-glucose glucoregulatory insulin sensitivity (IS<sub>Igly</sub>) and the suppression of NEFA (IS<sub>Inefa</sub>).

All statistical analyses were performed using SPSS Statistics version 17.0 for Windows (SPSS Inc, Chicago, IL). Several of the variables displayed skewed distributions. Spearman's rank correlation method was used to evaluate the relationships between ATBF and other variables. Partial correlation coefficients were calculated on log-transformed data.

Multiple regression analyses were performed using fasting ATBF and peak ATBF as dependent variables. Independent variables were chosen as those hypothesised to affect fasting or peak ATBF. Regression analysis was run as a backward elimination model, with stepwise removal of variables not achieving a significance of  $P < 0.10$  (SPSS default). Log-transformed data were used where appropriate.

So far as possible, analyses have been performed across the whole cohort. However, relationships with HRV and with IMT are necessarily limited to the subgroups in whom they were measured.

## RESULTS

The characteristics of the participants are given in Table 1.

### Fasting ATBF and univariate relationships with other variables

Fasting ATBF covered a wide range (3.2 – 10.2 ml.100g<sup>-1</sup>.min<sup>-1</sup>), and was significantly negatively related to glucoregulatory insulin resistance assessed with the HOMA index (Table 2, Fig. 2A). It was less strongly related to measures of post-glucose insulin sensitivity, ISIGly and ISInefa (Table 2).

Amongst indices of cardiovascular function, fasting ATBF was positively related to FMD (Fig. 2C, Table 3) but not to other indices. Fasting ATBF and FMD were each negatively related to both age and BMI. The relationship between fasting ATBF and FMD (Fig. 2C) gave the impression of two groups. Further exploration showed that this was age-dependent. If the group were split at the median age (42 years), then the correlation was strong in the younger group ( $r_s = 0.45$ ,  $P = 0.01$ ) but absent in the older group ( $r_s = 0.26$ ,  $P = 0.13$ ).

The relationships between fasting ATBF, HOMA index, and BMI were complex. In a partial correlation controlling for BMI, the relationship between fasting ATBF and HOMA-IR was not significant ( $P = 0.52$ ), while significance was also largely lost when the relationship between fasting ATBF and BMI was controlled for HOMA-IR ( $P = 0.07$ ).

In the sub-study of relationships with IMT, fasting ATBF and IMT were negatively related (Table 3).

### Peak ATBF and the ATBF response to glucose

Fasting and peak ATBF were related ( $r_s = 0.63$ ,  $P < 0.001$ ). ATBF response to glucose, the difference between fasting and peak ATBF, was not related to the fasting value ( $P = 0.90$ ) but was related to the peak value ( $r_s = 0.71$ ,  $P < 0.001$ ).

Peak ATBF and the ATBF response were strongly related to measures of post-glucose insulin sensitivity, both ISIGly and ISInefa (Table 2, Fig. 2B). Amongst cardiovascular function indices (Table 3), peak ATBF was positively related to FMD and to SDnn, a measure of HRV. Both peak ATBF and the ATBF response were related to the hyperaemic response to release of occlusion, and weakly negatively related to heart rate.

Peak ATBF and the ATBF response to glucose were negatively related to BMI, as expected (Table 3). Relationships with metabolic measurements (not shown) were generally not striking other than negative relationships of ATBF response with fasting NEFA and TG concentrations ( $r_s = -0.30$ ,  $P = 0.02$ ,  $n = 61$ ;  $r_s = -0.34$ ,  $P = 0.01$ ,  $n = 56$  respectively); peak ATBF did not relate to fasting NEFA but did to TG ( $r_s = -0.41$ ,  $P = 0.002$ ). Fasting NEFA concentrations were also positively related to both systolic blood pressure and to heart rate ( $r_s = 0.24$ ,  $P = 0.05$ ,  $n = 65$ ;  $r_s = 0.29$ ,  $P = 0.02$ ,  $n = 62$  respectively).

### Multiple regression analysis

In order to understand relationships between ATBF and other variables jointly, we conducted multiple regression analyses for fasting and for peak ATBF (Table 4). SDnn and fasting insulin contributed to a significant model for fasting ATBF, whereas SDnn and FMD were significant in the peak ATBF model, after allowing for the effect of other variables, which did not contribute substantially to these models.

### Sex-specific features

We looked for differences between men and women. In the whole cohort, age was well matched but BMI was significantly lower in women. We therefore truncated the BMI range to include only those with BMI greater than 22 and less than 33 kg/m<sup>2</sup>, which gave well-matched groups (Table 5). Adipose tissue blood flow (fasting, peak and response to glucose) was identical in men and women, whereas FMD and hyperaemic response were significantly greater in women (Table 5). Insulin sensitivity assessed by HOMA was identical, but NEFA concentrations were higher, and TG concentrations lower in women than in men (Table 5).

We then assessed whether the relationships amongst the measured variables differed in men and women. In general they were much stronger in men than in women (e.g. men, fasting ATBF vs FMD,  $r_s = 0.57$ ,  $P = 0.002$ ,  $n = 26$ ). This relationship was still significant in a partial correlation controlling for BMI ( $r = 0.45$ ,  $P = 0.024$ ), although in a partial correlation controlling for both age and BMI the significance was marginal ( $r = 0.39$ ,  $P = 0.060$ ). The ATBF response to glucose was generally less strongly related to other vascular parameters than the fasting ATBF, except that in women, the ATBF response correlated negatively with heart rate ( $r_s = -0.43$ ,  $P = 0.007$ ,  $n = 38$ ) and positively with SDnn ( $r_s = 0.46$ ,  $P = 0.02$ ,  $n = 26$ ).

## DISCUSSION

Adipose tissue is metabolically very active, playing an important role in the minute-to-minute regulation of the supply and removal of circulating lipid fuel [26]. Because TG and NEFA are not soluble in the plasma and require transport mechanisms for delivery to, or removal from, the tissue, it seems reasonable that in adipose tissue, more than in other tissues, there might be important interrelationships between ATBF and metabolism. Thus, there is a need for tissue-specific regulation of blood flow to meet the specific metabolic activities of adipose tissue. We showed previously [27] that adrenaline infusion causes a rise in ATBF with increased efflux of NEFA from adipose tissue, but also increased TG clearance. Appropriate regulation of ATBF may therefore have importance in metabolic physiology in the extraction and release of lipids by adipose tissue.

In this study, we explored relationships between the regulation of subcutaneous ATBF and a number of aspects of cardiovascular function. We found some relationship between fasting ATBF and FMD of the brachial artery, a widely-accepted assessment of endothelial function. This is perhaps not surprising given that NO is a major regulator of fasting ATBF [9]. Endothelial NO generation is usually assumed to be the major determinant of FMD, although formal assessment should include a test of glyceryl-trinitrate administration [7], which we did not do. Interestingly, a stronger relationship between fasting ATBF and FMD was seen in men than in women. This might indicate that endothelial function is influenced by other factors, perhaps including menstrual cycle, in women. It should be noted that we did not standardise the stage of the menstrual cycle for female participants, which is likely to have increased variability in some of our measurements: FMD is known to vary during the menstrual cycle [28, 29], although there is no information about ATBF. In addition, fasting ATBF was itself reasonably strongly negatively related to BMI and to insulin resistance, as we have found previously [4, 30]. Furthermore, fasting ATBF was also moderately strongly related to common carotid artery IMT, a surrogate marker of early atherosclerosis, suggesting that reduced ATBF is associated, directly or indirectly, with adverse functional cardiovascular consequences.

ATBF may increase several-fold in response to glucose (or mixed meal) ingestion [3, 19], although it does not respond to a pure fat load [31]. This response is mediated via sympatho-adrenal activation rather than via NO generation, and is largely blocked by local or systemic propranolol administration [9, 10]. We found no relationship between the ATBF response to glucose ingestion and FMD, but ATBF responsiveness was related to other aspects of cardiovascular function in which sympathetic activity may play a role, namely



systolic blood pressure and heart rate, and, in women, HRV measured as SDnn. Multivariate analysis in fact suggested that both fasting and stimulated ATBF were related to HRV.

We observed several differences in responses between men and women. The greater FMD in women is expected [32, 33] because of their smaller brachial artery diameter; FMD is highly dependent upon arterial diameter [33, 34]. However, this sex difference is not always observed [35] and may disappear after the menopause [32]. Our group, with a mean age of 45 years, were predominantly pre-menopausal. The hyperaemic response was also greater in women than in men. The physiological significance of this response is not clear, and this sex-difference has not been observed in other studies [32]. Post-occlusion hyperaemia was not different between two groups who differed considerably in remnant lipoprotein-cholesterol concentrations, and in FMD, after a fat load [22]. We observed a relatively strong relationship between hyperaemic response to occlusion, and the ATBF response to glucose ingestion. The hyperaemic response is not predominantly endothelium-dependent but is thought to relate to locally-generated factors including metabolites [36]. Possibly our results suggest that similar mediators affect the postprandial ATBF response. The greater plasma NEFA and lower TG concentrations that we found in women compared with men are to be expected from other studies; greater NEFA concentrations may relate to greater fat mass in women [37] and lower TG concentrations to faster removal [38, 39]. We did not find a sex-difference in ATBF, so this is unlikely to have contributed. The weak positive relationship that we noted between plasma NEFA concentrations and systolic blood pressure, which was seen equally in both sexes, confirms earlier findings (reviewed in [40]). It has been suggested recently that this may reflect sympathetic activation by fatty acids [41], although we did not see relationships with HRV.

The relationships that we observed tended to revolve around BMI and insulin sensitivity. The ATBF response to glucose was particularly related to measures of post-glucose insulin sensitivity, as we have shown previously [4]. Fasting ATBF and ATBF responsiveness, as well as FMD, were all negatively related to BMI. This makes it difficult fully to disentangle causal relationships, although the data carry a clear message that increasing BMI is associated with unfavourable cardiovascular changes. These are, in turn, reflected in the strong relationship observed between BMI and carotid artery IMT.

One possible common factor between ATBF regulation and endothelial function might be the presence of perivascular fat. Perivascular adipose tissue accumulates during expansion of fat depots [42] and is now recognized to secrete a variety of factors, including TNF- $\alpha$  [42], which may trigger vascular inflammation [43, 44] and interfere with vascular reactivity. Perivascular adipose tissue is associated with increased NO release at early stages of its accumulation in rodent models, suggesting an adaptive mechanism [45], and in some human studies has been shown to release vaso-relaxant factors [46]. However, in human vessels perivascular fat is also associated with increased responsiveness to constrictive influences such as that of angiotensin-II [47]. We did not assess perivascular fat in the present studies, but common relationships between BMI, perivascular adipose tissue accumulation and insulin sensitivity indices could explain some of the common regulation we observed in ATBF and FMD.

In order to elucidate the clinical significance of these peripheral vascular functions, in particular fasting ATBF and ATBF responsiveness, it would be of interest to investigate the effects of dietary intervention on these indices. A recent study showed that the DASH (Dietary Approaches to Stop Hypertension) diet altered neither FMD nor ATBF regulation [16] despite highly significant reduction in blood pressure and improvement in lipid parameters. However, the study was of short duration (30 days), and had no significant effect on insulin sensitivity and very little on adiposity (weight change < 1 kg). Therefore, further intervention should be addressed to clarify the clinical significance of these indices on cardiovascular atherosclerotic progression.

## AUTHOR CONTRIBUTIONS

JF introduced FMD measurements to the other authors, made the first measurements and wrote the first draft of the paper. ALD conducted most of the clinical procedures and commented on the paper. RR helped with clinical studies, collated data and commented on the paper. FK worked on data and contributed to the paper. KNF was responsible for project coordination, data analysis and revising the paper.

### **ACKNOWLEDGEMENTS**

We thank other members of the research group who helped with the studies, and especially Sandy Humphreys for statistical advice and Marje Gilbert for technical assistance.

### **FUNDING**

This work was supported by the Biotechnology and Biological Sciences Research Council (UK) (grant number BB/D008123/1).

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**Table 1 Characteristics of the participants: whole cohort**

Values are median (range).

	Women (n = 44)	Men (n = 35)
Age (yrs)	42 (22 – 56)	43 (19 – 62)
BMI (kg/m <sup>2</sup> )	24.1 (17.2 – 33.3)	27.3 (19.5 – 36.8)
Fasting glucose (mmol/l)	4.91 (4.00 – 5.70)	4.97 (4.30 – 6.20)
Fasting TG (mmol/l)	0.81 (0.29 – 1.66)	1.36 (0.57 – 3.26)
Total cholesterol (mmol/l)	4.64 (2.49 – 6.37)	4.41 (3.39 – 7.18)
HDL-cholesterol (mmol/l)	1.23 (0.76 – 1.88)	0.91 (0.64 – 1.59)
Systolic blood pressure (mm Hg)	110 (89 – 140)	111 (96 – 149)
Diastolic blood pressure (mm Hg)	69 (57 – 99)	73 (58 – 100)

**Table 2** Univariate inter-relationships between ATBF and insulin sensitivity indices

Data are Spearman rank correlation coefficients for bivariate correlations, shown as  $r_s$ ,  $P$ ,  $n$ . Only relationships significant at  $P \leq 0.10$  are shown; others are marked NS (non-significant). Note: HOMA-IR is a measure of insulin resistance, whereas IS<sub>Igly</sub> and IS<sub>Inefa</sub> are measures of insulin sensitivity.

ATBF, adipose tissue blood flow; HOMA-IR, homeostatic model assessment of insulin resistance (fasting glucoregulatory insulin resistance); IS<sub>Igly</sub>, a measure of post-glucose glucoregulatory insulin sensitivity; IS<sub>Inefa</sub>, a measure of post-glucose lipid regulatory insulin sensitivity.

	HOMA- IR	IS <sub>Igly</sub>	IS <sub>Inefa</sub>
ATBF fasting (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	-0.36 0.004 63	0.33 0.014 57	0.26 0.056 57
ATBF peak (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	-0.42 0.001 61	0.52 <0.001 57	0.52 <0.001 57
ATBF response (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	-0.22 0.08 61	0.46 <0.001 57	0.50 <0.001 57

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**Table 3** Univariate inter-relationships between ATBF, BMI and other indices of cardiovascular function

Data are Spearman rank correlation coefficients for bivariate correlations, shown as  $r_s$ ,  $P$ ,  $n$ . Only relationships significant at  $P \leq 0.10$  are shown; others are marked NS (non-significant).

ATBF, adipose tissue blood flow; FMD, flow-mediated vasodilatation; IMT, intima-media thickness of the common carotid artery; SDnn, standard deviation of the R-R intervals.

	BMI (kg/m <sup>2</sup> )	FMD (%)	Hyperaemic response (%)	IMT (mm)	Systolic BP (mm Hg)	Heart rate (min <sup>-1</sup> )	SDnn
ATBF fasting (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	-0.41 <0.001 77	0.32 0.008 66	NS	-0.51 0.023 20	NS	NS	0.26 0.062 53
ATBF peak (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	-0.57 <0.001 74	0.29 0.021 63	0.29 0.027 58	NS	NS	-0.20 0.090 71	0.35 0.010 53
ATBF response (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	-0.40 <0.001 74	NS	0.35 0.008 58	NS	-0.24 0.045 73	-0.26 0.029 71	NS
BMI (kg/m <sup>2</sup> )	-	-0.33 0.006 67	NS	0.63 0.003 20	NS	NS	-0.26 0.060 55



**Table 4 Multiple regression analysis of factors associated with fasting and peak ATBF**

For the method of analysis, see Calculations and statistical analysis.

FMD, flow-mediated vasodilatation; SDnn, standard deviation of the R-R intervals.

\* Variables log-transformed to improve normality

	<i>P</i> (overall model)	<i>P</i> (individual variables)
<i>Dependent variable: Fasting ATBF*</i>		
Variables included	0.125	
Age		
Sex		
BMI*		
Fasting insulin*		
sysBP*		
Heart rate*		
FMD*		
SDnn*		
Final model includes:	0.013	
Fasting insulin		0.079
SDnn		0.064
<i>Dependent variable: Peak ATBF*</i>		
Variables included	0.040	
Age		
Sex		
BMI*		
Fasting insulin*		
sysBP*		
Heart rate*		
FMD*		
SDnn*		
Final model includes:	0.003	
FMD		0.015
SDnn		0.013

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**Table 5 Differences between men and women (BMI range truncated to give matched groups)**

Values are median (range; *n*). Significance by Mann-Whitney test. NS, non-significant (all *P* > 0.15).

	Women	Men	Significance
Age (years)	45 (22 – 56; 33)	41 (19 – 62; 27)	NS
BMI (kg/m <sup>2</sup> )	24.8 (22.1 – 31.9; 33)	26.4 (22.3 – 31.4; 27)	NS
Fasting ATBF (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	2.48 (0.85 – 10.16; 31)	2.33 (0.87 – 7.49; 27)	NS
Peak ATBF (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	4.87 (0.88 – 13.17; 31)	3.99 (2.02 – 11.11; 27)	NS
ATBF response (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	2.02 (-1.28 – 6.30; 31)	1.62 (-0.24 – 5.50; 25)	NS
FMD (%)	6.11 (0.00 – 18.58; 30)	3.64 (0.59 – 12.12; 20)	0.027
Hyperaemic response (%)	1106 (149 – 3510; 28)	500 (126 – 1244; 17)	0.002
IMT (mm)	0.52 (0.44 – 0.61; 6)	0.59 (0.46 – 0.76; 6)	NS
Systolic blood pressure (mm Hg)	109 (89 – 140; 32)	110 (96 – 149; 27)	NS
Heart rate (min <sup>-1</sup> )	61 (44 – 77; 30)	57 (39 – 86; 27)	NS
SDnn	58 (18 – 147; 24)	55 (22 – 277; 20)	NS
Plasma glucose (mmol/l)	4.9 (4.0 – 5.5; 29)	4.9 (4.3 – 6.2; 23)	NS
Plasma insulin (mU/l)	8.8 (0.7 – 19.9; 29)	9.7 (6.2 – 16.9; 24)	NS
HOMA-IR	1.8 (0.1 – 4.3; 29)	2.0 (1.2 – 4.1; 23)	NS
Plasma NEFA (μmol/l)	576 (224 – 1034; 29)	434 (253 – 581; 23)	0.001
Plasma TG (μmol/l)	805 (419 – 1664; 27)	1225 (567 – 3263; 20)	0.001

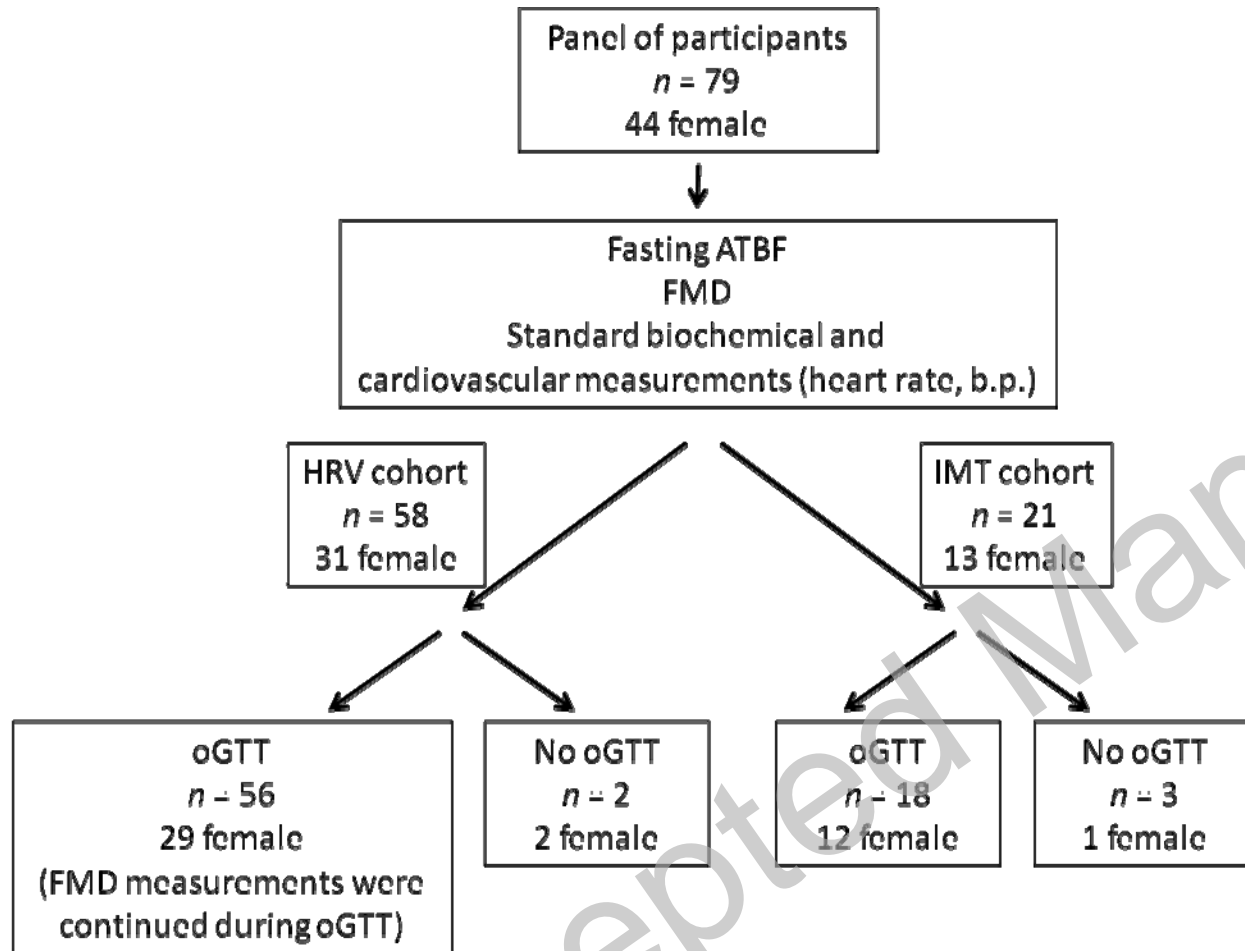
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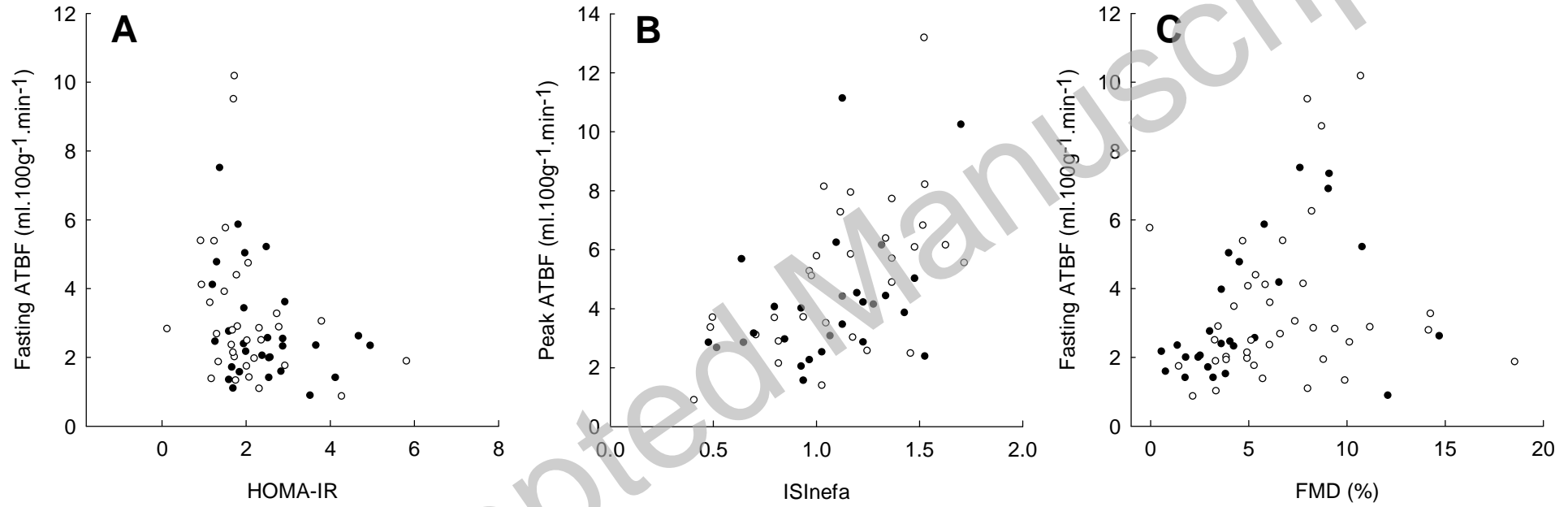
**Figure 1** Disposition of the participants amongst the various measurements made

**Figure 2** Relationships with fasting adipose tissue blood flow

Panel A, Relationship between fasting adipose tissue blood flow (ATBF) and insulin resistance, measured as HOMA-IR. One outlier for HOMA-IR (value 14.7) is not shown but is included in the statistics. The relationship was significant ( $r_s = -0.36$ ,  $P = 0.004$ ). Panel B, Relationship between peak ATBF and insulin sensitivity index for NEFA suppression (ISI<sub>nefa</sub>). The relationship was significant ( $r_s = 0.52$ ,  $P < 0.001$ ). Panel C, Relationship between fasting ATBF and flow-mediated vasodilatation (FMD). The relationship was significant ( $r_s = 0.32$ ,  $P = 0.008$ .) The relationship was affected by age (see Results section). For all panels: men, solid points; women, open points.



Funada et al. Revision 1. Figure 1



Funada et al. Revision 1. Figure 2