Association of adipose tissue blood flow with fat depot sizes and adipokines in women

Running title: ATBF, fat depot sizes, and adipokines

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Abstract

Objective: To explore possible associations between adipose tissue blood flow, adipose tissue depot sizes, and adipocyte-derived hormones (adipokines) in women. Subjects: Forty-three healthy women were divided into four groups: normal weight (n = 11) and obese (n = 11) premenopausal women and normal weight (n = 10) and obese (n = 11) postmenopausal women. **Methods:** Fasting levels of adipokines were obtained, and a single-slice computed tomography scan at the level of L4–L5 was used to estimate fat depot sizes. Adipose tissue blood flow was assessed by xenon washout while in a fasting state and after oral glucose load. We also measured glucose, insulin, and nonesterified fatty acids. **Results:** Total, subcutaneous, and visceral adipose tissue areas strongly correlated with adipose tissue blood flow (all $P < 0.001$). Circulating leptin levels strongly and inversely correlated with adipose tissue blood flow ($P = 0.001$), but this association did not remain after adjustment for BMI. Adiponectin was not associated with blood flow. **Conclusion:** Adipose tissue blood flow is closely linked to subcutaneous and visceral adipose tissue size. Further analyses are needed to determine possible mediators of this association, including mechanistic studies to assess a putative role for leptin as a significant modulator of blood flow.

Keywords: adipose tissue, blood flow, body fat distribution, leptin, women

Introduction: Adipose tissue blood flow (ATBF) is an important regulator of adipose tissue metabolism, because this tissue is highly vascularised and variations in blood flow facilitate storage and removal of lipids when needed (e.g., stress, exercise, and fasting).[1-2] Furthermore, postprandial ATBF may facilitate signaling between adipose tissue (AT) and other tissues, such as the liver and skeletal muscle.[3] The dynamics of ATBF is related to the degree of obesity, and obese subjects respond less to a mixed meal than lean subjects.[4-5] Notably, an attenuated postprandial ATBF may decrease glucose and triglyceride (TG) uptake, leading to postprandial hyperglycemia, hyperinsulinemia, and hyperlipidemia. In line with this, McQuaid et al. recently demonstrated that obese subjects have a postprandial reduction in chylomicron-TG–derived fatty acid storage.[6] This provides a possible pathophysiological basis for ectopic fat deposition and lipotoxicity and support for ATBF being a key player in the metabolic disturbances seen in obesity and obesity-related diseases. A putative regulator of ATBF is adipose tissue distribution. Regional metabolic differences in lipolysis and fatty acid uptake between upper- and lower-body fat have been described, as well as differences between subcutaneous and visceral fat depots.[7-8] It has been suggested that subcutaneous AT may act as a buffer for dietary lipids, i.e., a "metabolic sink," protecting other tissues from a lipid overflow with associated lipotoxicity.[7] Visceral adipocytes are phenotypically different from subcutaneous adipocytes,[9] and increased metabolic/cardiovascular risk is linked to visceral AT accumulation.

We recently found that ATBF is influenced by nitric oxide (NO) activity and autonomic nerve balance.[10] Putative modulators of a link between obesity, ATBF, NO activity, and autonomic activity include the adipokines leptin and adiponectin, which are secreted into the peripheral circulation from AT.[2, 11-13] These adipokines predict risk for type 2 diabetes and cardiovascular disease,[14-16] and in vitro studies have demonstrated the NO-mediated vasorelaxation effects of both leptin and adiponectin.[2, 11-13]

With menopause, women change their fat distribution to a more central (android) location.[17] On the basis of the metabolic differences between subcutaneous and visceral fat, and our recent demonstration of dysregulation of ABTF in postmenopausal overweight women,[10] we hypothesised that ATBF is closely related to the size of the abdominal fat depot. We also hypothesised that adipokines are potential independent regulators of ATBF.

Methods

Subjects

Details of the subjects included in this study were described previously.[10] Briefly, we recruited 43 healthy women and divided them into four groups: normal weight (n = 11) and obese (n = 11) premenopausal women and normal weight (n = 10) and obese (n = 11) postmenopausal women. Postmenopausal status was defined as the lack of menstrual periods for at least 12 continuous months. Basal characteristics are shown in Table 1. Exclusion criteria were pregnancy; thyroid disease; hypertension; known diabetes; cancer; previous stroke; known heart disease; psychiatric disorder; alcohol abuse; electrolyte disturbances; and treatment with glucocorticoids, systemic estrogens, or lipid-lowering agents. The premenopausal women were all studied in the follicular phase. The regional ethics committee for Northern Sweden approved the study, and written informed consent was obtained from all participants.

Clinical protocol

Clinical measurements were made at rest between 8 am and 1 pm after an overnight fast. Baseline anthropometry (weight, waist, hip, and blood pressure) was measured and an oral glucose tolerance test (OGTT) was performed as previously described.[10] A computed

tomography (CT) scan was performed on a separate day, within a month from the other measurements.

Adipose tissue blood flow

ATBF was measured by ^{133}Xe washout.[18] A dose of 1–2 MBq ^{133}Xe was injected subcutaneously into the para-umbilical area. Calculation of fasting ATBF was based on two independent readings taken 15 and 10 min before the OGTT and postprandial blood flow was calculated at 15, 30, 45, 60, 90, and 120 min after glucose loading, as previously described.[19-20]

Blood sampling and analyses

A cannula (Optiva 2 18–20; G Johnson & Johnson, New Brunswick, Canada) was inserted retrogradely into a distal forearm vein and kept patent by a continuous slow infusion of saline. The lower part of the forearm was heated in a chamber (Biomedical Engineering Department, Huddinge University Hospital, Sweden) to provide arterialised blood samples. A 10 mL aliquot of arterialised venous blood was collected twice prior to and then 15, 30, 45, 60, 90, and 120 min after glucose intake (Figure 1). At each time point, 1.5 mL of blood was placed in an EDTA (ethylenediaminetetraacetic acid) tube, immediately put on ice, and centrifuged at 4°C. The plasma was collected and stored at −80°C. The remaining blood volume was centrifuged and the serum stored at −80°C for later analyses.

Leptin and adiponectin levels were analysed with double-antibody radioimmunoassays (Linco Research, St. Louis, MO, USA). The total coefficient of variation was 4.7% at both low (2–4 ng/mL) and high (10–15 ng/mL) levels for leptin, and for adiponectin it was 15.2% at low levels (2–4 µg/mL) and 8.8% at high levels (26–54 µg/mL). Nonesterified fatty acids (NEFA) were analysed using reagent NEFA C (Wako Chemicals

GmbH) on Hitachi 912 (Roche). Insulin was analysed by a direct chemiluminiscent technique using an insulin reagent kit with a Modular E 170 immunoanalyzer (Roche). High-sensitivity C-reactive protein (CRP) and IL-6 solid chemiluminescent immunometric phase assays were performed using Immulite (Siemens). All other parameters were analysed by dry chemistry on a Vitros 950 (Ortho-Clinical Diagnostics, Raritan, NJ, USA).

Adipose tissue distribution

All participants underwent a CT examination with a General Electric (GE) LightSpeed 4 Channel CT scanner (GE, Milwaukee, WI, USA). A previously described method was used.[21-22] Image data were acquired as 5 mm slices at the L4–L5 level (120 mAs, 50 cm field of view). Anterior and lateral scanograms were used to identify the correct slice positions. The image data were used for calculation of total, subcutaneous, and visceral adipose tissue areas by using defined levels of Hounsfield units.

Statistical methods

Blood flow calculations were performed in an Excel sheet for Windows 2007 (Microsoft). The remaining statistical analyses were performed using SPSS Statistics 18 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered to be statistically significant. The Kruskal–Wallis nonparametric test was used to test for differences between groups, followed by the Mann–Whitney post-hoc test if significant differences were found. For bivariate correlation analyses, we used natural logarithm values to achieve approximate normality. Partial correlation analyses were used to assess the relation of ATBF to other parameters after adjustments were made as described in the text. Area under the curve (AUC) responses after glucose loads were estimated according to the trapezoid rule. Tertiles of adipose tissue areas were calculated using group-specific cut-off-values for pre-and postmenopausal women separately. The

homeostatic model assessment (HOMA) index was calculated as (fasting glucose [mmol/L] × fasting insulin [μU/mL])/22.5.[23]

Results

Basal characteristics

Pre- and postmenopausal obese women had significantly higher BMI, waist circumferences, waist-to-hip ratios, and intraabdominal adipose tissue areas compared to premenopausal normal weight women (Table 1). Anthropometric data were reported previously for this cohort.[10] Notably, both postmenopausal normal weight and postmenopausal obese women had an approximately 2-fold larger intraabdominal adipose tissue area compared to their premenopausal counterparts, despite similar BMI. The postmenopausal obese group had higher levels of glucose (AUC) and free fatty acids (FFA) (AUC) compared to the premenopausal groups. Fasting levels of glucose, insulin, and FFA were published previously.[10] Insulin resistance, expressed as a HOMA index, was highest in the postmenopausal obese group. Five individuals (two postmenopausal normal weight and three postmenopausal obese) had 2 h glucose levels ≥ 11.1, thus fulfilling the criteria of diabetes mellitus, despite normal fasting glucose levels.

The obese groups had higher leptin levels, and levels of adiponectin were lowest in obese premenopausal women. The ATBF (AUC) response to glucose was significantly lower in both obese groups compared to normal weight premenopausal women. Postmenopausal obese women had significantly lower ATBF (AUC) than their normal weight counterparts.

Adipose tissue blood flow and association with measures of obesity, biomarkers, and adipokines

ATBF (AUC) was significantly associated with all measures of obesity (BMI, waist, waist-to-hip ratio, total AT area, subcutaneous AT area, and intraabdominal AT area). These associations remained after adjustment for menopausal status and age (Table 2). ATBF was not associated with glucose and insulin levels. Associations were found between ATBF and FFA, and between ATBF and HOMA index; however, only the HOMA index association remained significant after adjustment for menopausal status and age. Leptin was strongly inversely associated with ATBF; the association remained after adjustment for menopausal status and age, but not after adjustment for BMI. By contrast, no association was seen between blood flow and adiponectin. Because baseline levels for ATBF differed among the groups, we also recalculated all data using centered cumulative response (CCR), but this did not significantly change the results. The highly significant negative association between leptin and ATBF and the nonsignificant association between adiponectin and ATBF are visualised with scatter plots in Figure 2a and 2b.

Adipose tissue blood during oral glucose tolerance test

ATBF was significantly lower at baseline in both obese groups compared to normal weight pre- and postmenopausal women ($P < 0.05$) (Figure 3). After glucose loading, at all time points, postmenopausal obese women had lower ATBF than premenopausal normal weight women ($P < 0.05$). The same pattern was seen between the two postmenopausal groups, except that the difference was nonsignificant at 60 and 90 min. Obese premenopausal women had significantly lower ATBF than their normal weight counterparts throughout the test ($P < 0.05$). There were no significant differences in blood flow between the two obese groups. The postmenopausal normal weight women had lower ATBF than premenopausal normal weight women at 60 and 120 min ($P < 0.05$).

Adipose tissue blood flow and association with total, subcutaneous, and visceral adipose tissue

We studied ATBF according to the amount of adipose tissue in each depot (expressed as tertiles with group-specific cut-offs) (Figure 4a–c). In each depot, we found highly significant differences in ATBF between the lowest and medium tertiles ($P < 0.01$) and between the lowest and highest tertiles ($P < 0.01$). We also found significant differences in ATBF between the medium and highest tertiles ($P < 0.05$) for subcutaneous and intraabdominal AT. In the subcutaneous depot, there was a trend (nonsignificant) toward a blood flow difference between the medium and highest tertile of the AT area.

Discussion

We found a strong association between ATBF and subcutaneous, as well as visceral, adipose tissue areas. This suggests that total adipose tissue mass, rather than specific sites of fat accumulation, is linked to dysregulation of ATBF. It was previously shown that both fasting ATBF and ATBF responsiveness to nutrients[5] are reduced in obesity, but the relationship of ATBF with different depots of abdominal fat had, to our knowledge, not been explored. The importance of our findings is underscored by recent data from obese study participants showing a reduced ability to store fat in adipose tissue after meals.[6] The absence of a difference between adipose tissue accumulation and ATBF in different fat depots suggests that subcutaneous adipose tissue accumulation has a relatively greater role than visceral adipose tissue in ectopic fat deposition and lipotoxicity, due to its larger volume. Our data therefore support the hypothesis of subcutaneous fat acting as the primary metabolic "sink," protecting other organs from excessive postprandial lipid levels.

Visceral fat depot size has been strongly linked to metabolic and cardiovascular diseases;[24] differences in insulin action, gene expression, metabolic responses, and adipokine

secretion[25-26] among fat depots could explain this difference. It has also been proposed that extrinsic factors, including depot-specific blood flow and/or innervation,[27] could contribute to distinct gene expression patterns and metabolic profiles in adipocytes in different anatomical regions. It is possible that lipid and glucose uptake in adipose tissue depots is regulated differently in people with obesity. Studies using positron emission tomography have elegantly demonstrated differences in perfusion and insulin-stimulated glucose uptake between visceral and abdominal subcutaneous adipose tissue in obese people.[28-29] Women are more protected from cardiovascular events than men, until their body fat distribution changes with menopause and takes on a more android (male) distribution.[30-31] If the menopausal transition also has differential effects on lipid and glucose uptake in different fat depots, this would clearly be an area of interest for further studies.

Notably, total perfusion through both abdominal subcutaneous and visceral depots can be up to 900 mL/min (~18% of average cardiac output) in obese people.[28] Thus, ATBF not only has an important impact on cardiac work load, but is also a powerful regulator of adipose tissue metabolism. Although the ATBF response to nutrient intake is thought to be of importance in the regulation of metabolism by facilitating signaling between adipose tissue and other tissues, such as skeletal muscle and liver,[3] the physiological significance of and mechanisms behind postprandial hyperaemia are not fully understood. Importantly, the rapid postprandial increase in ATBF and the rapid insulin-mediated suppression of adipocyte lipolysis changes the direction of FFA flux in tissues.[32] Related to this, we found an inverse correlation between insulin resistance and ATBF.

We also found a strong inverse association between circulating levels of leptin and ATBF. Hyperleptinemia has been linked to an increased risk of later development of type 2 diabetes and cardiovascular disease, including myocardial infarction and stroke.[33-35] Several mechanisms have been suggested to be behind these associations, including mediation of

platelet aggregation, increased sympathetic activity, and stimulation of inflammation.[36-37] The regulatory effects of leptin on vascular endothelial cells in rodents is also well established,[2, 11, 38] but less is known about putative effects on human endothelial function and blood flow regulation in vivo. The nonsignificant results after adjustments for BMI in this study should be interpreted with caution, because leptin and measures of obesity are strongly correlated and it is debatable whether models exploring the effect of leptin should be adjusted for obesity. . On the other hand we cannot rule out that the association between fat mass and ATBF could be determined by other factors than leptin. Furthermore, the links between leptin and later risk of diabetes, as well as cardiovascular outcomes, have been found mainly in males.[33-35] Studies are thus needed on possible differences between males and females regarding the vascular effects of leptin. This includes detailed mechanistic studies.

We found no association between adiponectin and ATBF. This suggests that adiponectin does not play a major role in ATBF regulation. In contrast, hypoadiponectinemia is linked to endothelial dysfunction in peripheral arteries,[39] and plasma total adiponectin concentrations are inversely related to the risk of myocardial infarction.

Our study has some limitations. We found a very high correlation between the size of subcutaneous and intraabdominal depots ($R = 0.77$, $P < 0.001$), in line with previous studies,[40-41] making it difficult to separate the effects of the different depots. Another weakness is the cross-sectional design, which makes it impossible to explore causality.

In summary, we found highly significant correlations between ATBF and total, visceral, and subcutaneous adipose tissue areas. We also show a strong inverse association between circulating levels of leptin and ATBF, although this was nonsignificant after adjustment for BMI. Further mechanistic studies are needed to assess the clinical significance of our findings.

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Conflicts of Interest The authors have no conflicts of interest to disclose.

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Figure legends

Figure 1. Study design.

Figure 2. Associations between circulating levels of leptin (a), adiponectin (b), and adipose tissue blood flow (area under curve).

Figure 3. Adipose tissue blood flow in the four groups of women during oral glucose tolerance test. Data presented as mean ± standard error of the mean (SEM). Pre NW, premenopausal normal weight; Post NW, postmenopausal normal weight; Pre obese, premenopausal obese; Post obese, postmenopausal obese.

Figure 4. Box plots of adipose tissue blood flow according to tertiles of total (a), subcutaneous (b), and intraabdominal (c) adipose tissue areas with separate group-specific cut-off values for pre- and postmenopausal women. The nonparametric Kruskal–Wallis test was used to determine whether means differed, followed by the Mann–Whitney post-hoc test if differences were significant. *$P < 0.05$; **$P < 0.01$; n.s., nonsignificant.

Table 1. Age, anthropometric data, adipose tissue distribution, and selected laboratory parameters of the study population.

	Premenopausal NW (*n*=11)	Premenopausal obese (*n*=11)	Postmenopausal NW (*n*=10)	Postmenopausal obese (*n*=11)	Significance
Age (y)	27 (24–31)	28 (25–32)	62 (58–67)	59 (55–63)	b, c, d, f
BMI (kg/m^2)	21 (20–22)	31 (28–34)	23 (22–25)	31 (30–33)	a, c, d, e
Waist (cm)	75 (70–79)	101 (91–111)	85 (78–92)	107 (98–116)	a, b, c, d, e
Waist–to–hip ratio	0.79 (0.75–0.83)	0.88 (0.77–1.00)	0.91 (0.78–1.04)	0.99 (0.87–1.11)	c
Total AT area (cm^2)	160 (140–180)	500 (430–560)	280 (210–340)	560 (460–660)	a, b, c, d, e
Subcutaneous AT area (cm^2)	130 (110–140)	420 (360–480)	190 (150–240)	390 (300–480)	a, b, c, d, e
Intraabd AT area (cm^2)	23 (18–27)	76 (57–95)	60 (40–79)	150 (120–180)	a, b, c, e, f
AUC glucose (min × mmol/L)	1100 (1000–1200)	1100(1000–1100)	1200 (1100–1300)	1200 (1100–1300)	c, f
AUC insulin (min × mU/L)	4800 (3600–6000)	6600 (3600–9700)	3900 (2700–5100)	5900 (4400–7400)	n.s
AUC FFA (min × mmol/L)	13 (10–15)	20 (15–24)	22 (15–28)	28 (22–33)	a, b, c, f
HOMA–index	1.4 (1.0–1.7)	1.9 (1.1–2.6)	1.1 (0.7–1.4)	2.8 (2.0–3.6)	c, e
Leptin (ng/mL)	9 (6–13)	31 (22–40)	11 (7–14)	29 (24–35)	a, c, d, e
Adiponectin (µg/mL)	14 (10–17)	12 (8.0–16)	20 (17–22)	16 (11–20)	b, d
AUC ATBF (min × mL/min/100g)	760 (500–1000)	310 (220–400)	520 (270–770)	260 (200–310)	a, c, f

Values are means (95% confidence intervals). NW, normal–weight; Intraabd AT area, intraabdominal adipose tissue area; AUC, area under curve; FFA, free fatty acids; HOMA, homeostasis model assessment; ATBF, adipose tissue blood flow. Kruskal–Wallis and Mann–Whitney post–hoc test, letters indicating significant differences (P<0.05) between groups: a, premenopausal obese versus premenopausal NW; b, postmenopausal NW versus premenopausal NW; c, postmenopausal obese versus premenopausal NW; d, postmenopausal NW versus premenopausal obese; e, postmenopausal obese versus postmenopausal NW; f, postmenopausal obese versus premenopausal obese; n.s., nonsignificant.

Table 2. Associations between adipose tissue blood flow and measures of adipose tissue, biomarkers, and adipokines.

	AUC adipose tissue blood flow	AUC adipose tissue blood flow (adjusted for age and menopausal status)	AUC adipose tissue blood flow (adjusted for age, menopausal status and BMI)
Age	−0.21	—	—
BMI	−0.71**	−0.71**	—
Waist	−0.67**	−0.64**	0.22
Waist/hip	−0.41**	−0.36*	−0.25
Total AT area	−0.75**	−0.73**	−0.32
Subcutan AT area	−0.73**	−0.72**	−0.30
Intraabd AT area	−0.69**	−0.70**	−0.27
AUC glucose	0.10	0.21	0.15
AUC insulin	−0.14	−0.18	0.00
AUC FFA	−0.36*	−0.29	0.06
HOMA-index	−0.48**	−0.47**	−0.19
Leptin	−0.68**	−0.69**	−0.23
Adiponectin	0.06	0.18	0.03

AUC, area under the curve; AT, adipose tissue; HOMA, homeostasis model assessment. *p < 0.05, **p < 0.01. Pearson correlation coefficients (AUC AT blood flow) and partial correlation analysis (r values after adjustment).

Figure 1.

Figure 1

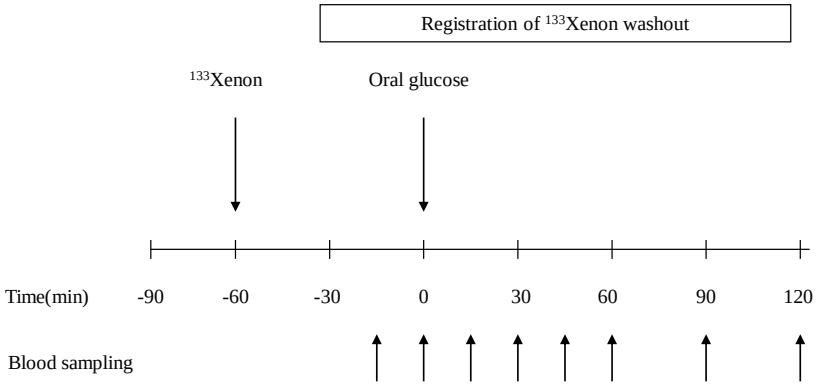

Figure 2a

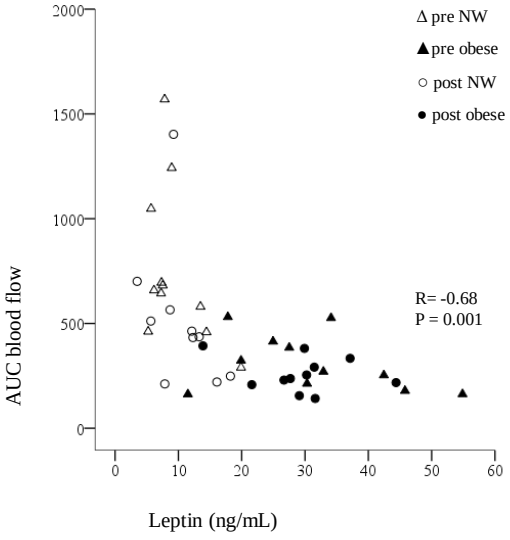

Figure 2b

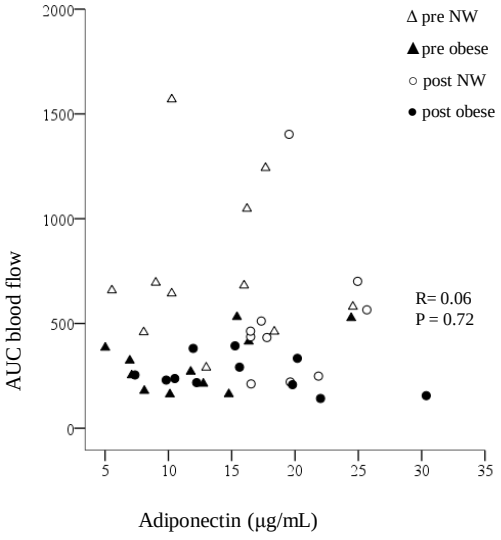

Figure 3

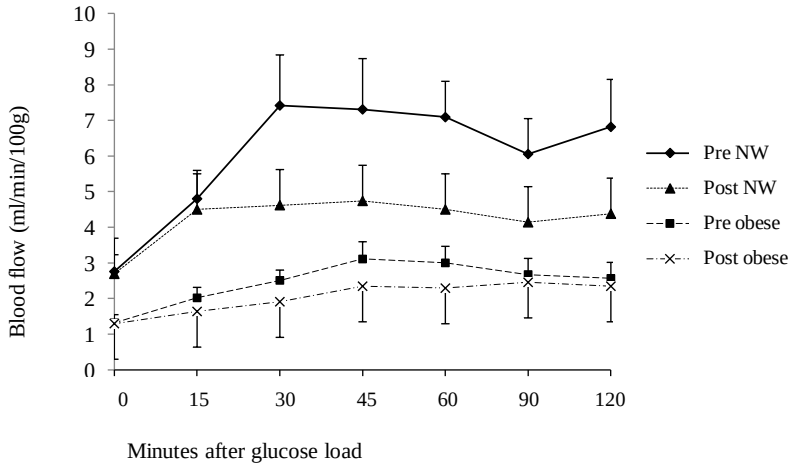

Fig 4a (tertiles of total AT area)

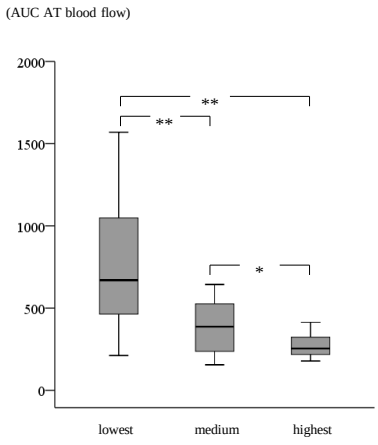

Fig 4b (tertiles of subcut AT area)

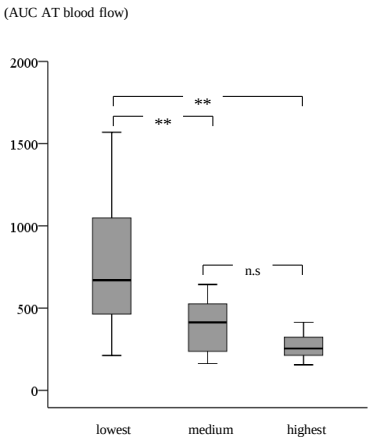

Fig 4c (tertiles of intraabd AT area)