# Transcriptional Control of Human Adipose Tissue Blood Flow

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**Adipose tissue is highly vascularized and expresses several genes involved in vasodilatory and vasoconstrictive regulation. We took a transcriptional approach to study the relationships between adipose tissue blood flow (ATBF) and genes involved in vasoactive processes. As ATBF is impaired in obesity, we tested whether body weight interfered with the transcriptional regulation of ATBF. The mRNA content (real-time PCR) of 26 genes was quantified in subcutaneous adipose tissue biopsies from 28 healthy men with a wide range of BMI. ATBF was measured by 133Xe washout. None of the transcripts was related to fasting ATBF (ATBFF). However, the expression levels of two transcripts involved in vasodilation (natriuretic peptide receptor A/guanylate cyclase A (*NPRA*) and endothelial nitric oxide synthase (*eNOS*)) were positively associated with postprandial ATBF ($r = 0.53$ and $r = 0.55$, $P < 0.01$, respectively). Although BMI was negatively related to the mRNA content of *NPRA* and *eNOS* ($r = -0.78$ and $r = -0.63$, $P < 0.01$, respectively), the strong associations found between postprandial ATBF and the two transcripts were not affected by obesity. Several genes were subject to coordinated regulation of expression. This study demonstrates for the first time that ATBF responsiveness to nutrient intake is related to the transcription of two genes expressed in adipose tissue and directly involved in vasodilatory actions (*eNOS* and *NPRA*), suggesting that part of the regulation of ATBF is at a transcriptional level. Interestingly, these associations were not secondary to changes in BMI. We also found that certain genes involved in the regulation of ATBF are subject to coordinate regulation of expression suggesting physiological autoregulation.**

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## INTRODUCTION

White adipose tissue is highly vascularized. In fact, there is an important interrelationship between blood flow and adipose tissue metabolism, as blood flow facilitates the storage and removal of lipids when needed (stress, fasting/feeding, exercise) (1).

It has been recognized that adipose tissue blood flow (ATBF) is not constant. Thus, there are several factors known to influence ATBF, such us exercise, skin temperature, and oxygen partial pressure (2,3). ATBF also seems to exhibit its highest degree of modulation in response to food intake, illustrated by either glucose (4,5) or mixed-meal ingestion (6,7), whereas fat alone does not elicit a blood flow response (8). Postprandial enhancement of ATBF may have importance in metabolic physiology as the extraction of plasma triglycerides increases with increasing blood flow (1). Furthermore, this ATBF response to nutrient intake may facilitate signaling between adipose tissue and other tissues, such as skeletal muscle and liver (9). Nitric oxide (NO), β-adrenergic activation, and the angiotensin system have been reported to be involved in the regulation of fasting and/or postprandial ATBF (10–13).

However, both fasting ATBF (ATBFF) and its responsiveness to nutrients are reduced in obesity (14,15). Obesity is typically associated with a number of metabolic derangements including insulin resistance, hypertriglyceridemia, and low high-density lipoprotein cholesterol concentrations. It is tempting to suggest that these disorders might be related to alterations in tissue perfusion (16). In this context, previous studies have reported that the degree of insulin sensitivity seems to be closely related to ATBF responsiveness to nutrients (14,17).

Despite increasing understanding of the regulation of ATBF (reviewed by (18), little is known about the transcriptional control of genes that may be involved in determining ATBF.

There is, however, one study from Tan *et al.* (7) where the impact of the Pro12Ala peroxisome proliferator-activated receptor-γ (PPAR-γ) polymorphism on integrative tissue-specific physiology and therefore, on adipose tissue metabolism and ATBF was investigated. Interestingly, Tan *et al.* (7) demonstrated that ATBF was affected by the Pro12Ala polymorphism. Thus, a higher ATBF was observed in the Ala12 carriers (suggested to be protected against type 2 diabetes (19–23)) compared with the Pro12 homozygotes (7), which also supports

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the previously described relationship between ATBF and insulin sensitivity (14,17).

These findings also raised the possibility that part of the regulation of ATBF could be at a transcriptional level. In this context, and as adipose tissue expresses a large number of genes involved in vasodilatory and vasoconstrictive regulation, the aim of this study was to provide novel insights into the relationships between ATBF and the transcriptional control of genes involved in several vasoactive processes. As obesity has been reported to impair the regulation of ATBF, we have also investigated the influence of body weight in the expression levels of these genes and, therefore, the effects of obesity on the transcriptional control of ATBF.

## METHODS AND PROCEDURES

This study was approved by the Oxfordshire Clinical Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. All participants gave their written informed consent prior to participation in this study.

### Subjects

Twenty-eight healthy men covering a wide range of BMI were recruited from the Oxford Biobank (7). They were initially recruited for a study of physiological phenotypes of the PPAR-γ Pro12Ala polymorphism including 24 participants (7) and 4 additional subjects from the same cohort and thus, consisted of 14 Ala12 heterozygotes and 14 Pro12 homozygotes. None of the subjects was taking any medication.

The subjects attended the Clinical Research Unit for a full-day metabolic investigation as described previously (7). Body fat was measured by bioimpedance using a Bodystat 500 (Bodystat, Douglas, UK). All the biochemical analysis were described previously (7,24).

During the study day, ATBF was measured by 133Xe washout (25). Three measures of ATBF were used: ATBFF, which was measured before the intake of a mixed meal containing 40 g carbohydrate and 40 g fat, postprandial ATBF averaged over 6 h following the meal (ATBFAVE) and finally, the maximum response of ATBF to the meal intake (ATBFRES) (17).

### Adipose tissue samples

After local anesthesia with 1% lignocaine, adipose tissue biopsies were taken from subcutaneous abdominal depots using a 12-gauge needle between 5 and 6 h after ingestion of the mixed meal (postprandial period). These tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C for later RNA extraction and quantification.

### RNA extraction and reverse transcription

Total RNA from 100 to 650 mg of adipose tissue biopsies was extracted using TrizolR reagent (GIBCO-Life Technologies Inc., Grand Island, NY) according to manufacturer's instructions. To avoid contamination with genomic DNA, DNA digestion and inactivation was assessed using the DNase I (RNase-free) kit (Ambion, Austin, TX). Quantification of RNA was determined using a Nanodrop ND-1000 spectrophotometer (Labtech Technologies UK, Ringmer, UK) (OD260/OD280 and OD260/OD230)) and quality assessment was achieved using the Agilent 2100 Bioanalyzer (Agilent Technologies UK, Stockport, UK). Between 0.5 and 1 µg of RNA were reverse transcribed to cDNA using random Hexamers Primers and Invitrogen Super Script III First-Strand Synthesis System (Invitrogen, Carlsbad, CA). The cDNA reaction was incubated for 10 min at 25 ºC followed by 50 min at 50 ºC and heated 5 min at 85 ºC (standard conditions for random hexamers).

### Real-time PCR analysis

For the real-time quantitative PCR analyses, 4.5 µl of 1 in 100 or 1 in 20 dilution of cDNA per reaction were used in a final reaction volume of 10 µl. Twenty-six genes were analyzed (**Supplementary Data S1** online) using predesigned TaqMan Assays-on-Demand (Applied Biosystems, Foster City, CA). The usage of adipose tissue biopsies allowed us to examine the expression of a large number of genes expressed in adipocytes, the most predominant cells in adipose tissue, as well as in other cell types also present in adipose tissue such as preadipocytes, endothelial cells, monocytes, or macrophages. Taqman Universal Master Mix was also provided by Applied Biosystems. The reaction conditions were followed according to the manufacturer's instructions. Amplification and detection of specific products were performed using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). All the samples were analyzed in quadruplicate.

Adipose tissue expression levels of each gene were normalized by three different housekeeping genes: *UBC* (ubiquitin C), *PPIA* (cyclophilin A), and *RPLP0* (ribosomal protein, large, P0), all obtained from Applied Biosystems and chosen as most stable out of a panel of 16 different genes (M.J. Neville, unpublished data).

A standard curve created with serial dilutions of a calibrator sample (a pool of all the samples used in the aforementioned experiments) was used to generate reaction efficiency values (E=10(−1/slope); slope generated by ABI software regression). $C_t$ values (the cycle where the emitted fluorescence signal is significantly above background levels and is inversely proportional to the initial template copy number) were generated by the ABI software. Expression values were calculated by the ΔCT transformation method ($\Delta CT = E[\min C_t - \text{sample} C_t]$) (26). Finally, the relative expression level of each gene was calculated as ΔΔCT ($\Delta C_t$ target gene/$\Delta C_t$ average of housekeeping genes) (26).

### Calculations and statistical analysis

133Xenon counts were recorded continuously, and blood flow was calculated as described previously (25). Three different ATBF measurements were used: ATBFF based on two independent readings taken at −30 and at time point 0 before the intake of the mixed meal; ATBFAVE, which is the area under the curve of the entire postprandial ATBF readings (nine readings over 360 min) and finally ATBFRES, calculated by the difference between fasting and postprandial peak blood flow. Peak blood flow values were calculated as the mean of the three contiguous points (including the maximum) that give the highest mean value (17).

Statistical analysis was performed using the SPSS/Windows 14.0 (SPSS, Chicago, IL) and statistical significance was set at $P < 0.05$ for all tests. Variables were presented as mean ± s.e.m.

BMI was used as a variable in two ways. In correlation analyses, it was used as a continuous variable. This included partial correlations between variables controlling for BMI (see below). In addition, BMI was used as a categorical variable, by dividing the subjects into two groups at the median BMI of 26.9 kg/m$^2$. For simplicity, subjects with a BMI above this value will be referred to as "overweight/obese" whereas those below will be referred to as "lean."

To check for normal distribution, two different methods (Kolmogorov–Smirnov and Shapiro–Wilk tests) were used. Differences between BMI groups and between PPAR-γ Pro12Ala and Pro12Pro groups were compared using either a Student's *t*-test or Mann–Whitney *U*-test, depending on the distribution followed by the data. Relationships between variables were analyzed by calculating Spearman's rank correlation coefficients. To perform partial correlations, data were logarithmically transformed if needed. Univariate General Linear Models were also carried out to corroborate results obtained from partial correlations controlled by BMI. To visualize the relationships between transcripts the Ward's Algorithm Clustering method with Euclidean distance measurement was carried out using the Genex 4.2.2 software (MultiD Analyses AB, Göteborg, Sweden).

## RESULTS

### Gene expression in PPARG Pro12Pro and Pro12Ala carriers

Only two of the 26 transcripts analyzed in this study were different between the Ala12 carriers and the Pro12 homozygotes.

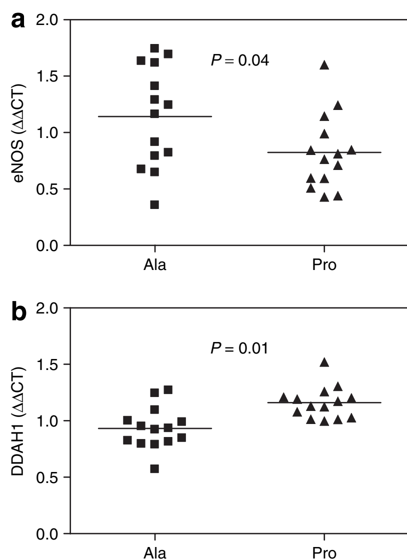


**Figure 1** Differential expression of eNOS and DDAH1 in subcutaneous adipose tissue of PPAR-γ Pro12Pro and Pro12Ala carriers. Relative expression levels of eNOS and DDAH1were presented as ΔΔCT (mean ± s.e.m.). DDAH1, dimethylarginine dimethylaminohydrolase 1; eNOS, endothelial nitric oxide synthase; PPAR-γ, peroxisome proliferator-activated receptor-γ.

Thus, endothelial nitric oxide synthase (*eNOS*) gene expression was significantly higher in the Ala12 group compared with the Pro12 group ($P = 0.04$). In contrast, dimethylarginine dimethylaminohydrolase 1 (*DDAH1*) mRNA content was significantly lower in the Ala12 subjects compared with the Pro12 group ($P = 0.01$) (**Figure 1**).

### Lean and overweight/obese subjects: characteristics

Because of the relative absence of differences in gene expression between Ala and Pro subjects, we decided to combine all the subjects into one group and then divide them into two groups according to their BMI, as described in Calculations and Statistical Analysis. Thus, of the 28 subjects, 14 were classified as overweight/obese (eight Pro12 homozygotes and six Ala12) and 14 as normal weight (six Pro12 homozygotes and eight Ala12 carriers). There were no significant differences in age, triglycerides, and total cholesterol levels between these two groups. However, fasting glucose and insulin levels together with blood pressure measurements (systolic and diastolic) were significantly increased in the overweight/obese group ($P = 0.03$, $P = 0.002$, $P = 0.03$, and $P = 0.008$, respectively). High-density lipoprotein cholesterol levels were slightly lower in the obese subjects, although this did not reach statistical significance ($P = 0.08$) (**Table 1**).

ATBFF was slightly lower in the overweight/obese group than in the lean subjects, although it did not reach statistical significance. However, a significantly lower postprandial averaged ATBF (ATBFAVE) was observed in the overweight/obese subjects when compared with the lean group ($P = 0.03$). Regarding the maximum response of ATBF to a mixed meal (ATBFRES), the lean subjects exhibited an increase (>37% baseline), although no response was observed in the overweight/obese group. When this response was compared between lean and overweight/obese subjects, a borderline difference was found between groups ($P = 0.053$) (**Table 1**).

**Table 1 Characteristics of subjects**

| | Lean ($n = 14$) | Overweight/obese ($n = 14$) | *P* value |
|---|---|---|---|
| Age | 45.4 ± 1.6 | 46.1 ± 1.6 | ns |
| BMI (kg/m$^2$) | 24.3 ± 0.4 | 31.3 ± 0.6 | $P < 0.0001$ |
| Fasting insulin (pmol/l) | 75.0 ± 6.1 | 123.2 ± 13.1 | $P < 0.01$ |
| Fasting glucose (mmol/l) | 5.2 ± 0.1 | 5.6 ± 0.1 | $P < 0.05$ |
| Fasting triglycerides (µmol/l) | 1,174 ± 195 | 1,383 ± 173.6 | ns |
| Total cholesterol (mmol/l) | 5.4 ± 0.2 | 5.6 ± 0.3 | ns |
| HDL-cholesterol (mmol/l) | 1.26 ± 0.07 | 1.10 ± 0.05 | $P = 0.08$ |
| Systolic blood pressure (mm Hg) | 121.4 ± 1.9 | 128.9 ± 2.6 | $P < 0.05$ |
| Diastolic blood pressure (mm Hg) | 77.0 ± 1.7 | 85.4 ± 2.4 | $P < 0.01$ |
| ATBFF (ml/min × 100 g of tissue) | 2.4 ± 0.2 | 2.1 ± 0.2 | ns |
| ATBFAVE (ml/min × 100 g of tissue) | 2.7 ± 0.2 | 2.1 ± 0.2 | $P < 0.05$ |
| ATBFRES (ml/min × 100 g of tissue) | 0.9 ± 0.3 | 0.002 ± 0.2 | $P = 0.053$ |

Values presented as mean ± s.e.m.
ATBFF, fasting adipose tissue blood flow; ATBFAVE, postprandial averaged adipose tissue blood flow; ATBFRES, adipose tissue blood flow response to a meal; HDL, high-density lipoprotein.

Finally, fifteen of the twenty-six transcripts analyzed in this study showed different levels of expression between the lean and the overweight/obese subjects (**Supplementary Data S2** online).

### Associations between transcripts and ATBF

None of the transcripts studied was related to ATBFF. However, the mRNA content of two transcripts involved in vasodilation (*eNOS* and natriuretic peptide receptor A/guanylate cyclase A (NPRA)) were positively associated with ATBFAVE ($r = 0.53$, $P < 0.01$ for *eNOS* and $r = 0.55$, $P < 0.01$ for *NPRA*) (**Table 2**).

Both transcripts, *NPRA* and *eNOS*, were negatively related to BMI ($r = -0.78$, $P < 0.001$, and $r = -0.63$, $P < 0.001$, respectively). A significant decrease in *eNOS* and *NPRA* mRNA content was observed in overweight/obese subjects ($P < 0.001$ for both transcripts) (**Supplementary Data S2** online). When partial correlation coefficients were calculated using BMI as covariate, the significant association between ATBFAVE and the previously mentioned genes remained significant ($r = 0.47$, $P < 0.05$ for *NPRA*, and $r = 0.53$, $P < 0.01$ for *eNOS*) (**Figure 2a,b**). Moreover, when general linear model/univariate was performed with obesity as fixed factor, the model was still significant ($P = 0.056$ for *NPRA*, and $P = 0.017$ for eNOS). These results suggest that ATBFAVE is regulated at a transcriptional level and that the associations observed between ATBFAVE and the mRNA content of *NPRA* and *eNOS* are independent of obesity.

**Table 2 Correlation analyses between ATBFRES and gene expression (ΔΔCT)**

| | ATBFAVE (ml/min × 100 g) | |
|---|---|---|
| Gene expression | Spearman's rho | Significance (two-tail) |
| *NPRA* | 0.53 | $P = 0.005$ |
| *eNOS* | 0.55 | $P = 0.004$ |

ATBFAVE, postprandial averaged adipose tissue blood flow; *eNOS*, endothelial nitric oxide synthase; *NPRA*, natriuretic peptide receptor A/guanylate cyclase A.

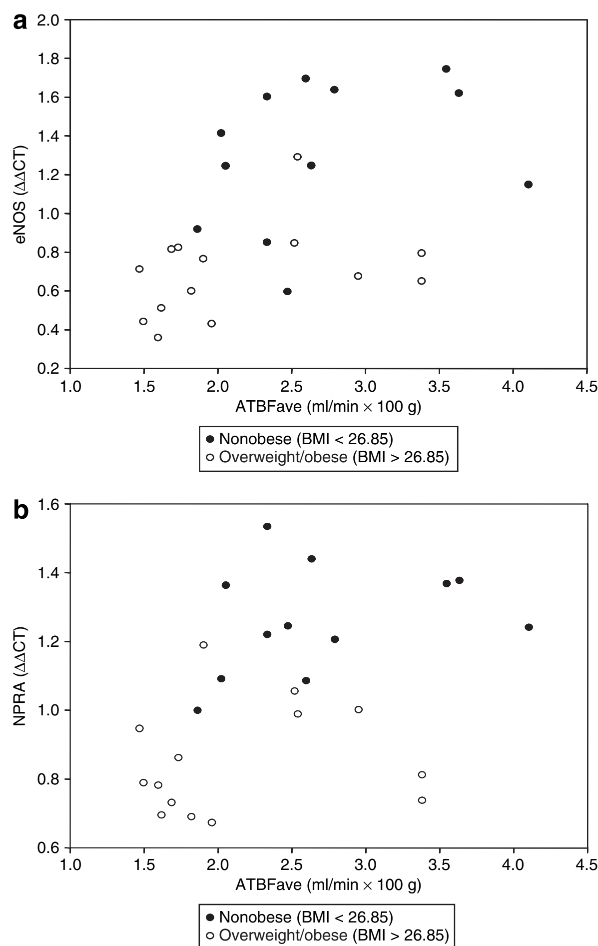


**Figure 2** Correlation analysis between averaged postprandial ATBF (ATBFAVE) (ml/min × 100 g) and *eNOS* (**a**) and *NPRA* (**b**) mRNA content (ΔΔCT) in subcutaneous adipose tissue of lean and overweight/obese subjects. ATBF, adipose tissue blood flow; ATBFAVE, postprandial averaged adipose tissue blood flow; eNOS, endothelial nitric oxide synthase; *NPRA*, natriuretic peptide receptor A/guanylate cyclase A.

Five different genes were found to be associated with ATBFRES. The mRNA content of adrenergic receptor β-1, angiotensin II receptor, type 1 (*AGTR1*), endothelin receptor type A (*EDNRA*), protein arginine methyltransferase 1, and vascular endothelial growth factor were positively related to the maximum response of ATBF to a meal (Table 3). When partial correlation coefficients with BMI as covariate were calculated, all these relationships became nonsignificant (Figure 3a,b). Several borderline associations were also

**Table 3 Correlation analyses between ATBFAVE and gene expression (ΔΔCT)**

| | ATBFRES (ml/min × 100 g) | |
|---|---|---|
| Gene expression | Spearman's rho | Significance (two-tail) |
| *ADRB1* | 0.43 | $P = 0.02$ |
| *AGTR1* | 0.45 | $P = 0.02$ |
| *EDNRA* | 0.45 | $P = 0.02$ |
| *PRMT1* | 0.42 | $P = 0.03$ |
| *VEGF* | 0.40 | $P = 0.04$ |
| *ADIPOQ* | 0.37 | $P = 0.06$ |
| *MCP1* | −0.35 | $P = 0.07$ |
| *eNOS* | 0.35 | $P = 0.08$ |
| *iNOS* | 0.34 | $P = 0.08$ |

*ADRB1*, adrenergic receptor β-1; *ADIPOQ*, adiponectin; *AGTR1*, angiotensin II receptor, type 1; ATBFRES, adipose tissue blood flow response to a meal; *eNOS*, endothelial nitric oxide synthase; *EDNRA*, endothelin receptor type A; *iNOS*, inducible nitric oxide synthase; MCP1, monocyte chemoattractant protein-1 (*CCL2*); *VEGF*, vascular endothelial growth factor.

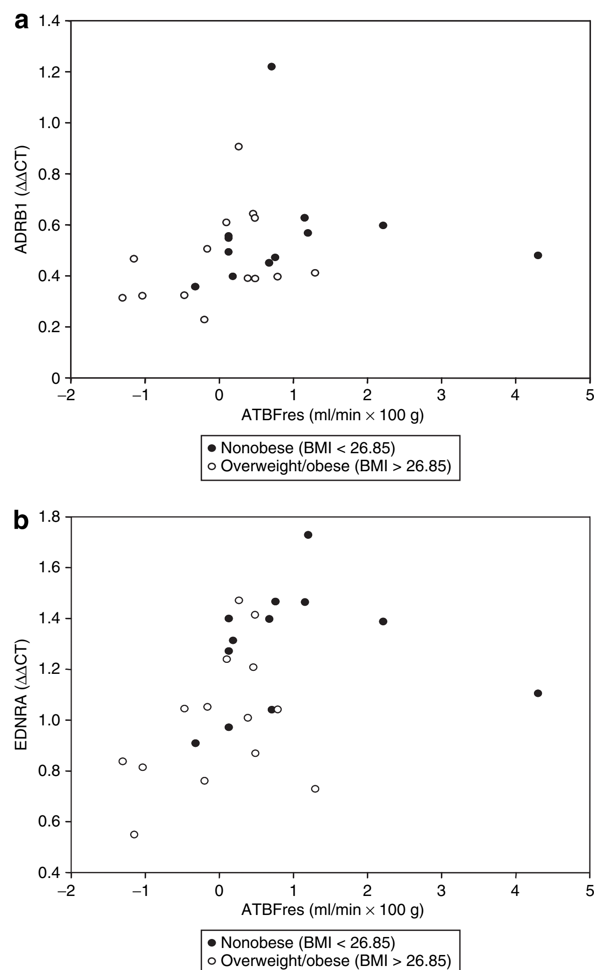


**Figure 3** Correlation analysis between ATBFRES (ml/min × 100 g) and *ADRB1* (**a**) and *EDNRA* (**b**) mRNA content (ΔΔCT) in subcutaneous adipose tissue of lean and overweight/obese subjects. *ADRB1*, adrenergic receptor β-1; ATBFRES, adipose tissue blood flow response to a meal; *EDNRA*, endothelin receptor type A.

found; the expression of two genes involved in the NO system (*eNOS* and inducible nitric oxide synthase (*iNOS*)) and adiponectin was positively associated with ATBFRES, while monocyte chemoattractant protein-1 (*CCL2*) (*MCP1*) was negatively related (**Table 3**). Again, when partial correlation coefficients with obesity as covariate were calculated, all these associations disappeared.

### Relationships between transcripts

The level of expression of the genes analyzed varied over a wide range (**Supplementary Data S1** online). Adipose tissue is comprised of adipocytes as well as many additional cell types such as preadipocytes, endothelial cells, pericytes, monocytes, macrophages, and others. In this sense, it has been demonstrated that many of the differences in gene expression in adipose tissue depot include changes in genes typically expressed by the stromal vascular cells (27). Because of that, the differences observed in gene expression could be due, at least in part, to the different sources of expression. Despite this, there were clear relationships between the expressions of genes, indicative of common regulatory mechanisms.

The gene expression of *NPRA* and *eNOS* (both positively related to ATBFAVE) was strongly related to each other ($r = 0.72$, $P < 0.001$). This association remained statistically significant when partial correlations controlled by BMI were calculated ($r = 0.52$, $P < 0.01$). The expression of *NPRA* and natriuretic peptide receptor C/guanylate cyclase C (*NPRC*), both atrial natriuretic peptide receptors, was negatively related to each other ($r = -0.43$, $P < 0.05$) suggesting opposite regulatory mechanisms. Thus and in contrast with the negative association found between *NPRA* and BMI ($r = -0.78$, $P < 0.001$), *NPRC* was positively related to obesity ($r = 0.40$, $P < 0.05$). However, when partial correlations were carried out using BMI as covariate, the aforementioned association between both receptors disappeared.

NPRA and adrenergic receptor β-2 (*ADRB2*) were strongly associated to each other ($r = 0.67$, $P < 0.001$). Furthermore, *ADRB2* and *eNOS* were also positively related to each other ($r = 0.60$, $P < 0.001$), and this association remained almost significant when controlled by BMI ($P = 0.07$). *EDNRA*, which was positively related to the response of ATBF to a meal, was also positively related to *NPRA*, *eNOS*, and *ADRB2* ($r = 0.55$,

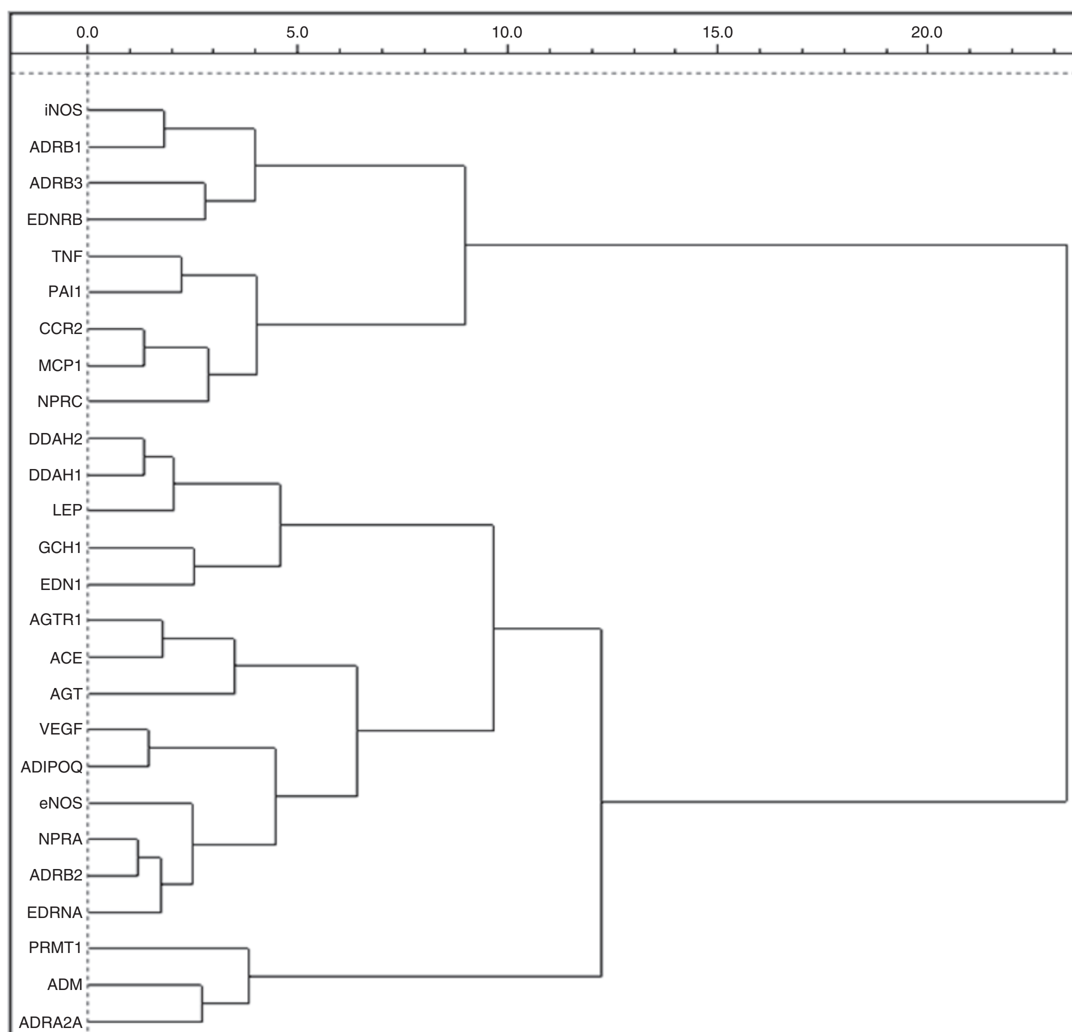


**Figure 4** Clustering model of relationships between transcripts. Ward's algorithm clustering method was performed.

*P* < 0.01; *r* = 0.50, *P* < 0.01; and *r* = 0.64, *P* < 0.001, respectively). The relationship between expression levels was further analyzed by clustering analysis, and it was observed that *NPRA*, *eNOS*, *ADRB2*, and *EDNRA* clustered together, indicating a coordinated regulation of expression (**Figure 4**). Interestingly, adiponectin, suggested to have protective effects in vascular walls, and vascular endothelial growth factor, involved in angiogenesis, were strongly related to each other (*r* = 0.74, *P* < 0.001), and this association remained significant after correcting by BMI (*P* < 0.001). Both genes also clustered with the previously mentioned genes (*eNOS*, *NPRA*, *ADRB2*, and *EDNRA*) (**Figure 4**).

Opposite actions of the Endothelin-1 (*EDN1*) and NO synthase system have been described. In this context, *EDN1* gene expression was negatively related to that of *eNOS* (*r* = −0.56, *P* < 0.01); however, this association largely disappeared after adjusting by BMI (partial correlations) (*P* = 0.06) suggesting that the association between both transcripts could be secondary to differences in BMI, especially because these genes were differently expressed in lean and overweight/obese subjects. In fact, *EDN1* mRNA levels were significantly higher in overweight/obese subjects whereas *eNOS* mRNA content was significantly decreased in the overweight/obese group (**Supplementary Data S2** online). *EDN1* was also negatively related to *NPRA* (*r* = −0.49, *P* < 0.01), and this association also disappeared when controlled by BMI. The expression pattern of three members of the angiotensin system, *AGTR1*, angiotensin-converting enzyme (*ACE*), and angiotensinogen were also studied. Unsurprisingly, all these genes clustered together (**Figure 4**), but *EDN1* was also found in this cluster (**Figure 4**).

*DDAH1* and dimethylarginine dimethylaminohydrolase2 (*DDAH2*), both involved in the elimination of asymmetric dimethylarginine (ADMA) (endogenous inhibitor of NO synthase) clustered together (**Figure 4**). Furthermore, *DDAH2* was positively and strongly related to protein arginine methyltransferase 1 (involved in the production of ADMA) (*r* = 0.69, *P* < 0.001), and this association remained statistically significant when partial correlations adjusted by BMI were carried out (*r* = 0.56, *P* < 0.05).

Finally, another strong association between transcripts that would suggest physiological autoregulation is that of *MCP1* and its receptor, chemokine (C-C motif) receptor 2 (*r* = 0.72, *P* < 0.001). *NPRC* was also related to these two transcripts (*r* = 0.47, *P* < 0.05 for *MCP1* and *r* = 0.48, *P* < 0.05 for chemokine (C-C motif) receptor 2). Tumor necrosis factor was strongly related to *PAI1* (*r* = 0.72, *P* < 0.001) and this association was still significant after correcting by BMI when partial correlation analysis was done. Again, when the relationship between expression levels was further analyzed by clustering analysis, it was observed that these genes: *TNF*, *PAI1*, *MCP1*, chemokine (C-C motif) receptor 2 and *NPRC* clustered together (**Figure 4**).

## DISCUSSION

We have demonstrated for the first time that postprandial ATBF is regulated at a transcriptional level. Thus, we found a direct relationship between ATBFAVE and the mRNA content of two genes involved in vasodilation (*NPRA* and *eNOS*), and these associations were independent of obesity. In addition, we also showed that certain genes involved in regulation of ATBF are subject to coordinate regulation of expression.

NO is known to be an important vasodilator and to determine the absolute level of ATBF (10). A decreased bioavailability of NO is a crucial factor for the development of endothelial dysfunction and insulin resistance (28,29). As the cohort we studied originally was selected for *PPARG* Pro12Ala status, and we previously observed a higher ATBF in Ala12 carriers (7), the constitutionally higher *eNOS* expression in Ala12 carriers observed here may help to explain the difference in ATBF. Apart from this finding, the *PPARG* Pro12Ala status did not seem to affect transcription of genes regulating ATBF. ATBFF has been described to be primarily under NO tone, and to some extent, under α-adrenergic and angiotensin II control (10,12,30). The lack of associations between ATBFF and any of the transcripts analyzed could be explained as fat biopsies were taken after 5–6 h of the ingestion of a mixed meal (postprandial period) and not during the fasting phase. Furthermore, this absence of associations could also be due to the fact that ATBFF would not be regulated at a transcriptional level and other mechanisms, such as posttranscriptional regulation, could be involved.

Postprandial enhancement of ATBF has been described to be controlled principally by the β-adrenergic system (10,11). However, no associations between any of the adrenergic receptors analyzed and ATBFAVE were found, despite the fact that ATBFAVE is a very robust measurement of the postprandial enhancement of ATBF. In contrast, a positive association was observed between adrenergic receptor β-1 mRNA content and ATBFRES suggesting that the maximum response of blood flow to a meal is associated, to some extent, with the activation of the adrenergic system. Furthermore, the loss of this association after correcting for BMI could corroborate previous findings that suggest that especially the adrenergic receptor β-1–mediated blood flow response is blunted in obesity (11). Moreover, because all the associations observed between the ATBFRES and the transcripts disappeared after correcting by BMI, it is tempting to suggest that the transcriptional control of ATBFRES (maximum response of ATBF to a meal) is secondary to differences in body weight.

To our knowledge, this is the first demonstration of a very strong association between the ATBFAVE and the mRNA content of the atrial natriuretic peptide receptor type A (*NPRA*), suggesting that part of the transcriptional control of postprandial ATBF is associated with the levels of expression of this receptor. In agreement with this positive association, previous studies showed, through subcutaneous adipose tissue microdialysis, that human atrial natriuretic peptide (*ANP*) stimulates local blood flow (31). Furthermore, *ANP* regulates vascular tone and stimulates lipolysis in human adipose tissue through *NPRA* supporting the finding that postprandial ATBF and lipolysis are co-regulated in the postprandial state (10). Furthermore, the lipolytic actions of *ANP* have been suggested

to be independent of the action of the sympathetic nervous system and also independent of obesity (32), which agrees with our observation that the association between ATBFAVE and *NPRA* is not modified by changes in BMI. In contrast, *NPRC*, known to mediate ANP clearance from circulation attenuating the lipolytic actions of ANP (33), was not related to ATBFAVE. Furthermore, a negative association was observed between the two receptors (*NPRA* and *NPRC*) and while *NPRA* mRNA levels were significantly decreased in overweight/obese, *NPRC* mRNA content was significantly increased, as described previously (34,35). These results suggest opposite autoregulation and function between both receptors.

Although the postprandial enhancement of ATBF is controlled principally by the β-adrenergic system, the level from which this enhancement takes place is influenced by the NO tone (10). Our results suggest that NO production is involved in the response of ATBF to nutrient intake because *eNOS* mRNA content was strongly and positively associated with ATBFAVE. These results could suggest that *eNOS* stimulates NO production which results in increasing ATBF. Moreover, the strong association found between ATBFAVE and *eNOS* mRNA content was not affected by body weight suggesting that part of the transcriptional control of postprandial ATBF is associated with the levels of expression of this transcript (*eNOS*). Furthermore, the expression of *eNOS* was decreased in the overweight/obese group, which could suggest a decreased NO production which leads to the development of several disorders related to obesity such as endothelial dysfunction and insulin resistance. However, these findings contrast with previous studies where an increased *eNOS* gene and protein expression was demonstrated in obese subjects (36,37). The disparity with our results is likely to be related to differences in the type of methodology used and also related to the fact that the obese subjects analyzed in Elizalde *et al.* study (36) had a bigger BMI than ours (39 vs. 31 $kg/m^2$), suggesting that the expression of *eNOS* is influenced by the degree of obesity with an increased expression when obesity is more established. Furthermore, Engeli *et al.* (37) used isolated adipocytes from postmenopausal women for measuring mRNA content whereas we used male adipose tissue biopsies, suggesting that gender and/or the type of sample (isolated adipocytes vs. whole adipose tissue) could influence the expression levels of *eNOS*. Finally, it is also worth mentioning that the lower expression of *iNOS* in comparison to *eNOS* under the same PCR conditions (**Supplementary Data S1** online) was not unexpected (37).

ADMA regulates vascular resistance and inhibits endothelium-dependent vasodilation together with NO synthesis (38). Our results suggest a well-balanced ADMA metabolism in human adipose tissue based on the associations found between protein arginine methyltransferase 1 and *DDAH2* (38,39). The strong associations between *DDAH2* and several vasodilator transcripts related to ATBF (*NPRA*, *eNOS*, adrenergic receptor β-1), together with the positive association observed between protein arginine methyltransferase 1 and ATBFRES, suggest a potential role of this system, and therefore, an involvement of NO in the postprandial regulation of ATBF, as stated previously.

The negative association observed between eNOS and the vasoconstrictor *EDN1* corroborates their opposite actions in the maintenance of vascular tone (40–42). Our results also suggest that both *EDN1* and its receptor *EDNRA* (type A endothelin receptor) are not co-regulated. Furthermore, the positive associations of *EDNRA* expression levels with the mRNA content of several vasodilator genes (*NPRA* and *eNOS*) contrast with previous studies that stated that *EDNRA* mediates the EDN1-induced vasoconstriction (43). Although the nature of these associations remains unknown, it is tempting to speculate that these discrepancies could be due to some compensatory events occurring at a posttranscriptional level. However, this has not been demonstrated yet.

All components of the angiotensin system are expressed in human adipose tissue, and ANG II, the vasoactive product of the system, clearly influences human ATBF *in vivo* (12). The lack of association between ATBFAVE and any of the components of this system, specially angiotensin-converting enzyme, corroborates previous findings that demonstrated that *in vivo* angiotensin-converting enzyme inhibition did not affect ATBF (12). These results suggest that the angiotensin system do not have a primary role in regulating ATBF at a transcriptional level. Finally, the strong association found between MCP1 and its receptor chemokine (C-C motif) receptor 2 or the relationships observed between tumor necrosis factor and adiponectin (negative) or tumor necrosis factor and PAI1 (positive) would suggest some co-regulation.

In summary, this study shows for the first time that ATBF responsiveness to nutrient intake is related to the transcription of two genes expressed in adipose tissue and directly involved in vasodilatory actions (*eNOS* and *NPRA*), demonstrating that part of the regulation of ATBF is at a transcriptional level. Interestingly, obesity does not interfere with these associations. Finally, we also showed that certain genes involved in the regulation of ATBF are subject to coordinate regulation of expression suggesting physiological autoregulation.

## SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

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## DISCLOSURE

The authors declared no conflict of interest.

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## REFERENCES

1. Samra JS, Simpson EJ, Clark ML *et al*. Effects of epinephrine infusion on adipose tissue: interactions between blood flow and lipid metabolism. *Am J Physiol* 1996;271:E834–E839.
2. Bulow J, Madsen J. Adipose tissue blood flow during prolonged, heavy exercise. *Pflugers Arch* 1976;363:231–234.
3. Hansen M, Jacobsen E, Madsen J. Influence of hypoxia and hyperoxia on subcutaneous adipose tissue blood flow in man. *Scand J Clin Lab Invest* 1976;36:655–660.

4. Bulow J, Astrup A, Christensen NJ, Kastrup J. Blood flow in skin, subcutaneous adipose tissue and skeletal muscle in the forearm of normal man during an oral glucose load. *Acta Physiol Scand* 1987;130:657–661.
5. Karpe F, Fielding BA, Ilic V, Humphreys SM, Frayn KN. Monitoring adipose tissue blood flow in man: a comparison between the (133)xenon washout method and microdialysis. *Int J Obes Relat Metab Disord* 2002;26:1–5.
6. Coppack SW, Evans RD, Fisher RM *et al*. Adipose tissue metabolism in obesity: lipase action *in vivo* before and after a mixed meal. *Metabolism* 1992;41:264–272.
7. Tan GD, Neville MJ, Liverani E *et al*. The *in vivo* effects of the Pro12Ala PPARγ2 polymorphism on adipose tissue NEFA metabolism: the first use of the Oxford Biobank. *Diabetologia* 2006;49:158–168.
8. Evans K, Clark ML, Frayn KN. Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism. *Am J Physiol* 1999;276: E241–E248.
9. Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW. Integrative physiology of human adipose tissue. *Int J Obes Relat Metab Disord* 2003;27:875–888.
10. Ardilouze JL, Fielding BA, Currie JM, Frayn KN, Karpe F. Nitric oxide and β-adrenergic stimulation are major regulators of preprandial and postprandial subcutaneous adipose tissue blood flow in humans. *Circulation* 2004;109:47–52.
11. Blaak EE, van Baak MA, Kemerink GJ *et al*. β-adrenergic stimulation and abdominal subcutaneous fat blood flow in lean, obese, and reduced-obese subjects. *Metabolism* 1995;44:183–187.
12. Goossens GH, McQuaid SE, Dennis AL *et al*. Angiotensin II: a major regulator of subcutaneous adipose tissue blood flow in humans. *J Physiol* 2006;571:451–460.
13. Karpe F, Fielding BA, Ardilouze JL *et al*. Effects of insulin on adipose tissue blood flow in man. *J Physiol* 2002;540:1087–1093.
14. Jansson PA, Larsson A, Lonnroth PN. Relationship between blood pressure, metabolic variables and blood flow in obese subjects with or without non-insulin-dependent diabetes mellitus. *Eur J Clin Invest* 1998;28:813–818.
15. Summers LK, Samra JS, Humphreys SM, Morris RJ, Frayn KN. Subcutaneous abdominal adipose tissue blood flow: variation within and between subjects and relationship to obesity. *Clin Sci (Lond)* 1996;91:679–683.
16. Summers LK, Samra JS, Frayn KN. Impaired postprandial tissue regulation of blood flow in insulin resistance: a determinant of cardiovascular risk? *Atherosclerosis* 1999;147:11–15.
17. Karpe F, Fielding BA, Ilic V *et al*. Impaired postprandial adipose tissue blood flow response is related to aspects of insulin sensitivity. *Diabetes* 2002;51: 2467–2473.
18. Frayn K, Macdonald I. Adipose tissue circulation. In: Bennett T, Gardiner S (eds). *Nervous Control of Blood Vessels*. Harwood Academic: Amsterdam, 1996, pp 505–539.
19. Altshuler D, Hirschhorn JN, Klannemark M *et al*. The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000;26:76–80.
20. Deeb SS, Fajas L, Nemoto M *et al*. A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nat Genet* 1998;20:284–287.
21. Ek J, Andersen G, Urhammer SA *et al*. Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) gene in relation to insulin sensitivity among glucose tolerant caucasians. *Diabetologia* 2001;44:1170–1176.
22. Hara K, Okada T, Tobe K *et al*. The Pro12Ala polymorphism in PPAR γ2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 2000;271:212–216.
23. Koch M, Rett K, Maerker E *et al*. The PPARγ2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of Type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* 1999;42:758–762.
24. Evans K, Burdge GC, Wootton SA, Clark ML, Frayn KN. Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes* 2002;51:2684–2690.
25. Larsen OA, Lassen NA, Quaade F. Blood flow through human adipose tissue determined with radioactive xenon. *Acta Physiol Scand* 1966;66: 337–345.
26. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;29:e45.
27. Urs S, Smith C, Campbell B *et al*. Gene expression profiling in human preadipocytes and adipocytes by microarray analysis. *J Nutr* 2004;134: 762–770.
28. Cooke JP. The endothelium: a new target for therapy. *Vasc Med* 2000;5: 49–53.
29. Sydow K, Mondon CE, Cooke JP. Insulin resistance: potential role of the endogenous nitric oxide synthase inhibitor ADMA. *Vasc Med* 2005;10(Suppl 1):S35–S43.
30. Galitzky J, Lafontan M, Nordenstrom J, Arner P. Role of vascular α-2 adrenoceptors in regulating lipid mobilization from human adipose tissue. *J Clin Invest* 1993;91:1997–2003.
31. Moro C, Polak J, Richterova B *et al*. Differential regulation of atrial natriuretic peptide-and adrenergic receptor-dependent lipolytic pathways in human adipose tissue. *Metabolism* 2005;54:122–131.
32. Galitzky J, Sengenes C, Thalamas C *et al*. The lipid-mobilizing effect of atrial natriuretic peptide is unrelated to sympathetic nervous system activation or obesity in young men. *J Lipid Res* 2001;42:536–544.
33. Sengenes C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J. Natriuretic peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 2000;14:1345–1351.
34. Engeli S, Sharma AM. The renin-angiotensin system and natriuretic peptides in obesity-associated hypertension. *J Mol Med* 2001;79:21–29.
35. Wang TJ, Larson MG, Levy D *et al*. Impact of obesity on plasma natriuretic peptide levels. *Circulation* 2004;109:594–600.
36. Elizalde M, Ryden M, van Harmelen V *et al*. Expression of nitric oxide synthases in subcutaneous adipose tissue of nonobese and obese humans. *J Lipid Res* 2000;41:1244–1251.
37. Engeli S, Janke J, Gorzelniak K *et al*. Regulation of the nitric oxide system in human adipose tissue. *J Lipid Res* 2004;45:1640–1648.
38. Cooke JP. Asymmetrical dimethylarginine: the Uber marker? *Circulation* 2004;109:1813–1818.
39. Sydow K, Mondon CE, Cooke JP. Insulin resistance: potential role of the endogenous nitric oxide synthase inhibitor ADMA. *Vasc Med* 2005; 10(Suppl 1):S35–S43.
40. Cardillo C, Kilcoyne CM, Cannon RO III, Panza JA. Interactions between nitric oxide and endothelin in the regulation of vascular tone of human resistance vessels *in vivo*. *Hypertension* 2000;35: 1237–1241.
41. Mather KJ, Lteif A, Steinberg HO, Baron AD. Interactions between endothelin and nitric oxide in the regulation of vascular tone in obesity and diabetes. *Diabetes* 2004;53:2060–2066.
42. Verhaar MC, Strachan FE, Newby DE *et al*. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation* 1998;97: 752–756.
43. Levin ER. Endothelins. *N Engl J Med* 1995;333:356–363.