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ORIGINAL ARTICLE

# Pattern of expression of adiponectin receptors in human adipose tissue depots and its relation to the metabolic state

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**Objective:** To investigate whether adiponectin receptor genes (*AdipoR1* and *AdipoR2*) expression in human subcutaneous (SAT) and visceral (VAT) adipose tissue in severely obese patients with or without diabetes is related to adiponectin gene (*APM1*) expression and *in vivo* metabolic parameters.
**Design:** Cross-sectional, clinical research study.
**Subjects:** Total RNA was extracted from SAT and VAT tissue obtained during surgery from 13 lean controls, 30 obese diabetic patients, 19 obese glucose-intolerant patients and 54 obese subjects with normal glucose tolerance.
**Measurements:** Tissue expression of *APM1*, *AdipoR1* and *AdipoR2*, tissue concentration of adiponectin (ApN), and metabolic variables.
**Results:** *APM1* expression was higher in SAT than VAT ($1.06 \pm 0.76$ vs $0.69 \pm 0.52$, $P<0.0001$) as was *AdipoR1* ($1.17 \pm 0.70$ vs $0.66 \pm 0.38$, $P<0.0001$) and *AdipoR2* ($7.02 \pm 6.19$ vs $0.75 \pm 0.64$, $P<0.0001$). In SAT, *APM1* and *AdipoR1* expression tended to be lower – by $0.38 \pm 0.22$ and $0.35 \pm 0.22$, respectively – and *AdipoR2* expression was markedly depressed – by $4.82 \pm 1.93$ – in association with obesity, whereas presence of diabetes had no additional effect. In VAT, *APM1* and *AdipoR1* expressions were also reduced – by $0.36 \pm 0.16$ and $0.30 \pm 0.11$, respectively – in association with obesity. Within both SAT and VAT, expression levels of *APM1*, *AdipoR1* and *AdipoR2* were all positively interrelated. Tissue ApN concentrations in SAT were similar across groups, whereas ApN levels in VAT were substantially lower in association with obesity (by an average of $63 \pm 12$ ng/mg total protein, $P<0.0001$). In multivariate models adjusting for sex, age and obesity, serum triglyceride concentrations were reciprocally related to *APM*1 ($r=-0.27$, $P<0.02$), *AdipoR1* ($r=-0.37$, $P<0.002$ and *AdipoR2* expression ($r=-0.37$, $P<0.002$) in VAT. Likewise, plasma insulin concentrations were inversely related only to *APM1* in VAT ($r=-0.25$, $P<0.03$).
**Conclusions:** Severe obesity is associated with suppressed expression of both ApN and its receptors in both SAT and VAT, the expression levels in VAT are specifically linked with hyperinsulinemia and dyslipidemia.
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**Keywords:** adiponectin gene; *AdipoR1* gene; *AdipoR2* gene; type 2 diabetes

## Introduction

Obesity, especially central obesity, is part of a cluster of abnormalities – including insulin resistance, type 2 diabetes (DM2), hypertension and dyslipidemia – that enhances the risk of cardiovascular disease. [1] Several molecules produced by adipose tissue, known as adipocytokines, circulate in increased concentration in several metabolic conditions.[2–6] Adiponectin (ApN), which is also exclusively produced by adipocytes,[7–9] is associated with improved insulin sensitivity through its stimulatory effect on fatty acid oxidation[10,11] and inhibitory action on hepatic glucose production.[12,13] Plasma ApN levels have been found to be significantly reduced in subjects with obesity, insulin resistance or DM2;[14–17] the mechanisms of this reduction are incompletely understood. A reduction in adiponectin gene (*APM1*) expression and secretion in adipose tissue from obese and diabetic subjects has been reported in some,[7,18–22] but not all

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studies.[23] Moreover, the role of omental vs subcutaneous adipose tissue (SAT) in ApN release and the relationship between the protein and its receptors in adipose tissue have not been fully investigated. Two adiponectin receptors (AdipoR1 and AdipoR2), G protein-coupled domains, have been identified and their genes (*AdipoR1* and *AdipoR2*) have been recently cloned.[24] AdipoR1 appears to have a high affinity for globular and a low affinity for full-length ApN, whereas AdipoR2 has intermediate affinity for both ApN isoforms. AdipoR1 has been reported to be more expressed in skeletal muscle, AdipoR2 in the liver.[24] Both receptors are reputed to mediate ApN effects on adenosine monophosphate kinase (AMPK), peroxisome proliferator-activated receptor-$\alpha$ ligands activities, fatty oxidation and glucose uptake.[15,25–27] Recently, Fasshauer *et al*.[28] have demonstrated the presence of both receptors in an adipocyte cell line, and Rasmussen *et al*.[29] have found that *AdipoR1* expression is reduced in adipose tissue of obese subjects.

The present study was undertaken to assess the separate role of obesity and DM2 in the expression of the *APM1* and both its receptors in visceral adipose tissue (VAT) and SAT and to explore the relationships between these expression levels and metabolic traits.

## Subjects and methods

*Subjects and study design*

The study involved 103 severely obese subjects undergoing bariatric surgery; 13 non-obese subjects undergoing elective abdominal surgery (cholecystectomy) served as the control group. All subjects gave informed, written consent, and the protocol was approved by the local Ethical Committee. The obese subjects were subgrouped according to their glucose tolerance status (as determined by a standard 75-g oral glucose tolerance test (OGTT) or a previous diagnosis of DM2): 54 subjects had normal glucose tolerance status (Obese-NGT), 19 had impaired glucose tolerance (Obese-IGT) and 30 had DM2 (Obese-DM2). All patients were asked to attend our Unit for the metabolic study 2 weeks before surgery. All subjects were instructed not to exercise for 48 h before the metabolic study or surgery. For the metabolic study, all subjects were examined in the morning after an overnight (12–14 h) fast. Peripheral blood samples were obtained for determination of lipid profile, glucose, insulin and ApN concentrations. Then a 75-g OGTT was performed, with sampling every 15 min within the first hour and every 30 min for the following 2 h, for measurement of plasma glucose and insulin. IGT and diabetes were classified according to the American diabetes association (ADA) criteria.[30] Thus, IGT was classified as a fasting plasma glucose concentrations $<7.0$ mmol/l and 2-h plasma glucose between 7.8 and 11.1 mmol/l. Diabetes was classified as a fasting glucose $\geqslant 7.0$ mmol/l or 2-h glucose $>11.1$ mmol/l. Diabetic subjects who were not taking insulin were considered to have DM2; insulin-taking diabetic subjects whose age of onset was $\geqslant 40$ years or whose body mass index (BMI) was $>30\,kg/m^2$ were also considered to have DM2. The remaining insulin-taking diabetic subjects were considered to have type 1 diabetes or to be unclassifiable, and were excluded from the study.

Anthropometric measurements, height, weight, waist and hip circumferences, and systolic and diastolic blood pressure, were measured as described elsewhere.[31] BMI was calculated as weight divided by height squared, and used as an index of overall adiposity. Subjects with a history of alcohol overconsumption, end-stage renal disease, cardiac failure, hepatitis virus B (HBV) or hepatitis virus C (HCV) chronic hepatitis were excluded.

Plasma glucose concentration was measured on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA, USA). Fasting concentrations of serum total cholesterol low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglycerides were determined as described elsewhere (Synchron CX4, Beckman Instruments Inc., Brea, CA, USA). Plasma insulin was measured by radioimmunoassay (Linco Research, St Charles, MO, USA) and ApN by enzyme-linked immunosorbent assay (ELISA) (Linco Research).

*Adipose tissue biopsies*

SAT and VAT fat depots (1–2 g) were collected during abdominal surgery in RNA-Later and stored at $-20^{\circ}C$ for total RNA extraction. In a subgroup of 20 Obese-NGT, six Obese-IGT, 11 Obese-DM2 and 10 controls, an additional 300-mg adipose tissue specimen from both SAT and VAT was collected and immediately transported in sterile containers to the laboratory (30 min after removal).

*Total RNA isolation and cDNA preparation*

The adipose tissue was cut into pieces of 600 mg, and after removing RNA-Later, total RNA was isolated using a mixed protocol: Trizol and RNeasy Midi Kit (Qiagen, Hilden, Germany) in order to obtain a larger amount of purified RNA. RNA was quantified by measuring absorbance at 260 and 280 nm. RNA integrity was then checked by visual inspection of the two ribosomal RNAs, 18S and 28S, on 1% agarose gel. Average yields of total RNA were $20\pm1\,\mu g/g$ of adipose tissue. Isolated RNA was stored at $-80^{\circ}C$ until quantification of the target mRNAs. For first-strand cDNA synthesis, $1\,\mu g$ total RNA was reverse-transcribed in a $20\,\mu l$ volume using random hexamers as primers, according to the manufacturer's instructions (First strand cDNA Synthesis Kit for RT-PCR, AMV, Roche, Indianapolis, IN, USA).

*Relative quantification of APM1, AdipoR1, AdipoR2 gene expression*

Relative quantification of the mRNAs was performed by real-time PCR using a Light-Cycler instrument (Roche Diagnostics,

Mannheim, Germany). Gene-specific primers for the target genes (*APM1*: forward 5′-TGGTGAGAAGGGTGAGAA-3′ and reverse 5′-AGATCTTGGTAAAGCGAATG-3′, 221 bp; *AdipoR1*: forward 5′-TTCTTCCTCATGGCTGTGATGT-3′, reverse 5′-AA GAAGCGCTCAGG-AATTCG-3′, 71 bp; *AdipoR2*: forward 5-′CCACCACCTTGCTTCATCTA-3′, reverse 5′-GATACTGAGGG GTGGCAAAC-3′, 97 bp) and the reference gene (hypoxantine-phosphorybosyl-transferase, *HPRT*: forward 5′-TGCTGACCT GCTGGATTACAT-3′, reverse 5′TTGCGACCTTG-ACCATCTTT-3′, 260 bp) were designed in our laboratory. *HPRT* mRNA was used for sample normalization. cDNA (1 $\mu$l) was brought to a final volume of 20 $\mu$l in a glass capillary containing 1 × LightCycler-FastStar DNA Master SYBR Green I mix (Roche Diagnostics), 4.5 mM $MgCl_2$ and 0.3 $\mu$M of primers (Proligo, Paris, France) for *APM1*, *AdipoR1* and *AdipoR2* and 0.5 $\mu$M for *HPRT*. PCR was performed with 10 min of initial denaturation and then 40 cycles with 10 s at 95°C (denaturation), 7 s at 58°C (annealing) and 13 s at 72°C (extension). The last cycle at 40°C for 15 s. Fluorescent data were acquired at the end of each extension phase. After amplification, a melting curve analysis from 65 to 95°C with heating rate of 0.1°C/s with a continuous fluorescence acquisition was constructed. To construct standard curves for each gene, total RNA from six control subjects (SAT and VAT) were pooled. In brief, after RNA reverse transcription of pool sample, the cDNA was diluted into threefold serial dilution (1:3–1:27) and both genes (target and reference) were amplified in triplicate. The standard curve was used to assess PCR efficiency. Standard curves were accepted only if the slope for each gene was around –3 with an $r$ value $>0.98$. Each cDNA was quantified in triplicate. For the relative quantification of samples, the 'Light Cycler Relative Quantification Software' was utilized. Results were expressed as the target/reference ratio of each sample, normalized by the target/reference ratio of the calibrator. The calibrator used for each quantification was the sample with a dilution of $10^{-3}$ utilized in the standard curve. In the present data, the coefficient of variation in measurements for the target gene in each sample ranged 0–10%.

We used *HPRT* as the reference gene because its expression was found to be relatively stable in different adipose tissue depots and showed no systematic differences between cases and controls. The mean $C_t$ ($\pm$s.d.) of *HPRT* was $25.72 \pm 1.42$ in obese subjects compared to $25.63 \pm 1.95$ in the control group for SAT, and $25.19 \pm 1.24$ in obese subjects compared to $25.47 \pm 1.73$ in the control group for VAT.

### *Adipocyte isolation and measurement of intracellular ApN concentrations*

Mature adipocytes were isolated as described by Grohmann *et al*.[32] The adipose tissue was washed three times in Hank's balanced salt solution, cut into 1-$mm^3$ pieces and digested with 3 ml of 4 mg/ml type II collagenase in Hank's balanced salt solution for 60 min at 37°C. Fragments of tissue still remaining after this treatment were removed with forceps. The mature adipocytes were separated from the stromal fraction by centrifugation at 90 *g* for 3 min and were carefully removed to a sterile Eppendorf containing 500 $\mu$l of lysis buffer (10 mM Tris-Cl, 50 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 15 mM sodium pyrophosphate, 50 mM sodium fluoride, 100 $\mu$M sodium orthovanadate and 1% Triton X-100 with freshly added 1% phenylmethylsulfonyl fluoride at the time of use). Cells were then lysed via repeated passaging through a 21-gauge needle and the fat cake at the top of the sample was carefully removed. Then the samples were frozen at −20°C. Samples were processed in batch, and ApN concentrations were determined using a standard ELISA assay; adipose tissue ApN concentrations were corrected for mg of total protein.

### *Statistical analysis*

Results are expressed as mean $\pm$ s.d. or median (interquartile range), for variables with normal or non-normal distribution, respectively. Categorical variables were compared by the $\chi^2$ test, continuous variables were analyzed by one-way analysis of variance or Kruskall–Wallis test. Univariate associations were tested with Spearman coefficient, *rho*. Multivariate analysis was performed by using general linear models including both continuous and categorical variables; results are given as the standardized regression coefficient (*sd.r*). For multivariate analysis, variables with non-normal distribution were log-transformed.

## Results

The clinical characteristics of the study population are reported in Table 1. The obese groups generally had similar clinical phenotypes, but blood pressure, serum triglycerides,

**Table 1** Baseline clinical and metabolic characteristics

| | *Obese-NGT* | *Obese-IGT* | *Obese-DM2* | *Controls* |
|---|---|---|---|---|
| Subjects | 54 | 19 | 30 | 13 |
| Sex (F/M)* | 48/6 | 14/5 | 21/9 | 7/5 |
| Age (years)* | 39 ± 11 | 40 ± 11 | 48 ± 8 | 51 ± 11 |
| BMI ($kg/m^2$)* | 47.8 ± 9.8 | 46.5 ± 6.5 | 45.1 ± 6.2 | 24.0 ± 2.9 |
| SBP (mm Hg)* | 127 ± 15 | 126 ± 17 | 139 ± 14 | 123 ± 10 |
| DBP (mm Hg)* | 76 ± 12 | 80 ± 11 | 86 ± 10 | 77 ± 7 |
| TG (mmol/l) | 1.26 [0.50] | 1.53 [1.02] | 2.10 [1.73] | 1.37 [0.13] |
| T-cholesterol (mg/dl) | 195 ± 33 | 207 ± 56 | 215 ± 52 | 192 ± 6 |
| HDL-cholesterol (mg/dl) | 49 ± 10 | 43 ± 11 | 45 ± 12 | 47 ± 2 |
| FPG (mmol/l)* | 4.9 ± 0.5 | 5.5 ± 0.6 | 8.8 ± 2.3 | 5.1 ± 0.3 |
| 2-h PG (mmol/l)* | 5.9 ± 1.2 | 8.9 ± 0.7 | 13.0 ± 1.4 | 6.2 ± 0.5 |
| FPI ($\mu$UI/ml)[a] | 4.62 [0.75] | 4.43 [0.79] | 4.82 [1.07] | 3.82 [0.57] |
| 2-h PI ($\mu$UI/ml) | 5.89 [1.15] | 6.41 [0.72] | 6.33 [1.37] | 6.00 [0.58] |
| Plasma ApN (mg/l)[a] | 3.77 [2.81] | 5.04 [2.39] | 3.74 [2.93] | 7.50 [5.49] |

Abbreviations: ANOVA, analysis of variance; ApN, adiponectin; BMI, body mass index; DBP, diastolic blood pressure; DM2, type 2 diabetes; F, female; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL, high-density lipoprotein; IGT, impaired glucose tolerance; M, male; NGT, normal glucose tolerance; PG, plasma glucose; PI, plasma insulin; SBP, systolic blood pressure; TG, triglyceride. *$P \leqslant 0.05$ by ANOVA. [a]Kruskall–Wallis test.

fasting and 2-h plasma glucose levels and fasting plasma insulin concentrations were higher in patients with DM2 than in obese NGT patients, while IGT patients showed intermediate values.

In the whole data set, *APM1* expression was higher in SAT than VAT ($1.06\pm0.76$ vs $0.69\pm0.52$, $P<0.0001$) as was *AdipoR1* expression ($1.17\pm0.70$ vs $0.66\pm0.38$, $P<0.0001$). For *AdipoR2* expression, levels were ~10-fold higher in SAT than VAT ($7.02\pm6.19$ vs $0.75\pm0.64$, $P<0.0001$). These differences were maintained across study groups (Table 2).

In SAT, *APM1* and *AdipoR1* expression tended to be lower – by $0.38\pm0.22$ and $0.35\pm0.22$, respectively – and *AdipoR2* expression was markedly depressed – by $4.82\pm1.93$ – in association with obesity, whereas presence of diabetes had no additional effect. Within SAT, expression levels of *APM1*, *AdipoR1* and *AdipoR2* were all positively interrelated.

In VAT, *APM1* and *AdipoR1* expression were significantly lower – by $0.36\pm0.16$ and $0.30\pm0.11$, respectively – in association with obesity, whereas *AdipoR2* expression tended to be reduced but the difference did not reach statistical significance. As was the case for SAT, expression levels were all positively interrelated and there was no independent influence of diabetes on them.

Plasma ApN concentrations were significantly lower in association with obesity (by $2.4\pm1.1$ mg/l on average, $P<0.03$), with no independent effect of diabetes. In the whole data set, *APM1* expression in SAT and VAT, *AdipoR1* expression in SAT and *AdipoR2* expression in VAT were each reciprocally related to BMI (with *rho* values of 0.19–0.21, all $P\leqslant0.05$). A positive correlation was also found between plasma ApN and *APM1* expression in VAT ($rho=0.32$, $P<0.001$), but not with receptors expression in VAT or *APM1* expression in SAT.

ApN concentrations in mature adipocytes of SAT were similar across groups, whereas ApN levels in VAT were substantially lower in association with obesity (by an average of $63\pm12$ ng/mg total protein, $P<0.0001$) (Table 2).

In a multivariate model adjusting for sex, age, and obesity, plasma insulin concentrations were inversely related only to *APM1* in VAT ($sd.r=-0.25$, $P<0.03$).

## Discussion

The major findings of the present study are that: (1) both *APM1* and its receptors, especially *AdipoR2*, are expressed at higher levels in SAT than in VAT; (2) circulating ApN levels are related to *APM1* but not receptor expression; (3) expression of *APM1* and both receptors is reduced in association with obesity but not diabetes and (4) adipocyte ApN concentrations are reduced in VAT of obese individuals. These findings are generally compatible with the notion that ApN is a link between visceral fat accumulation and insulin resistance,[33–35] but require specification.

Firstly, there is some disagreement about the absolute level of expression of *APM1* and its receptors in different fat depots. Thus, Tan *et al*.[36] could not find any correlation between *APM1* expression and receptor expression in subcutaneous abdominal fat of patients with DM2. Rasmussen *et al*.[29] on the other hand, reported higher expression of *AdipoR1* than *AdipoR2* in both SAT and VAT in a group of obese non-diabetic women. In our large group of subjects, we found that *AdipoR2* is the dominant receptor expressed in SAT but not in VAT regardless of gender and clinical condition (obesity or diabetes). Also, in our hands, the expression levels of *APM1* and its receptors were correlated with each other in both adipose depots. The reasons for the discrepancy in the relative abundance of *AdipoR1* and *AdipoR2* in different tissues remain unclear. Upon re-assaying a representative sample of our tissue specimens with the use of a different reporter gene (*GADPH*), we found a similar pattern of results (data not shown).

Secondly, in our study population, circulating ApN levels were weakly related to gene expression in VAT only. A lack of association between serum ApN levels and SAT *APM1* expression has been observed previously.[10,34] Hoffstedt *et al*.[37] have reported that ApN release by SAT is reduced in obese women when expressed per unit of fat tissue weight, but found a (weak) correlation between secretion and circulating level of the adipokine only in women with high insulin sensitivity. In general, it is difficult to extrapolate *APM1* expression or tissue secretion rates to circulating levels because (1) ApN has a fast plasma clearance rate, (2) at the

**Table 2** Adiponectin (APM1) and adiponectin receptors (AdipoR1 and AdipoR2) gene expression, and ApN concentrations in SAT and VAT[a]

| | *Obese-NGT* | *Obese-IGT* | *Obese-DM2* | *Controls* | $P_{ob}$ |
|---|---|---|---|---|---|
| *APM1* SAT | $0.94\pm0.68$ | $1.24\pm0.80$ | $1.02\pm0.78$ | $1.35\pm0.95$ | (0.07) |
| *AdipoR1* SAT | $1.02\pm0.59$ | $1.15\pm0.68$ | $1.29\pm0.82$ | $1.55\pm0.87$ | (0.09) |
| *AdipoR2* SAT | $6.20\pm5.93$ | $7.15\pm5.85$ | $6.94\pm5.80$ | $11.39\pm8.11$ | 0.014 |
| *APM1* VAT | $0.66\pm0.47$ | $0.72\pm0.38$ | $0.57\pm0.52$ | $1.01\pm0.73$ | 0.023 |
| *AdipoR1* VAT | $0.64\pm0.39$ | $0.67\pm0.25$ | $0.56\pm0.32$ | $0.93\pm0.56$ | <0.01 |
| *AdipoR2* VAT | $0.76\pm0.67$ | $0.74\pm0.49$ | $0.58\pm0.56$ | $1.03\pm0.79$ | ns |
| ApN SAT (ng/mg) | $19.9\pm12.1$ | $23.5\pm14.9$ | $20.9\pm12.9$ | $24.5\pm17.1$ | ns |
| ApN VAT (ng/mg) | $33.3\pm27.1$ | $17.9\pm14.3$ | $25.3\pm17.4$ | $91.7\pm63.2$ | <0.0001 |

Abbreviations: ANOVA, analysis of variance; ApN, adiponectin; DM2, type 2 diabetes; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. [a]Gene expression data are given as ratio of target gene to reference gene (*HPRT*). $P_{ob}=P$ values for the effect of obesity (BMI $>30\,kg\,m^{-2}$) by ANOVA.

whole body level, secretion depends on the product of tissue-specific secretion rate by total fat mass, and (3) different mixtures of globular or full-length protein could be released by different tissues and (4) gene expression level may not quantitatively translate into secretion rates if there are post-translational effects or autocrine/paracrine interactions. It is nevertheless important to note that, in the VAT depot of obese subjects, there was a consensual reduction in *APM1* expression and intracellular concentrations in association with lower circulating levels of ApN.

Suppressed plasma ApN concentrations and adipose tissue *APM1* expression in obese subjects have been reported previously,[7,21,38] and lower plasma ApN levels have been described in subjects with DM2.[10,11] However, the data in type 2 diabetic patients are conflicting.[19,34,39,40] Our results, while confirming lower expression and plasma concentrations in association with obesity, do not show an independent effect of hyperglycemia, in agreement with Koistinen *et al*.[40] On the other hand, our diabetic subjects were all severely obese, so that expression and circulating levels may have bottomed out (a 'floor effect'). Therefore, we cannot completely rule out that hyperglycemia in non-obese (or less obese) subjects may be associated with suppressed ApN.

Both *AdipoR1* and *AdipoR2* were found to be less expressed in the adipose tissue of our obese subjects, in SAT as well as VAT. This finding agrees with recent results by Rasmussen *et al*.[29] Importantly, we found a reciprocal association between the expression of *APM1* and its receptors in VAT and serum triglyceride levels that was independent of gender, age and degree of obesity. This result clearly suggests that the biology of ApN in VAT is relevant to the metabolic status of the subject. Thus, as the adipose mass expands, ApN and its receptors decrease in all adipose tissue depots, possibly as a result of changes in cellular phenotype (from small, insulin sensitive adipocytes to large, insulin-resistant cells). In line with the notion that insulin – or insulin resistance – may directly influence *APM1* receptors mRNA in adipose and muscle tissue,[28,41–44] we observed a negative association between plasma insulin concentrations and *APM1* expression. Whether the two receptors mediate different physiological actions in SAT and VAT or whether the relative abundance of the receptors in VAT vs SAT is a factor in the physiological actions of ApN are questions that require further investigation.

In summary, this study demonstrates that *APM1* expression is directly related to the expression of its receptors in abdominal adipose tissue, and that lower levels of *APM1* receptors mRNA are found in VAT than SAT. Furthermore, we found that obesity – but not diabetes – is associated with lower expression of *APM1* and its receptors in both abdominal fat depots, but that only VAT gene expression is related to metabolic parameters. Although our findings are compatible with the suggestion that the ApN system in visceral fat tissue is involved in the pathogenesis of obesity and insulin resistance, further studies are necessary to determine whether changes in gene expression translate into changes in protein levels and biological function.

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