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ORIGINAL ARTICLE

Regulation of adipokine production in human adipose tissue by propionic acid

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ABSTRACT

Background Dietary fibre (DF) has been shown to be protective for the development of obesity, insulin resistance and type 2 diabetes. Short-chain fatty acids, produced by colonic fermentation of DF might mediate this beneficial effect. Adipose tissue plays a key role in the regulation of energy homeostasis, therefore, we investigated the influence of the short-chain fatty acid propionic acid (PA) on leptin, adiponectin and resistin production by human omental (OAT) and subcutaneous adipose tissue (SAT). As PA has been shown to be a ligand for G-protein coupled receptor (GPCR) 41 and 43, we investigated the role of GPCR's in PA signalling.

Materials and methods Human OAT and SAT explants were obtained from gynaecological patients who underwent surgery. Explants were incubated for 24 h with PA. Adipokine secretion and mRNA expression were determined using ELISA and RT-PCR respectively.

Results We found that PA significantly stimulated leptin mRNA expression and secretion by OAT and SAT, whereas it had no effect on adiponectin. Furthermore, PA reduced resistin mRNA expression. Leptin induction, but not resistin reduction, was abolished by inhibition of Gi/o-coupled GPCR signalling. Moreover, GPCR41 and GPCR43 mRNA levels were considerably higher in SAT than in OAT.

Conclusions We demonstrate that PA stimulates expression of the anorexigenic hormone leptin and reduces the pro-inflammatory factor resistin in human adipose tissue depots. This suggests that PA is involved in regulation of human energy metabolism and inflammation and in this way may influence the development of obesity and type 2 diabetes.

Keywords Adiponectin, G-protein coupled receptors, human adipose tissue, leptin, propionic acid, resistin.

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Introduction

Obesity and its associated disorders, such as insulin resistance, type 2 diabetes and cardiovascular diseases, have become major public health issues. The aetiology of obesity and insulin resistance is complex and involves life style factors such as physical activity and diet. The dietary fibre content (DF) of the diet is one of the factors that may influence the development of these diseases. Several studies show that consumption of DF prevents accumulation of fat mass [1–3] and increases insulin sensitivity [4–6]. This may be attributable to fermentation of DF by the colonic-microbiota leading to the production of metabolites such as short-chain fatty acids (SCFA). SCFA are rapidly absorbed from the colonic lumen and drains through the visceral tissues into the portal vein [7].

Recently it has been shown that SCFA, including propionic acid (PA), are ligands for the G protein-coupled receptor GPCR41 and GPCR43. These receptors, like other GPCR, are linked to GTP-binding proteins (G-proteins). G-proteins are attached to the cytoplasmic side of the receptor, where they serve as relay molecules functionally coupling the receptors to their downstream targets. G-proteins are classified into four major classes, namely Gs, Gi/o, Gq/11 and G12/13. Each of them is specific for a particular set of GPCR's and a particular set of downstream targets [8]. Both GPCR41 and GPCR43 have been shown to be coupled to Gi/o-proteins and are present in adipose tissue [9,10], which suggests that adipose tissue is an important target for SCFA. As adipose tissue is an endocrine

organ that produces a great variety of adipokines, that influence metabolism in other organs, it could constitute a link between colonic fermentation and peripheral metabolic effects.

The principal SCFA that are produced in the colon are acetic acid, propionic acid and butyric acid. However, most of the studies investigated the effects of butyric or acetic acids, while very little is known about effects of PA. In addition, 90% of butyric acid is utilized by colonocytes and very little reaches the visceral tissues [11], while the majority of acetic acid and PA passes the colonocytes and visceral tissues and drains into the portal vein. Recently, it has been demonstrated that PA stimulates the production of the anorexigenic adipokine leptin in mouse and bovine adipocytes *in vitro* [12–14] and sheep adipose tissue *in vivo* [15]. However, these and most of earlier studies were performed in animals or animal tissue, namely ruminants [16]. Therefore, the aim of our study was to determine the effect of PA on the expression and secretion of adipokines by human adipose tissues. As different adipose tissue depots may vary in adipokine secretion patterns, we used omental as well as subcutaneous tissue. In addition to leptin, which is known to reduce food intake and increase energy expenditure [17] we examined the effect on adiponectin and resistin. Adiponectin has insulin sensitizing and anti-inflammatory effects [18]. Resistin is a pro-inflammatory factor [19–21] and recently, it has been shown to be inversely associated with insulin sensitivity [22–24]. PA is a ligand for GPCR41 and GPCR43. Consequently, we also studied the involvement of the Gi/o-proteins signalling pathway in the regulation of adipokine response to PA treatment.

Materials and methods

Materials

Gentamycin, glucose, PA and pertusis toxin (PTX) were purchased from Sigma (Zwijndrecht, The Netherlands). M199 media was purchased from Invitrogen (Breda, The Netherlands). GPCR43 and GPCR41 primers were purchased from Applied Biosystems (Nieuwerkerk a/d IJssel, The Netherlands); whereas the other primers were purchased from Biolegio (Nijmegen, The Netherlands).

Table 1 Characteristics of adipose tissue donors (N = 28)

Characteristics	Mean	Range
Age (years)	45·8 ± 9·3	26–68
Body mass index (BMI, kg m^{-2})	26·3 ± 3·3	20·8–33·3
Waist circumference (WC, cm)	87·3 ± 11·4	57–104
Hip circumference (HC, cm)	102·4 ± 9·7	82–120
Waist to hip ratio (WHR)	0·9 ± 0·08	0·54–0·93

Human adipose tissue culture

Human omental and abdominal subcutaneous adipose tissue explants as well as serum samples were obtained from 28 females, who underwent surgery for gynaecological disorders such as myoma, endometriosis and refertilization. None of the women had diabetes and their characteristics are summarized in Table 1. The study was approved by the local medical ethical committee.

Adipose tissue culture was performed as described previously [25] with slight modifications. In this study, 0·5 g tissue per 5 mL of M199 medium was used and after the last washing step, tissue explants were incubated for 24 h with different PA concentrations (0, 0·01, 0·1, 1, 3 and 10 mM). Regarding PTX treatment, adipose tissue explants were pre-incubated with PTX (100 ng mL^{-1}) for 2 h. Thereafter, 3 mM PA was added and incubated for 24 h. Subsequently, tissue was frozen immediately in liquid nitrogen and then stored at −80 °C until RNA was isolated. Media samples were stored at −80 °C prior to ELISA measurements.

Table 2 Primer sequences

Primer ID	Primer sequence (5′ → 3′)
Leptin forward	TCA CCA GGA TCA ATG ACA TTT CAC
Leptin reverse	AGC CCA GGA ATG AAG TCC AAA C
Leptin probe	CGC AGT CAG TCT CCT CCA AAC AGA AAG TCA
Adiponectin forward	AGG CCG TGA TGG CAG AGA T
Adiponectin reverse	GTC TCC CTT AGG ACC AAT AAG ACC T
Adiponectin probe	ATC TCC TTT CTC ACC CTT CTC ACC AGG G
Resistin forward	AAG CCA TCA ATG AGA GGA TCC A
Resistin reverse	CTC CAG GCC AAT GCT GCT TA
Resistin probe	CCC TAA ATA TTA GGG AGC CGG CGA CCT C
GAPDH forward	GGT GAA GGT CGG AGT CAA CG
GAPDH reverse	ACC ATG TAG TTG AGG TCA ATG AAG G
GAPDH probe	CGC CTG GTC ACC AGG GCT GC
GPCR41	Hs00271131_s1*
GPCR43	Hs00271142_s1*

*ID numbers of primer sets from Applied Biosystems.

Relative Q-PCR analysis

Total RNA was isolated from adipose tissue by the RNeasy lipid tissue mini kit and cDNA was synthesized using the Quantitect kit (Qiagen, Venlo, The Netherlands). Relative quantification of genes were performed in triplicate with the ABI 7900HT sequence detection system for relative real time polymerase chain reaction (Taqman; Applied Biosystems) using the ΔΔCT method. The primers pairs and probes used are displayed in Table 2. Stability of several housekeeping genes was assessed by geNorm analysis software (http://medgen.ugent.be/~jvdesomp/genorm/) [26]. GAPDH was chosen as the most stable housekeeping gene expressed in adipose tissue. PCR was performed using TaqMan Universal Master Mix in a total reaction mix volume of 10 μL. The PCR conditions were: 15 min at 95 °C, 40 cycles of 15 s at 95 °C followed by 1 min at 62 °C.

Adipokine protein quantification

Leptin and adiponectin concentrations were measured in culture media by Duoset ELISA kit in duplicate, according to the manufacturer's description (R&D Systems, Abingdon, UK); while resistin was measured in culture media and serum by Quantikine ELISA kit (R&D Systems).

Statistics

The average CV of measurements on triplicate incubations was 15·1% for mRNA and 28·1% for protein. All data are given as mean ± SEM. Comparison between two groups was performed by two-sided paired Student's *t*-test. The correlation between adipokine mRNA expression and protein level, age and anthropometric indices [body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist to hip ratio (WHR)], was calculated using the Pearson's product moment correlation coefficient test. Results were considered to be statistically significant when $P < 0{\cdot}05$.

Results

PA dose–effect relationship with adipokine mRNA expression in omental adipose tissue

OAT explants derived from 13 subjects were used to determine the dose–effect relationship of PA on adipokine mRNA expression. OAT explants of each subject were incubated in triplicate with each concentration of PA for 24 h. Figure 1 shows that leptin mRNA expression was significantly stimulated 65% and 100% by 1 mM ($P = 0{\cdot}04$) and 3 mM ($P = 0{\cdot}006$) PA treatments, respectively. By contrast, 10 mM did not influence leptin mRNA expression. This was also noticed by Le Poul *et al.* [10] who found that 10 mM did not influence neutrophils chemotaxis, while 1 mM did. On the other hand, resistin mRNA expression was significantly down-regulated 44% ($P = 0{\cdot}0026$) and 94% ($P < 0{\cdot}0001$) in adipose tissue treated with 3 and 10 mM PA respectively. None of the PA concentrations affected adiponectin mRNA expression.

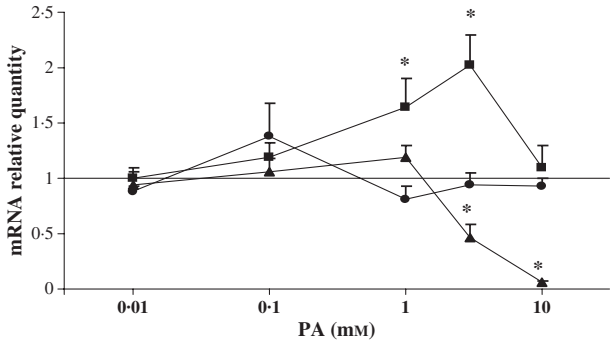

Figure 1 Dose–response curves of PA on adipokine production by human OAT *ex vivo*. OAT explants of each subject were incubated in triplicate with each concentration of PA for 24 h. Leptin (■), adiponectin (●) and resistin (▲) mRNA expression levels were determined using RT-PCR and depicted as relative quantities compared with controls (without PA). Error bars represent SEM. $*P < 0{\cdot}05$.

PA effect on adipokine mRNA expression and protein secretion by omental adipose tissue

Based on the results above, 3 mM PA was chosen for additional experiments in an extended set of explants to investigate the influence of age and anthropometric indices of the explant donors on the magnitude of the PA effect on adipokine mRNA expression. Moreover, mRNA response was validated on the protein level. Explants of each subject were incubated in triplicate with or without PA for 24 h. As represented in Fig. 2a, leptin mRNA ($N = 27$) and protein ($N = 12$) levels were significantly induced by ∼90% ($P < 0{\cdot}0001$ for both); whereas resistin expression was significantly reduced ($N = 27$; $P < 0{\cdot}0001$) on the mRNA level by 46%, but not on the protein level ($N = 12$; $P = 0{\cdot}61$). With respect to adiponectin, neither mRNA ($N = 22$) nor protein levels ($N = 12$) were changed. Induction of leptin mRNA expression after PA treatment of OAT was independent of age or anthropometric indices of the donors (Table 3). Basal resistin mRNA expression in OAT was positively correlated to the donors' age ($R = 0{\cdot}49$, $P = 0{\cdot}013$) but not to anthropometric indices, whereas the response of resistin to PA was negatively correlated to the donors' age only ($R = -0{\cdot}41$, $P = 0{\cdot}03$). While there was a positive correlation between basal resistin mRNA expression in adipose tissue with age, no correlation was observed between serum resistin and age of the explant donors.

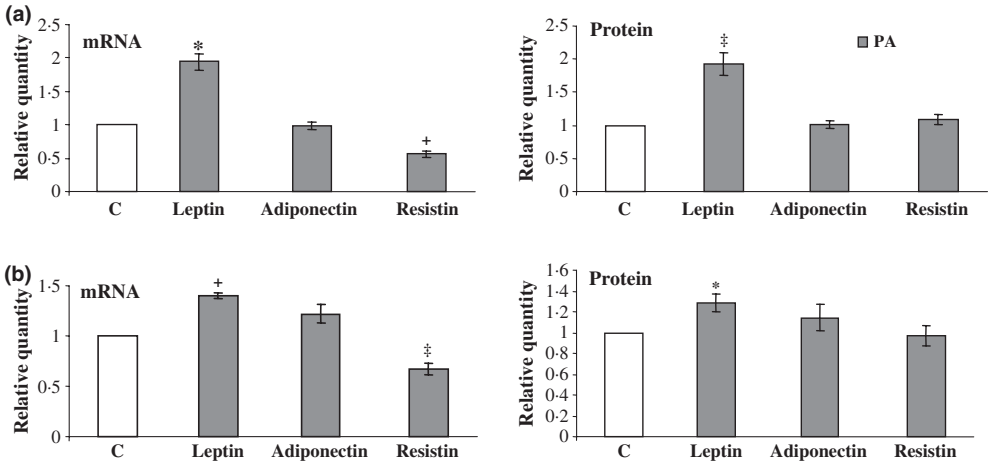

Figure 2 PA effects on adipokine production by OAT and SAT *ex vivo*. 3 mM PA (■) was chosen to validate the effect of PA on adipokine production by OAT (a) and SAT (b) on both mRNA and protein levels. OAT and SAT explants of each subject were incubated in triplicate with or without 3 mM PA for 24 h. mRNA expression levels were determined using RT-PCR and secreted adipokine levels in the media were determined using ELISA. Results are depicted as relative quantities compared with controls (without PA; □). Error bars represent SEM. $^{*}P < 0{\cdot}001$, $^{+}P < 0{\cdot}0001$ and $^{\ddagger}P < 0{\cdot}05$.

Table 3 Analysis of the correlations (r) in OAT between the mRNA expression levels of GPCR41, GPCR43 and resistin, resistin level in serum and resistin and leptin responses to 3 mM PA on the one hand and age and anthropometric indices on the other hand

	GPCR41 basal mRNA levels	GPCR43 basal mRNA levels	Resistin basal mRNA levels	Resistin basal serum levels	Resistin mRNA response to PA	Leptin mRNA response to PA
BMI	0·078	−0·23	0·088	0·29	−0·3	0·037
WC	0·099	−0·37	0·076	0·17	−0·14	0·11
HC	−0·15	−0·53*	0·22	0·17	−0·17	−0·17
WHR	0·48*	0·2	−0·083	0·035	−0·025	0·32
Age	−0·023	0·05	0·49*	0·25	−0·41*	−0·015

BMI, body mass index; HC, hip circumference; WC, waist circumference; WHR, waist-hip ratio.
$^{*}P < 0{\cdot}05$.

PA effect on adipokine mRNA expression and protein secretion by subcutaneous adipose tissue

The effect of PA was also investigated on adipokine mRNA and protein levels in SAT derived from 5 and 12 subjects respectively. Explants of each subject were incubated in triplicate with or without 3 mM PA for 24 h. As depicted in Fig. 2b, leptin protein and mRNA levels were induced by approximately 35% ($P = 0{\cdot}012$ and 0·027 respectively), resistin was reduced only on the mRNA level by 32% ($P = 0{\cdot}003$) and adiponectin was unaffected both on the mRNA and protein level.

GPCR41 and GPCR43 expression in human omental and subcutaneous adipose tissues

The SCFA are ligands for the G protein-coupled receptor GPCR41 and GPCR43 [9,10]. Therefore, we determined their expression in human OAT and SAT. As shown in Fig. 3, we found that GPCR41 was expressed at similar levels as GPCR43 in each adipose depot. However, levels of both receptors in SAT were approximately 10-fold higher than in OAT ($P = 0{\cdot}009$ and 0·021 for GPCR41 and GPCR43 respectively). We also determined whether GPCR41 and GPCR43 expression in OAT

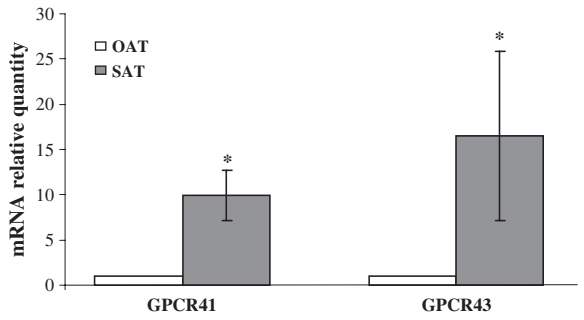

Figure 3 Gene expression levels of GPCR41 and GPCR43 in OAT and SAT *ex vivo*. mRNA expression of each receptor in SAT was determined using RT-PCR and depicted as a relative quantity compared with OAT. Error bars represent SEM. $^{*}P < 0{\cdot}05$.

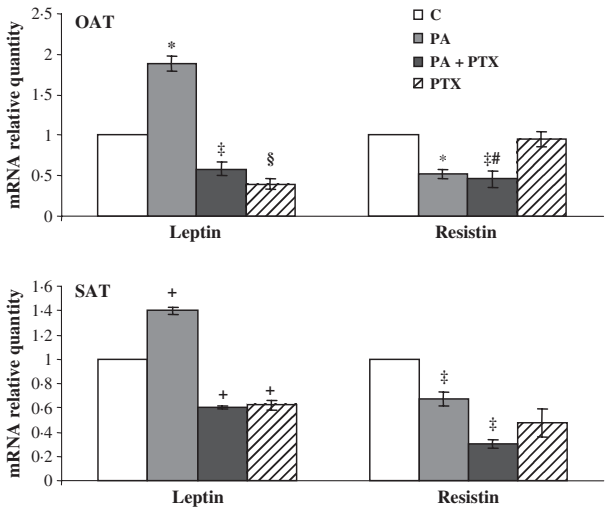

Figure 4 Involvement of G-protein coupled receptor(s). The involvement of G-protein coupled receptors in mediating PA effects on adipokines expression was determined by blocking the Gi/o signalling pathway with PTX. OAT and SAT explants of each subject were incubated in triplicate with PTX (100 ng/ml) for 2 h before incubation for 24 h with or without 3 mM PA. mRNA expression levels were determined using RT-PCR and depicted as relative quantities compared with controls (without PA). Error bars represent SEM. $^{*}P \leq 0{\cdot}001$ vs. control; $^{\ddagger}P < 0{\cdot}01$ vs. control; $^{\S}P < 0{\cdot}01$ vs. PA + PTX, $^{\#}P < 0{\cdot}05$ vs PTX and $^{+}P < 0{\cdot}05$ vs. control.

($N = 21$, 3 biopsies/subject) was correlated to age and anthropometric indices of the donors (Table 3). GPCR41 mRNA expression had a significant and positive correlation with WHR ($R = 0{\cdot}48$, $P = 0{\cdot}03$); whereas GPCR43 expression correlated negatively to HC ($R = -0{\cdot}53$, $P = 0{\cdot}013$).

Involvement of Gi/o-protein coupled receptors in the effect of PA on leptin and resistin mRNA expression

In mouse adipocytes, it has been shown that PA mediates its effect on leptin production via Gi/o-protein coupled receptor (Gi/o-PCR) [12]. To determine whether PA uses GPCR coupled to Gi/o proteins to affect leptin and resistin expression in human adipose tissue, OAT and SAT biopsies taken from six and five subjects respectively, were pretreated with PTX for 2 h to block the Gi/o pathway and then they were treated with or without 3 mM PA for 24 h. Figure 4 illustrates that induction of leptin expression by PA was completely abolished by PTX pretreatment in both OAT and SAT, while the PA-induced reduction in resistin expression was not affected by PTX. PTX treatment also reduced baseline leptin expression by approximately 60% and 40% in both OAT and SAT respectively ($P = 0{\cdot}004$ and $0{\cdot}01$ respectively). In OAT, PA plus PTX could not completely abolish leptin mRNA expression to the level observed with PTX alone.

Discussion

The present human *ex vivo* study reveals that PA influences adipokine production by both adipose tissue depots (OAT and SAT). Explants were derived from women without diabetes with BMI's ranging between 20·8 and 33·3 kg m^{-2}. PA induced leptin, both on the mRNA and protein level, while it almost completely abolished resistin mRNA expression. On the other hand, adiponectin expression was not influenced. The magnitude of the PA effect on leptin was 2·6-fold higher in OAT compared with SAT and was independent of the degree of adiposity and age of the explant donors. Induction of leptin by PA may indicate that PA reduces food intake and increases energy expenditure, as leptin is a potent anorexigenic hormone [17]. In sheep, PA has been shown to induce leptin expression in adipose tissue [15] and to reduce food intake [16,27]. The observed PA-induced reduction of resistin mRNA expression was slightly more pronounced in OAT compared with SAT and was negatively associated with age but independent of the degree of adiposity of the explant donors. However, on the protein level no significant change in resistin expression was seen. This discrepancy between the mRNA and the protein level may be explained by the fact that resistin is only released from the cell surface membrane or intracellular stores after an inflammatory trigger [27]. Therefore, a reduction in resistin protein only can be observed when cellular stores are depleted. Resistin is pro-inflammatory and, in human adipose tissue, is predominantly produced by macrophages [28]. Resistin is also a marker for insulin resistance in humans [21–23]. The PA-induced decrease in resistin expression may imply that PA is acting as

an anti-inflammatory agent. This is in agreement with other studies in which PA has been shown to have anti-inflammatory effects on human neutrophils and mouse colonic organ cultures [29]. Furthermore, PA inhibits proliferation of granulocyte/macrophage, haematopoietic progenitor cells and lymphocyte activation [30–33]. The observed improvement of insulin sensitivity in humans after high DF diets [4–6] may be attributed to this anti-inflammatory effect of PA as in obese persons adipose tissue inflammation and insulin resistance are closely linked [34]. In addition to PA, we investigated other SCFA. Our preliminary results show that other SCFA may also have an effect on adipokine expression (not shown). This is the focus of our future studies.

The expression of GPCR41 and GPCR43 by human adipose tissue was shown to be controversial. For example, Le Poul *et al.* [10] showed that GPCR41 and GPCR43 were expressed in human adipose tissue, while Hong *et al.* [13] showed it was not expressed. In addition, in these previous studies the adipose tissue depot was not specified. Our results show that both human adipose tissue depots expressed GPCR41 and GPCR43 mRNA. SAT expressed approximately 10-fold higher amounts of each receptor than OAT, suggesting that SAT is more responsive to PA than OAT. However, we found slightly higher responses to PA in OAT compared with SAT. Furthermore, we found that GPCR41 mRNA level in OAT was positively associated with WHR, as a measure of adiposity, while GPCR43 was negatively associated with HC (Table 3). However, no correlation with other measures of obesity (BMI and WC) was found. Therefore, the relevance of this observation remains unclear.

PTX abolished leptin induction by PA in both human OAT and SAT similar to what has been observed in mouse adipocytes [12]. This suggests that PA regulates leptin production through GPCR coupled to the Gi/o signalling pathway. However, the effect of PA on resistin expression was not inhibited by PTX. Le Poul *et al.* [10] showed that there is a unique Gi/o coupling for GPCR41 but for GPCR43, a dual coupling exists through Gi/o and Gq. Furthermore, in human adipose tissue, resistin is almost exclusively expressed in macrophages [35] while leptin expression is specific for adipocytes [36]. This may suggest that leptin induction by PA is mediated via GPCR41 on adipocytes, coupled to Gi/o, while the reduction of resistin by PA is mediated via GPCR43 on resident macrophages, coupled to Gq.

In *in vitro* studies [30–33], including our study, relatively high PA concentrations (1–10 mM) are needed for the observed effects. The question rises whether colonic fermentation can provide such concentrations. As a result of the absence of blood flow, the effective concentration in tissue cultures probably is substantially higher than *in vivo*. The PA concentration in the human colon is approximately 20 mmol kg^{-1} [7]. In ruminants, 50% of absorbed PA reaches the portal vein [37], indicating that the remainder is utilized by tissues it encounters such as the colonic wall and possibly OAT. *In vivo*, the exposure time of adipose tissue to PA may be an important factor. Furthermore, effects of different SCFA may be additive as acetate, propionate and butyrate all share the same receptors [9,10]. Further *in vivo* measurements have to confirm this.

In conclusion, this study demonstrates for the first time that PA influences adipokine secretion by human adipose tissue. We show that leptin and resistin, but not adiponectin production is affected by PA and involves GPCR signalling. These findings suggest that modulation of PA quantity through e.g. dietary manipulation, prebiotics and probiotics, or dietary supplementation may have potential in preventing obesity and its associated complications such as inflammation and insulin resistance.

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References

1 Higgins JA, Higbee DR, Donahoo WT, Brown IL, Bell ML, Bessesen DH. Resistant starch consumption promotes lipid oxidation. *Nutr Metab* 2004;**1**:8.
2 Higgins JA, Brown MA, Storlien LH. Consumption of resistant starch decreases postprandial lipogenesis in white adipose tissue of the rat. *Nutr J* 2006;**5**:25.
3 So PW, Yu WS, Kuo YT, Wasserfall C, Goldstone AP, Bell JD *et al.* Impact of resistant starch on body fat patterning and central appetite regulation. *PLoS One* 2007;**2**:e1309.

4 Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr* 2005;**82**:559–67.
5 Weickert MO, Mohlig M, Schofl C, Arafat AM, Otto B, Viehoff H *et al*. Cereal fiber improves whole-body insulin sensitivity in overweight and obese women. *Diabetes Care* 2006;**29**:775–80.
6 Keenan MJ, Zhou J, McCutcheon KL, Raggio AM, Bateman HG, Todd E *et al*. Effects of resistant starch, a non-digestible fermentable fiber, on reducing body fat. *Obesity* 2006;**14**:1523–34.
7 Cummings JH, Pomare EW, Branch WJ, Nayor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987;**28**:1221–7.
8 Hur EM, Kim KT. G protein-coupled receptor signalling and cross-talk: achieving rapidity and specificity. *Cell Signal* 2002;**14**:397–405.
9 Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D *et al*. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem* 2003;**278**:11312–9.
10 Le Poul E, Loison C, Struyf S, Springael JY, Lannoy V, Decobecq ME *et al*. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem* 2003;**278**:25481–9.
11 Cook SI, Sellin JH. Review article: short chain fatty acids in health and disease. *Aliment Pharmacol Ther* 1998;**12**:499–507.
12 Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM *et al*. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proc Natl Acad Sci USA* 2004;**101**:1045–50.
13 Hong YH, Nishimura Y, Hishikawa D, Tsuzuki H, Miyahara H, Gotoh C *et al*. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* 2005;**146**:5092–9.
14 Soliman M, Kimura K, Ahmed M, Yamaji D, Matsushita Y, Okamatsu-Ogura Y *et al*. Inverse regulation of leptin mRNA expression by short- and long-chain fatty acids in cultured bovine adipocytes. *Domest Anim Endocrinol* 2007;**33**:400–9.
15 Lee SH, Hossner KL. Coordinate regulation of ovine adipose tissue gene expression by propionate. *J Anim Sci* 2002;**80**:2840–9.
16 Allen MS. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J Dairy Sci* 2000;**83**:1598–624.
17 Friedman JM. Obesity: causes and control of excess body fat. *Nature* 2009;**459**:340–2.
18 Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 2008;**93**(Suppl. 1):S64–73.
19 Kusminski CM, da Silva NF, Creely SJ, Fisher FM, Harte AL, Baker AR *et al*. The *in vitro* effects of resistin on the innate immune signaling pathway in isolated human subcutaneous adipocytes. *J Clin Endocrinol Metab* 2007;**92**:270–6.
20 Bertolani C, Sancho-Bru P, Failli P, Bataller R, Aleffi S, DeFranco R *et al*. Resistin as an intrahepatic cytokine: overexpression during chronic injury and induction of proinflammatory actions in hepatic stellate cells. *Am J Pathol* 2006;**169**:2042–53.
21 Qatanani M, Szwergold NR, Greaves DR, Ahima RS, Lazar MA. Macrophage-derived human resistin exacerbates adipose tissue inflammation and insulin resistance in mice. *J Clin Invest* 2009;**119**:531–9.
22 Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB Sr, Wilson PW *et al*. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. *J Clin Endocrinol Metab* 2008;**93**:3165–72.
23 Frankel DS, Vasan RS, D'Agostino RB Sr, Benjamin EJ, Levy D, Wang TJ *et al*. Resistin, adiponectin, and risk of heart failure the Framingham offspring study. *J Am Coll Cardiol* 2009;**53**:754–62.
24 Butler J, Kalogeropoulos A, Georgiopoulou V, de Rekeneire N, Rodondi N, Smith AL *et al*. Serum resistin concentrations and risk of new onset heart failure in older persons: the health, aging, and body composition (Health ABC) study. *Arterioscler Thromb Vasc Biol* 2009;**29**:1144–9.
25 Alvarez-Llamas G, Szalowska E, de Vries MP, Weening D, Landman K, Hoek A *et al*. Characterization of the human visceral adipose tissue secretome. *Mol Cell Proteomics* 2007;**6**:589–600.
26 Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A *et al*. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;**3**:RESEARCH0034.1–0034.11.
27 Farningham DA, Whyte CC. The role of propionate and acetate in the control of food intake in sheep. *Br J Nutr* 1993;**70**:37–46.
28 Boström EA, Tarkowski A, Bokarewa M. Resistin is stored in neutrophil granules being released upon challenge with inflammatory stimuli. *Biochim Biophys Acta* 2009;**1793**:1894–900.
29 Tedelind S, Westberg F, Kjerrulf M, Vidal A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. *World J Gastroenterol* 2007;**13**:2826–32.
30 Stork LC, Ambruso DR, Wallner SF, Sambrano JE, Moscinski LC, Wilson HL *et al*. Pancytopenia in propionic acidemia: hematologic evaluation and studies of hematopoiesis *in vitro*. *Pediatr Res* 1986;**20**:783–8.
31 Wajner M, Santos KD, Schlottfeldt JL, Rocha MP, Wannmacher CM. Inhibition of mitogen-activated proliferation of human peripheral lymphocytes *in vitro* by propionic acid. *Clin Sci* 1999;**96**:99–103.
32 Hutchinson RJ, Bunnell K, Thoene JG. Suppression of granulopoietic progenitor cell proliferation by metabolites of the branched-chain amino acids. *J Pediatr* 1985;**106**:62–5.
33 Curi R, Bond JA, Calder PC, Newsholme EA. Propionate regulates lymphocyte proliferation and metabolism. *Gen Pharmacol* 1993;**24**:591–7.
34 Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;**444**:860–7.
35 Curat CA, Wegner V, Sengenès C, Miranville A, Tonus C, Busse R *et al*. Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia* 2006;**49**:744–7.
36 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;**372**:425–32.
37 Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 1990;**70**:567–90.