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ORIGINAL ARTICLE

Propionic acid affects immune status and metabolism in adipose tissue from overweight subjects

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ABSTRACT

Background Adipose tissue is a primary site of obesity-induced inflammation, which is emerging as an important contributor to obesity-related diseases such as type 2 diabetes. Dietary fibre consumption appears to be protective. Short-chain fatty acids, e.g. propionic acid, are the principal products of the colonic fermentation of dietary fibre and may have beneficial effects on adipose tissue inflammation.

Materials and methods Human omental adipose tissue explants were obtained from overweight (mean BMI 28·8) gynaecological patients who underwent surgery. Explants were incubated for 24 h with propionic acid. Human THP-1 monocytic cells were differentiated to macrophages and incubated with LPS in the presence and absence of propionic acid. Cytokine and chemokine production were determined by multiplex-ELISA, and mRNA expression of metabolic and macrophages genes was determined by RT-PCR.

Results Treatment of adipose tissue explants with propionic acid results in a significant down-regulation of several inflammatory cytokines and chemokines such as TNF-α and CCL5. In addition, expression of lipoprotein lipase and GLUT4, associated with lipogenesis and glucose uptake, respectively, increased. Similar effects on cytokine and chemokine production by macrophages were observed.

Conclusion We show that propionic acid, normally produced in the colon, may have a direct beneficial effect on visceral adipose tissue, reducing obesity-associated inflammation and increasing lipogenesis and glucose uptake. Effects on adipose tissue as a whole are at least partially explained by effects on macrophages but likely also adipocytes are involved. This suggests that, *in vivo*, propionic acid and dietary fibres may have potential in preventing obesity-related inflammation and associated diseases.

Keywords Adipose tissue, dietary fibre, inflammation, propionic acid, short-chain fatty acids.

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Introduction

During obesity, adipose tissue can be a site of low-grade inflammation [1]. In obese adipose tissue, the production of several inflammatory cytokines and chemokines and the infiltration of inflammatory cells is increased [2]. Adipose tissue inflammation is associated with the pathogenesis of obesity-related diseases such as type 2 diabetes and cardiovascular diseases [3,4]. A large body of evidence indicates that dietary fibre consumption has a profound effect on human health. This includes the increase in postmeal satiety and the decrease in body weight, fat mass and the severity of type 2 diabetes [5–10]. These effects may be caused via colonic fermentation of dietary fibre. Various metabolites are produced, such as short-chain fatty acids (SCFA), which are absorbed by the host and influence its energy homoeostasis [6,11,12]. Colonic metabolism can influence the development of obesity and its associated diseases through different mechanisms, recently reviewed [11,13].

It has been demonstrated that SCFA in particular acetate, butyrate and propionate inhibit inflammation. However, most of the studies focused on butyrate and to a lesser extent on acetate, while the effects of propionic acid (PA) in humans have remained largely unexplored [14,15]. Therefore, we focused on PA. Fermentation of dietary fibre and/or resistant starch by the colonic microbiota is the primary source of PA. Despite the technical and ethical issues involved, a few studies measured the quantities of different SCFA in human portal vein and peripheral blood. PA quantity in the human colon was reported to be 20 mmol/kg and to depend on the balance between production and absorption [16,17], the type of microbiota, the quantity and the type of the substrate and the gut transit time [18]. The majority of the PA produced in the colon is absorbed, passes the colonocytes and the viscera, and drains into the portal vein. PA can be partially metabolized by colonocytes; however, the quantity that is utilized by visceral tissues, e.g. visceral adipose tissue, has not been examined yet. The quantity of PA in the portal vein in nonfasting humans was demonstrated to be approximately 0·1 mM; while it was threefold lower in blood derived from fasting humans [16,19,20]. Around 90% of PA in the portal vein is metabolized by the liver and the rest is transported into peripheral blood [18], where a concentration of 6 μM in humans was reported [19,21–23], far in excess of that of butyrate, but lower than that of acetate.

Propionic acid and other SCFA are potent and efficacious ligands for the G-protein-coupled receptors 41 (GPCR41) and 43 (GPCR43) [24]. GPCR43 knockout mice showed exacerbated inflammation in models of inflammatory diseases, i.e., colitis, arthritis and asthma [25]. These experiments strongly support the role of colonic PA production as an important anti-inflammatory mechanism. In addition, in previous experiments, we have shown *ex vivo*, in human adipose tissue explants, that PA influences adipokine secretion by stimulating leptin and reducing resistin expression [26], suggesting a role in satiety and inflammation. As adipose tissue is a major contributor to obesity-induced low-grade inflammation [27] and as both GPCR receptors of PA are present on adipose tissue [26], one could envision that colonic microbiota metabolism, via PA, influence obesity-induced low-grade inflammation in adipose tissue. Therefore, in this study, we investigated the influence of PA on several inflammatory and metabolic parameters in human omental adipose tissue (OAT) explants and in macrophages. We show that, in OAT, PA reduced the production of a panel of chemokines and cytokines and adipose tissue macrophage markers as well as expression of genes involved in glucose and lipid metabolism. Thus, providing evidence that a microbial metabolite, *in casu* PA, is able to modulate the inflammatory reaction in human adipose tissue as well as glucose and lipid metabolism. This suggests that PA and dietary fibres may have potential in preventing obesity-related inflammation and associated diseases.

Materials and methods

Materials

Gentamycin, glucose, LPS and PA were purchased from Sigma (Zwijndrecht, The Netherlands). M199 media was purchased from Invitrogen (Breda, the Netherlands). CD16A, CD31, CD163 and MMP-9 primers were purchased from Applied Biosystems (Nieuwerkerk a/d IJssel, the Netherlands), whereas the rest of the primers were purchased from Biolegio (Nijmegen, the Netherlands).

Human adipose tissue culture

Omental adipose tissue explants were obtained from women who underwent surgery for gynaecologic disorders such as myoma and prolapse. None of the women had diabetes and their anthropometric indices are presented in Table 1. The study was approved by the local medical ethical committee. Adipose tissue culture was performed as described previously [26] with slight modifications. Briefly, adipose tissue explants were transported from the operating room to the laboratory in transport buffer (PBS, 5·5 mM glucose, 50 μg/mL gentamicin). Immediately upon arrival, tissue was transferred to a Petri dish containing 20 mL of PBS and was finely minced in 20–80 mg pieces using scissors. Tissue pieces were extensively washed with PBS over a filter. The pieces were transferred to a tube containing 50 mL of PBS and centrifuged for 1 min at 277 *g* to remove red blood cells and debris. The weight of the tissue was determined, and pieces were distributed over 6-well plates (0·5 g/5 mL). The dishes were incubated at 37 °C at 5% CO_2. The medium was renewed after 1, 18·5, 22·5 and 26·5 h to remove serum proteins. After the last washing step, tissue explants were incubated for 24 h with or without 3 mM PA. Subsequently, tissue pieces were snap frozen in liquid nitrogen and then stored at −80 °C until RNA was isolated. Media samples were stored at −80 °C prior to ELISA measurements.

Cell culture

Human THP-1 monocytic cell line was maintained in RPMI-1640 phenol red-free media supplemented with 10% FBS,

Table 1 Anthropometric indices of adipose tissue donors

Subject ID	BMI	WHR	WC
S-1	30·44	0·82	95
S-2	27·46	0·86	90
S-3	30·85	0·93	95
S-4	31·89	0·91	104
S-5	23·59	0·84	82

BMI, body mass index; WHR, waist hip ratio; WC, Waist circumference.

100 U/mL of penicillin and 100 μg/mL of streptomycin in humid atmosphere containing 5% CO_2 at 37 °C. To induce monocyte-macrophage differentiation, THP-1 cells were seeded at a concentration of 5×10^5 cells/mL and were differentiated with 10 ng/mL PMA for 48 h. THP-1-derived macrophages were treated for 2 h, in triplicate, with 1 μg/mL LPS alone or in combination with various concentrations of PA (0·001, 0·01, 0·1, 1 and 10 mM).

Relative Q-PCR analysis

Total RNA was isolated by the RNeasy lipid tissue mini kit, and cDNA was synthesized using the Quantitect kit (both from 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 $\Delta\Delta C_T$ 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/) [28]. 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.

Protein quantification

Secreted chemokines and cytokines were measured in culture media by multiplex-ELISA according to the manufacturer's description (Bio-Rad, Hercules, CA, USA). TNF-α was also determined by ELISA (Duoset; R&D Systems, Minneapolis, MN, USA). If necessary, samples were concentrated ten times using ultra filtration (Sartorius Stedim Biotech, Goettingen, Germany).

Statistics

Comparison between two groups was performed by two-sided paired Student's *t*-test. Results were considered to be statistically significant at $P < 0{\cdot}05$.

Table 2 Primers sequences

Primer ID	Primer sequence (5′-3′)
GAPDH forward	GGT GAA GGT CGG AGT CAA CG
GAPDH backward	ACC ATG TAG TTG AGG TCA ATG AAG G
GAPDH probe	CGC CTG GTC ACC AGG GCT GC
GLUT4 forward	GCT GTG GCT GGT TTC TCC AA
GLUT4 backward	CCC ATA GCC TCC GCA ACA TA
GLUT4 probe	CGA GCA ACT TCA TCA TTG GCA TGG GTT
LPL forward	TGG AGA TGT GGA CCA GCT AGT G
LPL backward	CAG AGA GTC GAT GAA GAG ATG AAT G
LPL probe	CTC CCA CGA GCG CT
SREBP1c forward	GGA TTG CAC TTT CGA AGA CAT G
SREBP1c backward	AGC ATA GGG TGG GTC AAA TAG G
SREBP1c probe	CAG CTT ATC AAC AAC CAA GAC AGT GAC TTC CC
CD163 forward	TGC AGA AAA CCC CAC AAA AAG
CD163 backward	CAA GGA TCC CGA CTG CAA TAA
CD163 probe	AAC AGG TCG CTC ATG CCG TCA GTC A
CD16A	Hs01569121_m1*
CD31	Hs01065282_m1*
MMP-9	Hs00234579_m1*

LPL, lipoprotein lipase; SREBP-1c, sterol regulatory-element-binding protein-1c. *ID numbers of primer sets from Applied Biosystems.

Results

Propionic acid inhibits cytokine and chemokine secretion by human adipose tissue

We previously showed that 3 mM PA is optimal for inducing leptin production in human adipose tissue [26]. Because resistin has been associated with an inflammatory response [29], we investigated the effect of PA on the basal levels of several chemokines and cytokines in human OAT explants derived from five women with BMI's ranging from 23·6 to 31·9 (Table 1). Treatment with 3 mM PA for 24 h significantly inhibits its basal secretion of interleukin-4 (IL-4), interleukin-10 (IL-10), tumour necrosis factor-α (TNF-α), granulocyte colony-stimulating factor (G-CSF), interferon-gamma-induced protein (IP-10), macrophage inflammatory proteins-1α and -1β (MIP-1α and MIP-1β) and CCL5 (RANTES). Interleukin-1β (IL-1β) and interleukin-8 (IL-8) and monocyte chemo tactic protein-1 (MCP-1) were not influenced, while interleukin-12 (IL-12) and interleukin-13 (IL-13) were not detectable (Fig. 1). Absolute concentrations are shown in Table 3. The largest decrease was found for IL-10 (68·1%) followed by IP-10 (60·1%), TNF-α (59·9%) and RANTES (58·7%). G-CSF, MIP-1β and MIP-1α were reduced by 50·4%, 46·3% and 35·7%, respectively. Only a marginal decrease in IL-4 (14·8%) was detected.

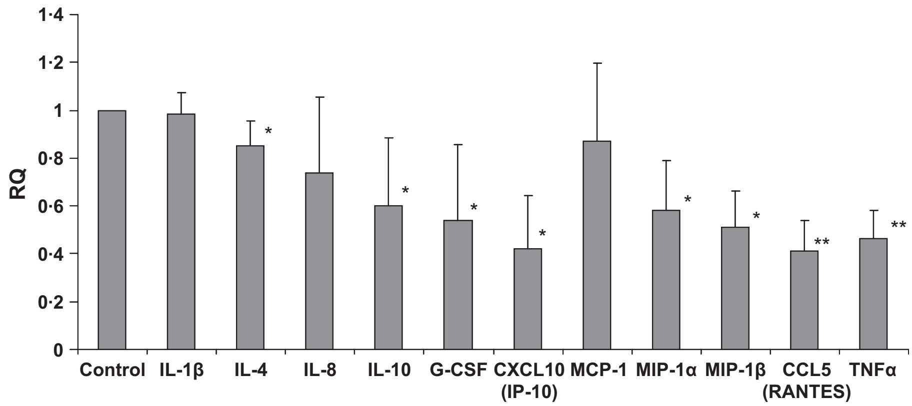

Figure 1 The effect of propionic acid (PA) on the secretion of chemokines and cytokines by human omental adipose tissue (OAT). OAT explants of five overweight subjects were incubated in triplicate with or without 3 mM PA for 24 h. The secretion of the listed chemokines and cytokines were determined in the media by multiplex-ELISA. Results are depicted as relative quantities (RQ) compared with the control (without PA). The measured concentrations are listed in Table 3. Error bars represent ± SD. *$P < 0{\cdot}05$ vs. control.

Propionic acid stimulates expression of sterol regulatory-element-binding protein-1c and key metabolic genes in human adipose tissue

Glucose and lipid uptake are key metabolic processes executed by adipose tissue. Therefore, we measured the effect of PA on the expression of the insulin responsive glucose transporter (GLUT-4), lipoprotein lipase (LPL) as well as the transcription factor sterol regulatory-element-binding protein-1c (SREBP-1c) in four explants in triplicate (Fig. 2a). Both GLUT-4 and LPL are regulated by SREBP-1c [30,31]. We found that PA up-regulates GLUT-4, LPL and SREBP-1c mRNA expression by 54·4%, 55·5% and 36·1%, respectively.

Table 3 Effect of PA on cytokine and chemokine secretion by human omental adipose tissue

	Control	PA
IL-1β	6·62 ± 0·59	6·49 ± 0·68
IL-4	1·42 ± 0·12	1·21 ± 0·21
IL-8	64254 ± 21049	48711 ± 31752
IL-10	30·1 ± 42·1	9·60 ± 5·87
IL-12	N.D.	N.D.
IL-13	N.D.	N.D.
G-CSF	256 ± 222	127 ± 150
CXCL10 (IP-10)	1510 ± 911	589 ± 566
MCP-1	5439 ± 4386	36710 ± 1198
MIP-1α	84·6 ± 32·5	54·4 ± 34·4
MIP-1β	382 ± 101	205 ± 89
CCL5 (RANTES)	252 ± 120	104 ± 61·1
TNF-α	12·2 ± 17·1	4·89 ± 6·69

G-CSF, granulocyte colony-stimulating factor; N.D., not detectable; PA, propionic acid. Concentrations in pg/mL in 10-fold concentrated media.

Propionic acid inhibits expression of macrophage-specific genes in human adipose tissue

To determine whether the effects of PA on chemokine and cytokine production could involve adipose tissue macrophages, we determined the effect of PA on established macrophage markers. Human adipose tissue explants obtained from four women were incubated with or without 3 mM PA in triplicate, and mRNA expression of the human macrophage-specific markers CD163, CD16A and metalloproteinase-9 (MMP-9) as well as the endothelial marker CD31 was determined (Fig. 2b). PA down-regulated these genes by 44·7%, 29·7%, 59·3% and 14·5%, respectively.

Propionic acid inhibits cytokine and chemokine secretion by human macrophages

The inhibition of expression of macrophage marker genes in adipose tissue by PA suggests that adipose tissue macrophages are a target for PA in adipose tissue. Therefore, we studied the effect of PA on cytokine and chemokine production by THP-1-derived human macrophages. First, we determined expression of the SCFA receptors GPCR41 and GPCR43 in these macrophages (Fig. 3a). Both receptors are expressed. GPCR43 is 3·34-fold higher expressed compared with GPCR41. Next, we

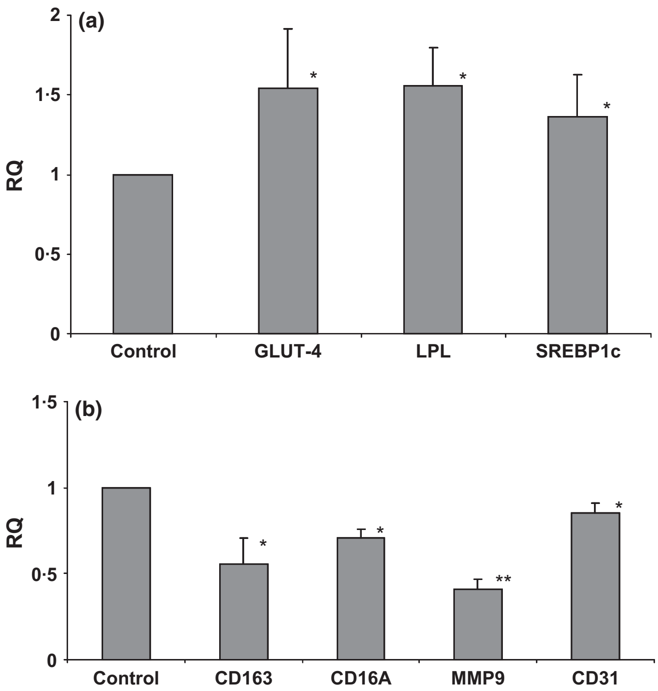

Figure 2 The effect of propionic acid (PA) on the expression of key metabolic genes and macrophage-specific markers in adipose tissue. Omental adipose tissue explants of five overweight subjects were incubated in triplicate with or without 3 mM PA for 24 h. mRNA expression levels were determined by RT-PCR and depicted as relative quantities (RQ) compared with the control (without PA). (a) The effect of PA on mRNA expression of GLUT4 (glucose metabolism) and LPL (lipogenesis) and the transcription factor SREBP1c. (b) The effect of PA on mRNA expression of the macrophage markers CD163, CD16A and MMP-9 and the endothelial marker CD31 (PECAM-1) in adipose tissue. Error bars represent ± SD. *$P < 0{\cdot}05$ **$P < 0{\cdot}001$ vs. control.

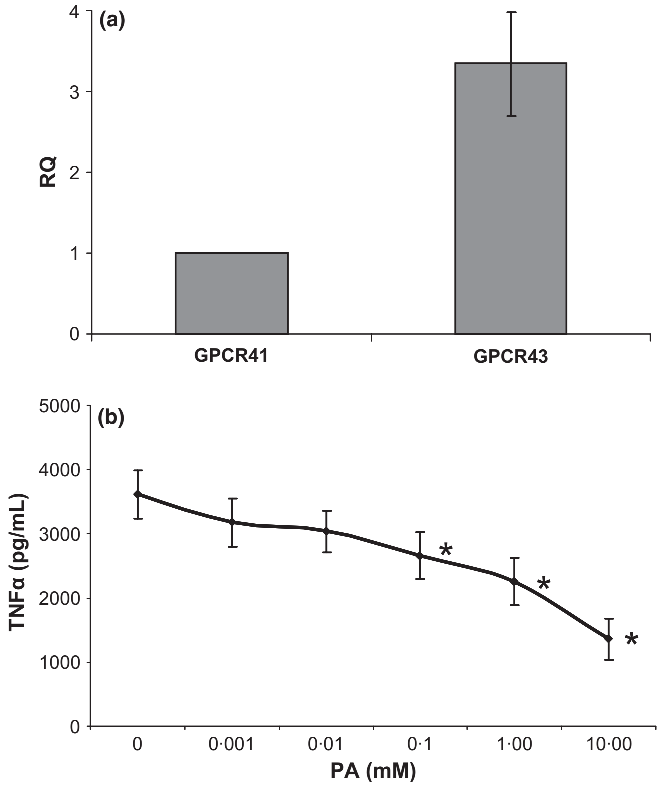

Figure 3 Expression of short-chain fatty acid receptors in human macrophages and inhibition of LPS-induced TNF-α secretion by propionic acid (PA). (a) The level of mRNA expression of G-protein-coupled receptor 43 (GPCR43) in THP-1-derived macrophages was determined by RT-PCR and depicted as a relative quantity (RQ) compared with GPCR41. (b) THP-1-derived macrophages were incubated in triplicate with 1 μg/mL LPS alone or in combination PA (0·001–10 mM) for two hours. Dose–response curves of PA on TNF-α secretion from THP-1-derived macrophages. TNF-α in the media was determined by ELISA. Error bars represent ± SD. *$P < 0{\cdot}05$ vs. control (LPS alone).

determined the effect of PA on LPS-induced TNF-α production. Macrophages were incubated in triplicate with 1 μg/mL LPS, to induce TNF-α, and with different concentrations of PA (0·001, 0·01, 0·1, 1 and 10 mM) for 2 h. A gradual decrease in TNF-α secretion was observed with increasing PA concentrations, which became significantly different with 100 μM PA (26·6% inhibition) (Fig. 3b). A maximal inhibition of TNF-α production of 62·5% was found with 10 mM PA. The latter condition was used in a new experiment where we determined the effect of PA on the production of a number of cytokines and chemokines by macrophages (Fig. 4). PA significantly inhibited the secretion of IL-10, G-CSF, MCP-1, CCL5 and TNF-α. while IL-1β, IL-8, IP-10, MIP-1α and MIP-1β were not influenced. IL-12 and IL-13 were not detectable. Absolute concentrations are shown in Table 4. The largest decrease was found for IL-10 (77·5%) followed by G-CSF (46·9%) MCP1 (44·7%) TNF-α (36·0%) and CCL5 (28·6%). In contrast to adipose tissue (Fig. 1), an increase in IL-4 (61·2%) was detected. However, absolute IL-4 concentrations were low (Table 4).

Discussion

It is becoming clear that especially (prebiotic) diets, favouring the production of SCFA by the colonic microbiota, are associated with a reduction in obesity-related diseases [11,14,15,32] suggesting that adipose tissue may be a target for

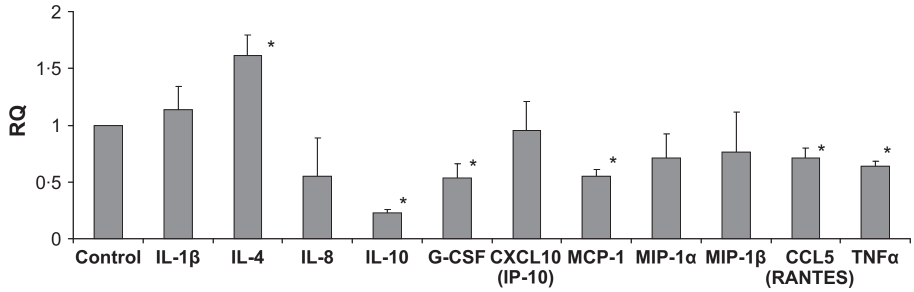

Figure 4 The effect of propionic acid (PA) on the LPS-induced secretion of chemokines and cytokines by human macrophages. THP-1-derived macrophages were incubated in triplicate with 1 μg/mL LPS alone or in combination with 10 mM PA for 2 h. Chemokines and cytokines in the media were determined by multiplex-ELISA. Results are depicted as relative quantities (RQ) compared with control (LPS alone). Measured concentrations are listed in Table 4. Error bars represent ± SD. *$P < 0{\cdot}05$ vs. control.

colon-produced SCFA. Indeed, we previously showed that both human subcutaneous and OAT express the SCFA receptors GPCR41 and GPCR43 and that PA is able to induce leptin and reduce resistin production while leaving adiponectin unaffected [26]. In the present study, we studied the immune-modulating effect of PA in explants of human OAT from overweight persons (average BMI 28·8), which produce measurable amounts of cytokines and chemokines and show that PA is able to inhibit their production. PA could inhibit the production of inflammatory (TNF-α) as well as anti-inflammatory (IL-4, IL-10) cytokines. Furthermore, production of a number of chemokines (IL-8, MIP-1α and MIP-1β, CCL5 and CXCL10) was decreased by PA. Chemokines are crucial for the attraction of mononuclear cells from the circulation into adipose tissue [33,34]. The PA-induced inhibition of chemokines may reduce the infiltration of immune cells into adipose tissue and therefore may suppress the propagation of adipose tissue inflammation in obese adipose tissue [35]. Therefore, although some anti-inflammatory cytokines are also affected, results suggest that the net effect of PA on adipose tissue is anti-inflammatory. Besides this immune-modulating effect of PA, we also show that PA affects two major metabolic pathways in adipose tissue namely lipogenesis and glucose metabolism because both LPL and GLUT4 expression were found to be up-regulated by PA. Expression of LPL and GLUT4 is known to be regulated by SREBP1c [30,31]. Indeed, we found an increased SREBP1c expression after PA stimulation, suggesting that SREBP1c is responsible for the increased expression of LPL and GLUT4. These data suggest that besides an anti-inflammatory effect, PA also has an, insulin like, anabolic effect, stimulating two important metabolic pathways that are also stimulated by insulin. In fact, although speculative, PA may possibly stimulate glucose and lipid uptake independently of the insulin signalling pathway via direct stimulation of SREBP1c. This would implicate that PA may be able to stimulate glucose and lipid uptake in insulin resistant cells/tissues. If so, this might improve blood glucose levels in insulin resistant/patients with type 2 diabetes.

We also show that the expression of CD163, CD16A and MMP-9, specific macrophage marker genes, is affected by PA suggesting that macrophages are target cells for SCFA within adipose tissue. Therefore, we conducted studies with *in vitro* differentiated human THP-1 macrophages. Although it would be better to use isolated human adipose tissue macrophages, these are very difficult to isolate with sufficient purity and quantity to perform these experiments. We show that THP-1-derived macrophages express both SCFA receptors with a

Table 4 Effect of PA on cytokine and chemokine secretion by human THP-1 macrophages

	LPS	LPS + PA
IL-1β	171 ± 267	194 ± 403
IL-4	0·25 ± 0·23	0·41 ± 0·13
IL-8	761 ± 4590	420 ± 870
IL-10	4·26 ± 6·70	0·96 ± 0·46
IL-12	N.D.	N.D.
IL-13	N.D.	N.D.
G-CSF	1·55 ± 1·68	0·82 ± 0·98
CXCL10 (IP-10)	26·7 ± 43·5	25·4 ± 82·4
MCP-1	8·46 ± 7·47	4·68 ± 1·00
MIP-1α	134 ± 445	95·3 ± 215
MIP-1β	207 ± 712	158 ± 286
CCL5 (RANTES)	255 ± 310	182 ± 50·6
TNF-α	2046 ± 1428	1308 ± 494

G-CSF, granulocyte colony-stimulating factor; N.D., not detectable; PA, propionic acid. Concentrations in pg/mL.

3·3-fold higher expression of GPCR43 compared with GPCR41. Furthermore, we found a dose-dependent inhibition of LPS-induced TNF-α secretion by PA. A significant inhibition of 26·6% was already obtained with 100 μM PA. Inhibition was maximal with 10 mM PA. Also with an extended set of cytokines and chemokines, PA shows similar reductions in IL-10, G-CSF, CCL5 and TNF-α compared with experiments with adipose tissue. In addition, in macrophages, a reduction in MCP-1 was seen, which was not observed in adipose tissue. In contrast with adipose tissue, CXCL10, MIP-1α and MIP-1β were not changed in macrophages. Furthermore, IL-4 was increased by PA treatment in macrophages, while a slight decrease was observed in adipose tissue. The relevance of this observation is unclear because levels of IL-4 produced by adipose tissue and macrophages were very low (Tables 3 and 4). Anti-inflammatory properties of PA have also been observed in a colon carcinoma cell line and a mechanism involving inhibition of the NF-κB transcription factor was suggested [36], possibly via suppressed proteasome function which prevents breakdown of the inhibitory IkB protein [37]. The differences between effects of PA on macrophages and on adipose tissue as a whole may be explained by additional effects of PA on adipocytes, the major cell type in adipose tissue. We recently showed that adipocytes may act as immune cells and produce a whole spectrum of cytokines and adipokines upon an inflammatory challenge [38] under which the set of inflammatory mediators measured in the current study. Therefore, the net effect of PA on adipose tissue may likely consist of effects on adipocytes as well as macrophages.

In summary, our data show that PA has anti-inflammatory effects on OAT from overweight subjects, accompanied by improved expression of LPL and GLUT4, associated with lipogenesis and glucose uptake, respectively. These effects are partially explained by effects on macrophages but likely also adipocytes are involved. We hypothesize that PA, produced in the colon, may have a direct beneficial effect on visceral adipose tissue by reducing obesity-associated inflammation and increasing lipogenesis and glucose uptake. These data correspond well with studies that show that diets that are likely to enhance the production of PA by the microbiota correlate with lower incidence and better outcome of metabolic syndrome and other obesity-related diseases [15,39]. The physiological relevance of these findings has to be determined in future studies.

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Disclosures

The authors have not financial conflicts of interest.

Contributions

SA designed and performed the experiments, analysed the data and participated in drafting the manuscript; DW assisted with the experiments; AH provided human adipose tissue and anthropomorphic data and critically revised the manuscript. FR assisted with data interpretation and critically revised the manuscript. HR, RV and KV assisted with the experimental design, data interpretation and drafting the manuscript.

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