International Journal of Obesity (2013) **37,** 874–880

www.nature.com/ijo

ORIGINAL ARTICLE

Endocannabinoids regulate adipokine production and the immune balance of omental adipose tissue in human obesity

Q Ge[1], E Maury[1], L Rycken[1], J Gérard[1], L Noël[1], R Detry[2], B Navez[2] and SM Brichard[1]

OBJECTIVES: (1) To investigate whether modulation of the cannabinoid type 1 receptor (CB1R) directly regulates the production of adiponectin (ApN) and other adipokines in omental adipose tissue (OAT) of obese subjects, (2) to establish in which cellular fraction of OAT the effects of CB1R blockade take place and (3) to unravel the underlying mechanisms.
SUBJECTS AND METHODS: OAT was obtained from 30 obese subjects (body mass index: $40.6 \pm 1.3\,kg\,m^{-2}$) undergoing abdominal surgery. Primary cultures of explants or of freshly isolated adipocytes or stromal-vascular cells (SVCs) were used.
RESULTS: In OAT explants, the CB1R blocker Rimonabant upregulated ApN gene expression. mRNA abundance of omentin that exhibits insulin-sensitizing properties was upregulated as well. Conversely, mRNA levels of two pro-inflammatory cytokines, macrophage inflammatory protein (MIP)-1β and interleukin (IL)-7 were downregulated. We next examined where these effects took place within OAT. CB1R expression was similar in both cellular fractions. In isolated mature adipocytes, blockade of CB1R reproduced the increase of ApN mRNA and the decrease of IL-7 mRNA, while inducing a rise of ApN secretion into the medium. In isolated SVC, gene expression of omentin, which is restricted to this fraction, was augmented, while that of MIP-1β was diminished. Finally, we deciphered the mechanisms leading to ApN regulation by the endocannabinoid system (ES). We first established that ApN regulation was actually mediated by the CB1R: ApN gene expression was upregulated by Rimonabant and downregulated by the CB1R agonist arachidonyl-2-chloroethylamide (ACEA). Upregulation of ApN by Rimonabant was unaltered by inhibiting cAMP production. However, downregulation of ApN by ACEA was fully reversed by an inhibitor of p38 mitogen-activated protein kinase (p38MAPK) and ACEA increased p38MAPK phosphorylation.
CONCLUSIONS: Blockade of CB1R attenuates the inflammatory state in both cellular fractions of OAT either by increasing ApN and omentin production or by decreasing mRNAs of MIP-1β and IL-7. ApN regulation by the ES partly involves p38MAPK.

International Journal of Obesity (2013) **37,** 874–880; doi:10.1038/ijo.2012.123; published online 7 August 2012

Keywords: endocannabinoid; rimonabant; adipokines; adipose tissue; adiponectin

INTRODUCTION

Obesity is locally characterized by adipose tissue inflammation and dysregulation of adipokine production with oversecretion of deleterious regulatory peptides and hyposecretion of defensive ones, like adiponectin (ApN).[1,2] Such a dysregulation triggers the development of a systemic low-grade pro-inflammatory state, which is considered to build the common soil for the development of obesity comorbidities and the metabolic syndrome.[1,3,4] Preferential accumulation of central-omental rather than subcutaneous fat appears to be a stronger risk factor for this adverse health profile.[1]

The endocannabinoid system (ES), consisting of the cannabinoid type 1 receptor (CB1R) and of endogenous lipid-derived ligands, modulates energy homeostasis through central orexigenic effects and peripheral metabolic effects on several organs including adipose tissue where energy storage is increased.[5,6] Obese subjects exhibit higher endocannabinoid levels in visceral fat and serum than lean controls[7,8] and also show higher endocannabinoid levels in visceral than in subcutaneous fat.[9] A strong activation of the ES might thus be considered as a driving force leading to—or associated with—central obesity.[10]

Treatment of obese patients with the selective CB1R blocker Rimonabant promoted significant decreases of body weight and waist circumference (a marker of abdominal adiposity) as well as improvements in cardiovascular and metabolic risk factors.[11] Parts of these improvements have been ascribed to a rise of circulating ApN levels. This rise was partly independent of weight loss and suggested specific improvement of adipose tissue function.[12]

In line with this observation, Rimonabant increased the expression of ApN both in adipose tissue of rodents treated *in vivo* and in murine 3T3-F442A adipocyte line *in vitro*.[13] However, the peripheral effects of cannabinoids have been poorly investigated in human adipose tissue. Few reports yielded controversial data on adipokine release by human fat cells.[14–16] These experiments were carried out on adipocytes differentiated *in vitro* from stromal precursors, which were isolated mainly from subcutaneous fat of non-obese subjects,[15,16] then submitted to pharmacological doses of adipogenic cocktails. However, omental rather than subcutaneous fat, as well as obesity are linked to the metabolic syndrome and abnormal endocannabinoid tone. Additionally, differentiation of fat cells within an *in-vivo* context together with preserved cell interactions may be a prerequisite for optimal adipokine gene expression.[17]

[1]Endocrinology, Diabetes and Nutrition Unit, Institute of Experimental and Clinical Research, Medical Sector, University of Louvain, Brussels, Belgium and [2]Surgery Unit, Institute of Experimental and Clinical Research, Medical Sector, University of Louvain, Brussels, Belgium. Correspondence: Dr SM Brichard, IREC – Pôle d'Endocrinologie, Diabétologie and Nutrition, UCL/EDIN B1.55.06 – Avenue Hippocrate 55, B-1200 Brussels, Belgium.
E-mail: Sonia.Brichard@uclouvain.be
Received 4 April 2012; revised 7 June 2012; accepted 8 June 2012; published online 7 August 2012

Besides downregulation of ApN, we like others have shown that several adipokines are oversecreted by omental adipose tissue (OAT) of obese subjects and could contribute to metabolic and cardiovascular disorders as well.[18] Both adipocytes and stromal-vascular cells (SVCs; that is, non-fat cells) contribute to such adipokine deregulation in human obesity.[18] Yet, the potential direct role of the ES on the SVC fraction has never been addressed. Moreover, the mechanisms underlying a potential regulation of adipokines by the ES are still unexplored.

The aims of this work were (1) to assess whether modulation of CB1R directly regulates the production of ApN and other adipokines in OAT of obese subjects, (2) to establish in which cellular fraction of OAT the effects of CB1R blockade take place and (3) to unravel the underlying mechanisms.

SUBJECTS AND METHODS

Subjects

OAT was obtained from 30 obese subjects undergoing bariatric surgery (vertical banded gastroplasty) after an overnight fast (Table 1). Patients were not treated with hormones (for example, glucocorticoids; except for insulin for one of them), non-steroid anti-inflammatory drugs or any medications known to influence fat mass or metabolism (for example, thiazolidinediones, systemic and non-specific modulators of adrenergic receptors).

For each patient, blood was collected in the fasting state before surgery. As shown in Table 1, these patients were severely obese, had higher systolic blood pressure, fasting glycemia, low-density lipoprotein cholesterol and C-reactive protein levels than the recommended or normal values and women had lower high-density lipoprotein cholesterol concentrations. Eight patients were diagnosed as type 2 diabetic and were treated by diet and/or oral anti-diabetic agents or by insulin for one of them. Two-thirds of the patients met criteria of the metabolic syndrome as defined by a joint scientific statement.[19]

For each culture, explants or cells from only one subject were used. Due to the limited tissue availability, not all data could be generated from all patients.

The study protocol had the approval of the local Ethical Committee of the Cliniques Universitaires Saint-Luc.

Cell or adipose tissue culture

We used established protocols to study the production of adipokines by fragments or isolated cells from OAT.[18,20,21] Under those experimental conditions, neither tissue fractionation nor culturing *per se* did alter adipokine expression levels.[18]

Briefly, OAT biopsies were transported to the laboratory within 5–10 min after sampling.[20] Samples were fractionated into adipocytes and SVC by collagenase treatment, as reported.[18,20] This yields two high purity fractions.[18] Adipocytes (150 μl packed cells) and SVCs (150 μl per 3.6 g initial tissue) were cultured in MEM supplemented with 10% fetal calf serum and 1/500 (v/v) antibiotics (Primocin InvivoGen, Toulouse, France), as described.[18] Cells were allowed to stabilize for 1 h in this medium; next, the medium was renewed and cells were cultured for another 24 h with or without SR141716 (Rimonabant, a selective CB1R antagonist; Sanofi-Aventis, Diegem, Belgium) or a selective CB1R agonist ACEA (arachidonyl-2-chloroethylamide, Tocris Bioscience, Ellisville, MO, USA). In some experiments, adipocytes were cultured for 25 h with specific inhibitors of kinases (c-Jun NH_2-terminal protein kinase (JNK), SP600125; p38 mitogen-activated protein kinase (p38MAPK), SB203580; Extracellular Signal-Regulated kinases 1/2 (ERK1/2), PD 98059) or of adenylate cyclase (MDL-12,330A), before the addition of ACEA or SR141716 for the last 24 h. All inhibitors were from Calbiochem (La Jolla, CA, USA); the concentrations used were based on those recommended by the manufacturer or previously published in the literature.[21–24] Because SR141716, SP600125, SB203580 and PD 98059 were dissolved in dimethylsulfoxide and ACEA was dissolved in ethanol, dimethylsulfoxide or ethanol was used as vehicle for the control conditions. The final concentrations of dimethylsulfoxide or ethanol in medium were $<0.1\%$. In our first experiments, adipose tissue explants (2–3 mm^3 fragments, 400 mg tissue) were also cultured for 24 h, as described for cellular fractions.[20,25] After culturing, cells or explants were harvested and frozen at $-80\,^{\circ}C$. Aliquots from adipocyte culture medium were also saved and stored at $-20\,^{\circ}C$.

Table 1. Clinical and laboratory characteristics of the obese subjects

		Reference values	
		Cut-points for the MS[19]	*Normal laboratory values*
Clinical parameters			
Age (years)	43 ± 2		
Sex ratio (Men/Women)	11/19		
BMI ($kg\,m^{-2}$)	40.6 ± 1.2	$\geqslant 30$[a]	
Systolic blood pressure (mm Hg)	137 ± 3	$\geqslant 130$ (or drug treatment) and/or	
Diastolic blood pressure (mm Hg)	84 ± 2	$\geqslant 85$ (or drug treatment)	
Glucose homeostasis			
Glucose ($mg\,dl^{-1}$)	110 ± 8	$\geqslant 100$ (or drug treatment)	
Diabetes (%)	27%		
Lipids			
Cholesterol total ($mg\,dl^{-1}$)	194 ± 8		<190
LDL cholesterol ($mg\,dl^{-1}$)	124 ± 7		<115
HDL cholesterol ($mg\,dl^{-1}$)	44 ± 2 (men)/ 46 ± 3 (women)	$<40/<50$	
Triglycerides ($mg\,dl^{-1}$)	131 ± 12	$\leqslant 150$	
Inflammation			
CRP ($mg\,dl^{-1}$)	2.5 ± 0.5		<0.1
Metabolic syndrome (%)	67%		

Abbreviations: BMI, body mass index; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Clinical and laboratory parameters were obtained in the fasted state before surgery. Reference values take into account cut-points for the metabolic syndrome (MS)[19] or our normal laboratory values. [a]It is usually assumed that central obesity is present when the BMI is $\geqslant 30\,kg\,m^{-2}$ (Alberti *et al.*[19]). Values are means ± s.e.m. for 30 obese subjects.

RNA extraction and real-time quantitative PCR (RTQ-PCR)

Total RNA from cells or tissue was extracted by using TriPure Isolation Reagent (Roche Diagnostics, Vilvoorde, Belgium). In all, 0.2–2 μg of total RNA was reverse transcripted as described. RTQ-PCR primers were designed by using Primer Express Software (Applied Biosystems, Carlsbad, CA, USA; see Table 2). In all, 4–40 ng of total RNA equivalents was amplified with iQSyber Green Supermix (Bio-Rad Laboratories, Nazareth, Belgium) containing 200 nM of each specific primer using iCycler iQ Real Time PCR detection System (Bio-Rad Laboratories). Briefly, the threshold cycles (CT) were measured in duplicate. ΔCT values were calculated in every sample for each gene of interest as follows: $CT_{gene\ of\ interest} - CT_{reporter\ gene}$ with TATA box-binding protein (TBP) as the reporter gene. Relative changes in the expression level of one specific gene (ΔΔCT) were calculated as ΔCt of the test group minus ΔCT of the reference group, and then presented as $2^{-\Delta\Delta CT}$ (Delaigle *et al.*[26]).

Determination of ApN concentration in medium by RIA

ApN concentrations were measured in medium from cultured adipocytes by using a commercially available RIA kit (Linco Research, Nuclilab, The Netherlands). This kit does not discriminate between the different forms of ApN. Samples from diluted medium (1:1) were run in duplicate.

Table 2. Human gene sequences used as forward and reverse primers for RTQ-PCR

Gene	*Forward primer*	*Reverse primer*	*Amplicon (bp)*
Adiponectin	GCAGAGATGGCACTCCTGGA	CCCTTCAGTTCCTGTCATTCC	101
CB1R	CCCTCTTGTGAAGGCACTGC	GCGGCCCTGTGAACACTG	101
GRO	GCCAGTGCTTGCAGACCCT	GGCTATGACTTCGGTTTGGG	101
IL-7	GCACTGGTGATTTTGATCTCCA	CAGGGCAGCTGGTTTTCTTC	101
Leptin	AAGCTGTGCCCATCCAAAAA	GGAGGAGACTGACTGCGTGTG	101
MIP-1β	GCTGCTTTTCTTACACCGCG	GGTTTGGAATACCACAGCTGG	101
Omentin	CCGGTGATCCCTGTGGTCTA	AACTGAACAAATCCCGCAGTG	101
RANTES	AGCCCTCGCTGTCATCCTC	GGGCAATGTAGGCAAAGCAG	101
TIMP-1	TGGCATCCTGTTGTTGCTGT	TGATGACGAGGTCGGAATTG	101
TNF-α	CTCTTCTGCCTGCTGCACTTT	GATGATCTGACTGCCTGGGC	101
TPO	ATGGAGGAGACCAAGGCACA	GATGAGAGGCAAGTGGGTCC	101
TBP	CCCCATGACTCCCATGACCC	ACGAAGTGCAATGGTCTTTAGGT	136

Abbreviations: CB1R, CB1 receptor; GRO, growth-related oncogen factor; IL, interleukin; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated upon activation normal T cells expressed and secreted; RTQ-PCR, real-time quantitative PCR; TBP, TATA Box-binding protein; TIMPs, tissue inhibitor of metalloproteinases; TNF-α, tumor necrosis factor-α; TPO, thrombopoietin.

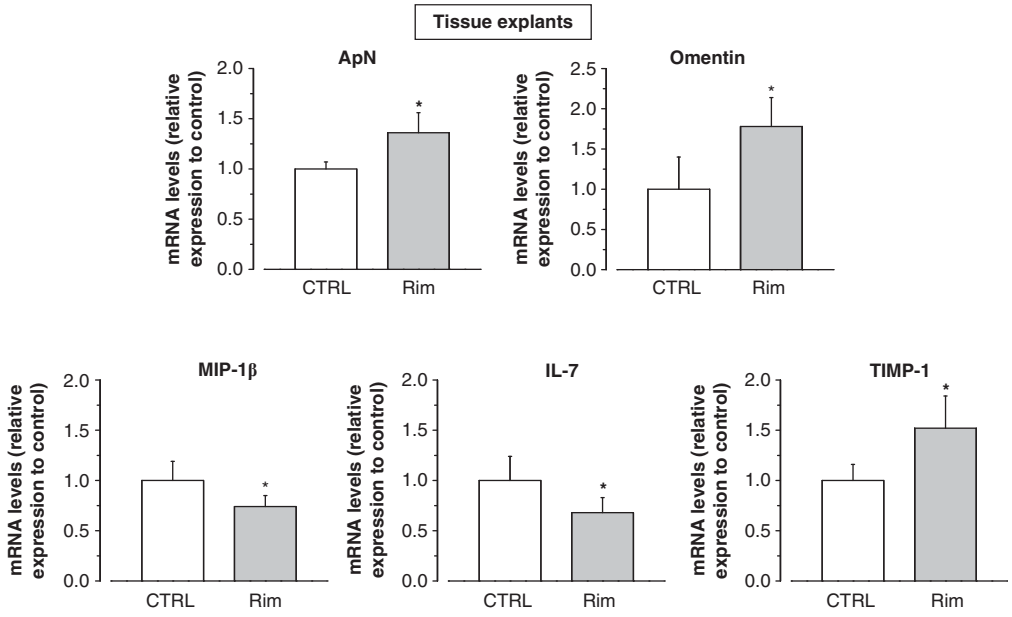

Figure 1. Gene expression of adipokines in explants of human OAT treated with Rimonabant. Explants of OAT form obese subjects were cultured with or without 10–100 nM Rimonabant (Rim) for 24 h. Adipokine mRNA levels were quantified by RTQ-PCR, normalized to the levels of TBP (used as reporter gene) and are presented as relative expression compared with control (untreated) explants. Results are mean ± s.e.m. for 8–12 obese subjects. *$P < 0.05$ vs control conditions.

cAMP immunoassay

Cellular cAMP concentrations were measured by a competitive ELISA kit (R&D Systems Europe Ltd., Abingdon, UK) according to manufacturer's instructions. cAMP levels were normalized to cellular protein levels as determined by the Bradford method.

Western blot

Adipocytes were homogenized in a lysis buffer (Cell Signaling Technology, BIOKÉ, Leiden, The Netherlands) supplemented with 1% protease inhibitor cocktail (Roche Diagnostics). In all, 20 μg proteins were dissolved in Laemmli buffer, subjected to SDS-PAGE under reducing and heat-denaturating conditions and then transferred onto PVDF membranes. The following antibodies were used for immunodetection: anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), anti-phospho-SAPK/JNK (Thr183/Tyr185) and anti-phospho-p38MAPK (Thr180/Tyr182). Each antibody was used according to manufacturer's instructions (Cell Signaling Technology, BIOKÉ). Signals were revealed by enhanced Chemiluminescence. Band intensities were quantified by scanning densitometry (Gel-Doc2000, Bio-Rad Laboratories, Ltd, Hemel Hempstead, UK), analyzed with Quantity One (Bio-Rad Laboratories Ltd) and normalized to Actin (Sigma-Aldrich, Bornem, Belgium) band intensity.

Presentation of the results and statistical analysis

The results are presented as mean values ± s.e.m. for the indicated numbers of patients. Comparisons between different conditions within a same group were made using two-tailed paired Student's *t*-test (two conditions) or repeated analysis of variance (several conditions) followed by the Dunnett's or Newman–Keuls' test, when appropriate. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Effect of CB1R blockade on adipokine gene expression in cultured explants

We first investigated the effects of blocking CB1R with Rimonabant on whole adipose tissue of obese patients. Rimonabant for 24 h upregulated ApN gene expression by ~40% in omental

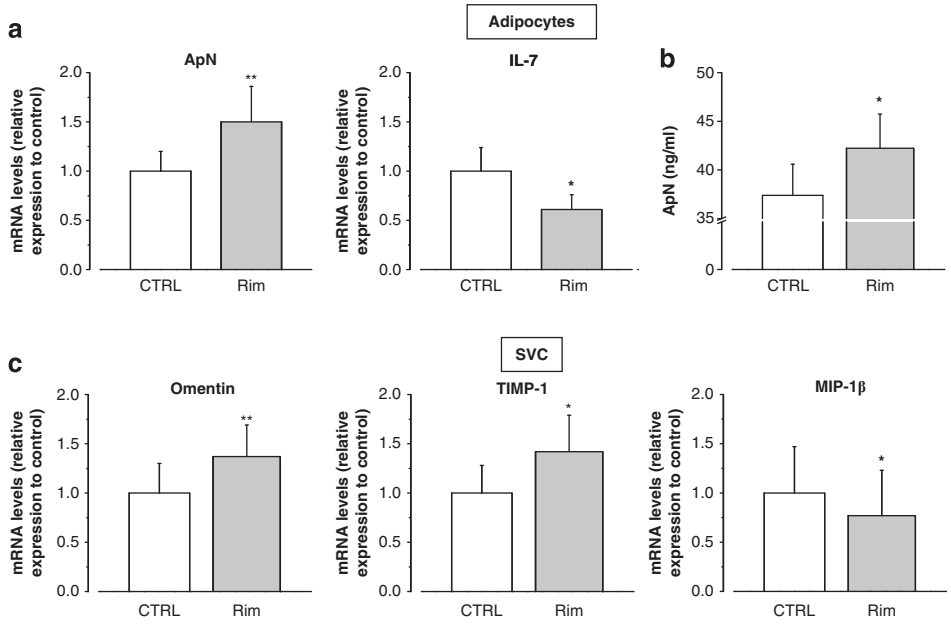

Figure 2. Adipokine production in human adipocytes and SVCs treated with Rimonabant. Mature adipocytes and SVC isolated from OAT of obese subjects were cultured with or without 100 nM Rimonabant (Rim) for 24 h. Adipokine mRNA levels (**a**, **c**) were quantified by RTQ-PCR, normalized to the levels of TBP and presented as relative expression compared with control cells. The concentration of ApN in medium (**b**) was measured by RIA and expressed as ng ml^{-1}. Results are mean values ± s.e.m. for 12–15 (mRNAs) or 18 (protein secretion) obese subjects. *$P<0.05$, ** $P<0.01$ vs control conditions.

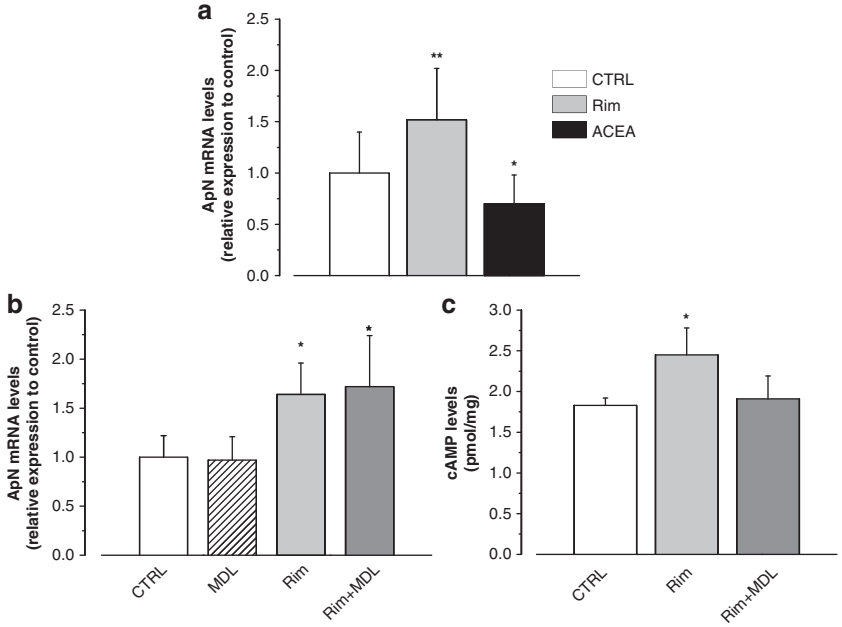

Figure 3. ApN regulation by CB1R modulation (**a**) is independent of cAMP levels (**b**, **c**). (**a**) CB1R modulation on ApN gene expression in adipocytes. Adipocytes were cultured with 100 nM Rimonabant (Rim) or with 1 μM ACEA for 24 h. ApN mRNA levels are presented as relative expression compared with control adipocytes. (**b**) Inhibition of cAMP production on Rimonabant-stimulated ApN gene expression in adipocytes. Adipocytes were cultured with or without MDL-12330A (20 μM) for 25 h, while Rim (100 nM) was added during the last 24 h. ApN mRNA levels are presented as relative expression compared with control adipocytes. (**c**) Increase of cAMP production by Rim and prevention by MDL. For cAMP measurement, adipocytes were cultured in serum-free medium (2% BSA) with or without 100 nM Rim for 10 min. In all, 20 μM MDL-12330A was added 1 h before Rim treatment. cAMP levels were measured by ELISA and normalized to adipocyte protein content. Results are mean values ± s.e.m. for seven (**a**), eight (**b**) and five (**c**) obese subjects. *$P<0.05$, **$P<0.01$ vs respective control conditions.

adipose explants (Figure 1). mRNA abundance of omentin which, like ApN, exhibits insulin-sensitizing properties and is decreased in obesity[27] was upregulated as well (approximately +78%; Figure 1). We further examined the effects of Rimonabant on several other adipokines that we have previously identified as overproduced by OAT in human obesity[18]: three chemokines (Growth-Related Oncogen factor, Regulated upon Activation Normal T cells Expressed and Secreted, macrophage

inflammatory protein (MIP)-1β), one interleukin (IL-7), one tissue inhibitor of metalloproteinases (TIMP-1) and one hematopoietic growth factor (thrombopoietin). Gene expression of MIP-1β and IL-7 was downregulated by Rimonabant (approximately −26% and approximately −32%, respectively; $P<0.05$) while TIMP-1 was upregulated (approximately +40%, $P<0.05$; Figure 1). mRNA levels of the other adipokines (Regulated upon Activation Normal T cells Expressed and Secreted, Growth-Related Oncogen factor and thrombopoietin) were not modified by CB1R blockade. Likewise, gene expression of leptin and tumor necrosis factor-α was not affected (data not shown, $n=7$–8 patients/group).

Effect of CB1R blockade on adipokines in mature adipocytes and SVC

We next examined where the effects of Rimonabant on adipokine production took place within OAT. It is of note that CB1R was similarly expressed in both cellular fractions of OAT (relative mRNA abundance: 1.0 ± 0.5 in adipocytes vs 1.1 ± 0.6 in SVC, $n=9$). In isolated mature adipocytes, blockade of CB1R reproduced the increase of ApN mRNA and the decrease of IL-7 mRNA while inducing a moderate but significant increase of ApN secretion into the medium (+20%; Figures 2a and b). In isolated SVC, gene expression of omentin, which is known to be restricted to this fraction[27] was augmented like that of TIMP-1, while MIP-1β mRNA was diminished (Figure 2c).

Mechanisms involved in ApN regulation by the ES

To decipher the mechanisms leading to ApN regulation by the ES, we investigated candidate signaling pathways in adipocytes.

We first established that ApN regulation by the ES was actually mediated by the CB1R. Indeed, while ApN gene expression was upregulated by the selective CB1R antagonist Rimonabant, its expression was downregulated by using a specific agonist of CB1R, ACEA (Figure 3a).

Next, we investigated post-CB1R signaling pathways. The classic post-receptor signal transduction pathways of CB1R activation include inhibition of cAMP production and stimulation of MAPKs, including p38MAPK, JNK and ERK1/2 in a variety of cell types.[28]

We thus investigated whether the stimulation of ApN gene by Rimonabant resulted from a rise of cAMP levels. The upregulation of ApN mRNA by Rimonabant was unaltered by using the adenylate cyclase inhibitor MDL-12330A (Figure 3b). Yet, this inhibitor was effective as it prevented the rise of cAMP levels caused by Rimonabant in adipocytes (Figure 3b). Taken together, these data suggest that cAMP may not be involved in ApN regulation by the ES. We next blocked components of MAP kinase signaling by using specific inhibitors of p38MAPK (SB203580), ERK1/2 (PD98059) and JNK (SP60025). These inhibitors were tested during stimulation of CB1R signaling. This strategy was chosen because basal phosphorylation of MAPK components was rather low and it thus appeared to be easier to disclose an increase (rather than a decrease) of phosphorylation levels. None of the

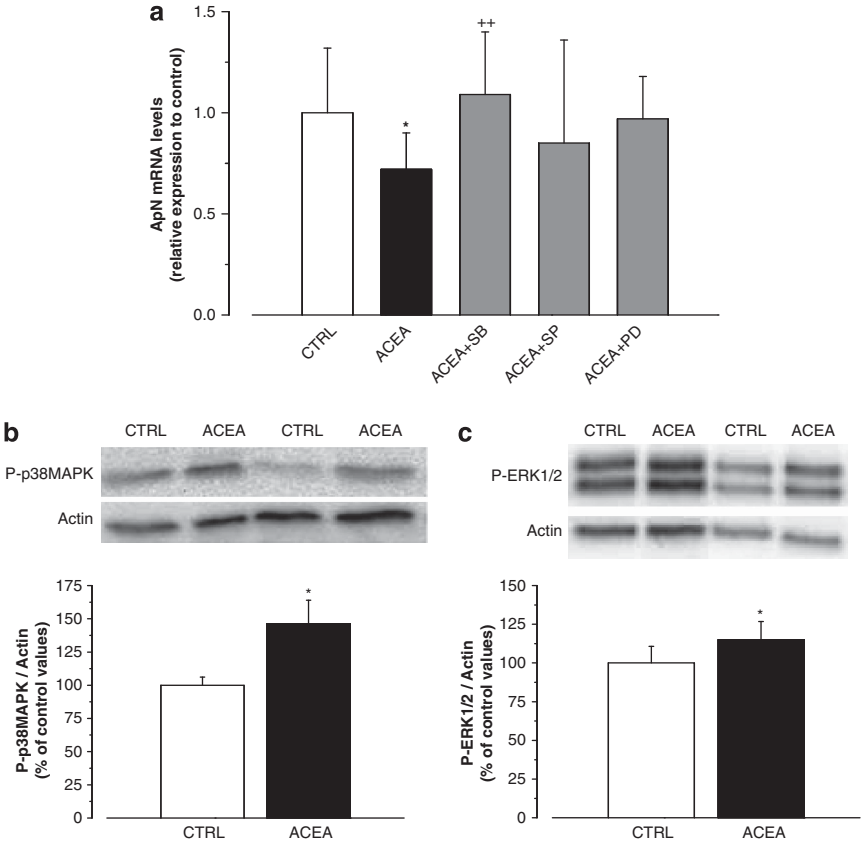

Figure 4. p38MAPK-mediated ApN regulation by the endocannabinoid system. (**a**) Blockade of different MAPK signaling pathways (p38MAPK, ERK1/2, JNK) on ApN gene expression in adipocytes treated by the CB1R agonist, ACEA. Adipocytes were cultured with or without p38MAPK, ERK1/2, JNK inhibitors (20 μM SB203580, 20 μM PD98059 and 10 μM SP60025, respectively) for 25 h in the presence of ACEA (1 μM), which was added during the last 24 h. ApN mRNA levels are presented as relative expression compared with control adipocytes. Results are mean values ± s.e.m. for seven obese subjects. *$P<0.05$ vs control, $^{++}P<0.01$ vs ACEA. (**b**, **c**) Phosphorylation of p38MAPK and ERK1/2 in response to CB1R stimulation was measured by western blot analysis. Adipocytes were cultured in serum-free medium (2% BSA) with or without 1 μM ACEA for 1 h. Representative blots are shown as inserts. p38MAPK and ERK1/2 phosphorylation was normalized to actin levels and these ratios were expressed as percentages of control values in untreated adipocytes. Results are mean values ± s.e.m. for four obese subjects. *$P<0.05$ vs control.

inhibitors used alone altered ApN gene expression (not shown). Downregulation of ApN expression by ACEA was fully reversed by the selective inhibitor of p38MAPK ($P<0.01$ for ACEA vs ACEA + SB), while the differences between ACEA alone vs ACEA + SP or ACEA + PD did not reach statistical significance (Figure 4a). This suggests that p38MAPK pathway could mediate CB1R-regulated ApN expression. Western blot analysis further showed that stimulation of CB1R with 1 μM ACEA did actually increase by ~50% p38MAPK phosphorylation ($P<0.05$; Figure 4b) and only slightly increase ERK1/2 phosphorylation in adipocytes (~15%, $P<0.05$; Figure 4c). JNK phosphorylation was unaffected (data not shown).

DISCUSSION

We show that CB1R modulation alters adipokine production both in explants and in each cellular fraction isolated from OAT of obese subjects. Previous work on human adipocytes differentiated *in vitro* (mainly from the subcutaneous depot and from non-obese subjects) yielded controversial data. On one hand, neither treatment with a CB1R agonist nor with a CB1R blocker affected ApN expression,[14] in line with the lack of relationship between human adipose tissue CB1R expression and ApN production.[29] On the other hand, a synthetic cannabinoid upregulated pro-inflammatory adipokines in human fat cells,[15] while the selective CB1R blocker Rimonabant restored to basal levels the secretion of ApN, which was downregulated by lipopolysaccharide co-treatment.[16] Herein, we unambiguously show that a selective CB1R agonist (ACEA) decreased ApN expression, while a selective CB1R blocker upregulated ApN and concurrently downregulated the expression of several pro-inflammatory adipokines in both cellular fractions of OAT. The fact that we were able to disclose an effect of CB1R blockade even in basal conditions may be explained by the already enhanced cannabinoid tone in omental fat of obese subjects. We further demonstrated that CB1R blockade alters the adipokine production by SVC as well. This is novel information as all the studies performed so far have focused on the adipocyte fraction. In line with our data, the mRNA abundance of CB1R was similar in both cellular fractions of human OAT. It should be mentioned that ACEA may have a weak affinity for CB2R[30] and that Rimonabant may be an agonist of the orphan G protein-coupled receptor GPR55.[31] However, action of these compounds through these alternate receptors is unlikely in our study for two reasons. First, such action requires much higher concentrations of each compound that those used herein.[32] Second, the inverse regulation of ApN by ACEA and Rimonabant strongly supports the concept that ApN production is actually CB1R regulated.

Besides ApN, other adipokines were regulated by CB1R blockade. We found that Rimonabant upregulated the expression of omentin and TIMP-1 in SVC, while downregulating that of MIP-1β and IL-7 in SVC and adipocytes, respectively. All these adipokines are over-produced in obesity[18] except for omentin, which is decreased like ApN.[33] Omentin is a novel adipokine preferentially produced by visceral adipose tissue, with a main contribution of SVC.[27] This adipokine exhibits insulin-like properties in human adipocytes,[27] inhibits vascular inflammation[34] and may exert cardiovascular protection.[35] The increase of omentin may thus represent a novel benefit of CB1R blockade in the context of the metabolic syndrome. TIMP-1 was also upregulated by CB1R blockade. Matrix metalloproteinases and their tissue inhibitors (TIMPs) may have a role in development of obesity by contributing to adipogenesis and extracellular matrix degradation. However, the exact role of TIMP-1 on fat mass development and adipogenesis is still unclear: some studies reported enhanced adipocyte differentiation and hypertrophy,[36,37] while others did not observe any effect[38] or reported impaired differentiation.[39] CB1R blockade also downregulated two adipokines: IL-7 and MIP-1β. IL-7 is a potent pro-inflammatory cytokine, known for its critical role in T-cell development and survival and its role in B-cell development.[40] MIP-1β is a chemokine, which is crucial in recruiting macrophages and other pro-inflammatory cells.[41] Although the function of these adipokines on adipose tissue is still poorly documented, both may have a role in initiating and shaping the immune-inflammatory responses in this tissue. The adipokines regulated by CB1R modulation may thus represent novel mechanisms involved in the peripheral metabolic, immune and cardiovascular effects of ES in human obesity.

Major mediators of CB1R involve inhibition of adenylyl cyclase and regulation of ion channels as well as phosphorylation of MAPKs, such as p38MAPK, ERK1/2 and JNK.[28] However, the mechanisms by which CB1R regulates ApN have never been studied in human adipocytes. There is only one report indicating that Rimonabant increased ApN expression and concurrently decreased ERK1/2 activity in a murine preadipocyte cell line.[42] We therefore examined whether cAMP and MAPKs were involved in the regulation of ApN in human mature adipocytes. CB1R blockade increased cAMP production, while CB1R stimulation increased the phosphorylation of p38MAPK and also possibly that of ERK1/2 without affecting JNK. cAMP is a well-known potent inhibitor (not a stimulator) of ApN production in human visceral adipose tissue.[43] This fact may be *a priori* difficult to reconcile with the observation that CB1R blockade increased both cAMP and ApN levels. However, the upregulation of ApN was unaltered by inhibiting cAMP production, ruling out a potential involvement of cAMP in this observation. Thus, CB1R blockade upregulated ApN in spite of rising cAMP. We also found that CB1R activation downregulated ApN gene expression and that this effect was reversed by specific inhibitors of p38MAPK. These data were confirmed by CB1R-induced phosphorylation of p38MAPK. Our finding is consonant the report that ACEA reduced mitochondrial biogenesis also through activation of the same component (that is, p38MAK) in mouse adipocytes.[44] Taken together, these data indicate that p38MAPK could mediate the effects of CB1R on ApN regulation.

In summary, the ES directly regulates the immune balance of OAT in human obesity. Specifically, blockade of CB1R attenuates the inflammatory state in both adipocytes and SVC either by increasing ApN and omentin production or by decreasing MIP-1β and IL-7. ApN regulation by the ES partly involves p38MAPK.

CONFLICT OF INTEREST

SMB received grants from Sanofi-Aventis, Diegem, Belgium. The remaining authors declare no conflict of interest.

REFERENCES

1 Matsuzawa Y. Therapy Insight: adipocytokines in metabolic syndrome and related cardiovascular disease. *Nat Clin Pract Cardiovasc Med* 2006; **3**: 35–42.
2 Guerre-Millo M. Adipose tissue and adipokines: for better or worse. *Diabetes Metab* 2004; **30**: 13–19.
3 Jernas M, Palming J, Sjoholm K, Jennische E, Svensson PA, Gabrielsson BG *et al.* Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. *FASEB J* 2006; **20**: 1540–1542.
4 Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab* 2007; **92**: 1023–1033.
5 Scheen AJ. The endocannabinoid system: a promising target for the management of type 2 diabetes. *Curr Protein Pept Sci* 2009; **10**: 56–74.
6 Muccioli GG, Naslain D, Backhed F, Reigstad CS, Lambert DM, Delzenne NM *et al.* The endocannabinoid system links gut microbiota to adipogenesis. *Mol Syst Biol* 2010; **6**: 392.
7 Engeli S, Bohnke J, Feldpausch M, Gorzelniak K, Janke J, Batkai S *et al.* Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 2005; **54**: 2838–2843.
8 Bluher M, Engeli S, Kloting N, Berndt J, Fasshauer M, Batkai S *et al.* Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 2006; **55**: 3053–3060.

9 Matias I, Gonthier MP, Orlando P, Martiadis V, De PL, Cervino C *et al.* Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocrinol Metab* 2006; **91**: 3171–3180.
10 Bellocchio L, Cervino C, Pasquali R, Pagotto U. The endocannabinoid system and energy metabolism. *J Neuroendocrinology* 2008; **20**: 850–857.
11 Ruilope LM, Despres JP, Scheen A, Pi-Sunyer X, Mancia G, Zanchetti A *et al.* Effect of rimonabant on blood pressure in overweight/obese patients with/without co-morbidities: analysis of pooled RIO study results. *J Hypertens* 2008; **26**: 357–367.
12 Despres JP, Golay A, Sjostrom L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N Engl J Med* 2005; **353**: 2121–2134.
13 Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F *et al.* The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 2003; **63**: 908–914.
14 Pagano C, Pilon C, Calcagno A, Urbanet R, Rossato M, Milan G *et al.* The endogenous cannabinoid system stimulates glucose uptake in human fat cells via phosphatidylinositol 3-kinase and calcium-dependent mechanisms. *J Clin Endocrinol Metab* 2007; **92**: 4810–4819.
15 Gonzalez-Muniesa P, Bing C, Trayhurn P. Upregulation of the expression of inflammatory and angiogenic markers in human adipocytes by a synthetic cannabinoid, JTE-907. *Horm Metab Res* 2010; **42**: 710–717.
16 Murumalla R, Bencharif K, Gence L, Bhattacharya A, Tallet F, Gonthier MP *et al.* Effect of the Cannabinoid Receptor-1 antagonist SR141716A on human adipocyte inflammatory profile and differentiation. *J Inflamm (Lond)* 2011; **8**: 33.
17 Hwang CS, Loftus TM, Mandrup S, Lane MD. Adipocyte differentiation and leptin expression. *Annu Rev Cell Dev Biol* 1997; **13**: 231–259.
18 Maury E, Ehala-Aleksejev K, Guiot Y, Detry R, Vandenhooft A, Brichard SM. Adipokines oversecreted by omental adipose tissue in human obesity. *Am J Physiol Endocrinol Metab* 2007; **293**: E656–E665.
19 Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA *et al.* Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640–1645.
20 Halleux CM, Declerck PJ, Tran SL, Detry R, Brichard SM. Hormonal control of plasminogen activator inhibitor-1 gene expression and production in human adipose tissue: stimulation by glucocorticoids and inhibition by catecholamines. *J Clin Endocrinol Metab* 1999; **84**: 4097–4105.
21 Maury E, Noel L, Detry R, Brichard SM. *In vitro* hyperresponsiveness to tumor necrosis factor-alpha contributes to adipokine dysregulation in omental adipocytes of obese subjects. *J Clin Endocrinol Metab* 2009; **94**: 1393–1400.
22 Hsu HF, Tsou TC, Chao HR, Shy CG, Kuo YT, Tsai FY *et al.* Effects of arecoline on adipogenesis, lipolysis, and glucose uptake of adipocytes-A possible role of betel-quid chewing in metabolic syndrome. *Toxicol Appl Pharmacol* 2010; **245**: 370–377.
23 Patel NG, Holder JC, Smith SA, Kumar S, Eggo MC. Differential regulation of lipogenesis and leptin production by independent signaling pathways and rosiglitazone during human adipocyte differentiation. *Diabetes* 2003; **52**: 43–50.
24 Xu A, Wong LC, Wang Y, Xu JY, Cooper GJ, Lam KS. Chronic treatment with growth hormone stimulates adiponectin gene expression in 3T3-L1 adipocytes. *FEBS Lett* 2004; **572**: 129–134.
25 Halleux CM, Servais I, Reul BA, Detry R, Brichard SM. Multihormonal control of ob gene expression and leptin secretion from cultured human visceral adipose tissue: increased responsiveness to glucocorticoids in obesity. *J Clin Endocrinol Metab* 1998; **83**: 902–910.
26 Delaigle AM, Jonas JC, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: *in vivo* and *in vitro* studies. *Endocrinology* 2004; **145**: 5589–5597.
27 Yang RZ, Lee MJ, Hu H, Pray J, Wu HB, Hansen BC *et al.* Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *Am J Physiol Endocrinol Metab* 2006; **290**: E1253–E1261.
28 Turu G, Hunyady L. Signal transduction of the CB1 cannabinoid receptor. *J Mol Endocrinol* 2010; **44**: 75–85.
29 Lofgren P, Sjolin E, Wahlen K, Hoffstedt J. Human adipose tissue cannabinoid receptor 1 gene expression is not related to fat cell function or adiponectin level. *J Clin Endocrinol Metab* 2007; **92**: 1555–1559.
30 Hillard CJ, Manna S, Greenberg MJ, DiCamelli R, Ross RA, Stevenson LA *et al.* Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J Pharmacol Exp Ther* 1999; **289**: 1427–1433.
31 Moreno-Navarrete JM, Catalan V, Whyte L, Diaz-Arteaga A, Vazquez-Martinez R, Rotellar F *et al.* The L-alpha-lysophosphatidylinositol/GPR55 system and its potential role in human obesity. *Diabetes* 2012; **61**: 281–291.
32 Henstridge CM, Balenga NA, Schroder R, Kargl JK, Platzer W, Martini L *et al.* GPR55 ligands promote receptor coupling to multiple signalling pathways. *Br J Pharmacol* 2010; **160**: 604–614.
33 de Souza Batista CM, Yang RZ, Lee MJ, Glynn NM, Yu DZ, Pray J *et al.* Omentin plasma levels and gene expression are decreased in obesity. *Diabetes* 2007; **56**: 1655–1661.
34 Tan BK, Adya R, Farhatullah S, Chen J, Lehnert H, Randeva HS. Metformin treatment may increase omentin-1 levels in women with polycystic ovary syndrome. *Diabetes* 2010; **59**: 3023–3031.
35 Yamawaki H, Tsubaki N, Mukohda M, Okada M, Omentin Hara Y. a novel adipokine, induces vasodilation in rat isolated blood vessels. *Biochem Biophys Res Commun* 2010; **393**: 668–672.
36 Alexander CM, Selvarajan S, Mudgett J, Werb Z. Stromelysin-1 regulates adipogenesis during mammary gland involution. *J Cell Biol* 2001; **152**: 693–703.
37 Lijnen HR, Demeulemeester D, Van Hoef B, Collen D, Maquoi E. Deficiency of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) impairs nutritionally induced obesity in mice. *Thromb Haemost* 2003; **89**: 249–255.
38 Scroyen I, Cosemans L, Lijnen HR. Effect of tissue inhibitor of matrix metalloproteinases-1 on *in vitro* and *in vivo* adipocyte differentiation. *Thromb Res* 2009; **124**: 578–583.
39 Meissburger B, Stachorski L, Roder E, Rudofsky G, Wolfrum C. Tissue inhibitor of matrix metalloproteinase 1 (TIMP1) controls adipogenesis in obesity in mice and in humans. *Diabetologia* 2011; **54**: 1468–1479.
40 Hartgring SA, Bijlsma JW, Lafeber FP, van Roon JA. Interleukin-7 induced immunopathology in arthritis. *Ann Rheum Dis* 2006; **65**(Suppl 3): iii69–iii74.
41 Maurer M, von Stebut E. Macrophage inflammatory protein-1. *Int J Biochem Cell Biol* 2004; **36**: 1882–1886.
42 Gary-Bobo M, Elachouri G, Scatton B, Le Fur G, Oury-Donat F, Bensaid M. The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits cell proliferation and increases markers of adipocyte maturation in cultured mouse 3T3 F442A preadipocytes. *Mol Pharmacol* 2006; **69**: 471–478.
43 Delporte ML, Funahashi T, Takahashi M, Matsuzawa Y, Brichard SM. Pre- and post-translational negative effect of beta-adrenoceptor agonists on adiponectin secretion: *in vitro* and *in vivo* studies. *Biochem J* 2002; **367**(Part 3): 677–685.
44 Tedesco L, Valerio A, Dossena M, Cardile A, Ragni M, Pagano C *et al.* Cannabinoid receptor stimulation impairs mitochondrial biogenesis in mouse white adipose tissue, muscle, and liver: the role of eNOS, p38 MAPK, and AMPK pathways. *Diabetes* 2010; **59**: 2826–2836.