Upregulation of the Expression of Inflammatory and Angiogenic Markers in Human Adipocytes by a Synthetic Cannabinoid, JTE-907

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Abstract
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Inflammation in adipose tissue is a characteristic of obesity and the metabolic syndrome. It is suggested that the endocannabinoid system is involved in the regulation of inflammatory and angiogenic processes within the tissue. Human subcutaneous preadipocytes (Zen Bio) were used as the source of human preadipocytes or adipocytes. Gene expression was examined by RT-PCR and real-time PCR. The secretion of inflammation-related proteins was determined by an ELISA array. In experiments on adipocytes treated at day 14 post-differentiation, JTE-907, a synthetic cannabinoid, upregulated the expression of key inflammatory markers – IL-6, MCP-1 and IL-1β – and angiogenic factors – VEGF and ANGPTL4 – at 10 μM after 20 h of treatment, having also increased the expression of TRPV1 at 10 μM. JTE-907 showed no effect after 4 h. The ELISA array showed a 2.6-fold increase in IL-6 protein release. The effect of JTE-907 was inhibited by AM251 (CB_1 antagonist), and partially by arachidonyl serotonin (TRPV1 and FAAH antagonist). The CB_2 antagonist, AM630, partially upregulated the effect of JTE-907. Preadipocytes fed 14 days after 100 % confluence exhibited downregulation of CB_1, MCP-1, and IL-1β, 20 h after having been exposed to JTE-907. CB_1 and TRPV1 receptors participate in the regulation of several inflammatory and angiogenic factors in human adipocytes, indicating their potential value as targets for the treatment of disorders related to obesity.

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Abbreviations
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AM251	N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
AM630	6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl) methanone
Arachidonyl serotonin (A.S.)	N-[2-(5-Hydroxy-1H-indol-3-yl)ethyl]-5,8,11,14-eicosatetraenamide
JTE-907	N-(1,3-Benzodioxol-5-ylmethyl)-1,2-dihydro-7-methoxy-2-oxo-8-(pentyloxy)-3-quinolinecarboxamide

Introduction
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The medical properties of marijuana (*Cannabis sativa*) led to the discovery of the endocannabinoid system (ECS) in the early 1990s, with the cloning of the cannabinoid receptors CB_1 and CB_2 (nomenclature follows Alexander, Mathie, and Peters [1]), and subsequently the discovery of the first endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG) [2–4]. More recently, other putative ECS receptors, such as TRPV1 (transient receptor potential cation channel, subfamily V, member 1) and GPR55 (G protein-coupled receptor 55), have been recognized [5,6]. The tissues and organs where the various ECS receptors are expressed is still unclear, but in humans the CB_1 receptor is mainly expressed in the brain with some expression in a range of other tissues, including kidney, liver, lung, skeletal muscle, and adipose tissue [7–9]. The TRPV1 receptor is highly expressed in human sensory neurons [10], but it is also expressed in human adipose tissue [11]. On the other hand, the CB_2 receptor is expressed in macrophages [12] and expression is also reported in the rat central nervous system [13], while there is some controversy about its presence in human adipose tissue depots [9,11].

The list of ligands of the cannabinoid receptors is extensive and is divided into 3 categories: phytocannabinoids (from plants), endocannabinoids (endogenous cannabinoids), and synthetic cannabinoids (developed as putative drugs). JTE-907, which is in this last category, binds to CB_1 and CB_2 cannabinoid receptors with a higher affinity for the latter [14]. This synthetic compound appears to be an anti-inflammatory or antiallergic agent [15–17]. The ECS and its receptors CB_1, TRPV1, and CB_2 have been linked to a wide range of functions, including: vascular and endocrine responses, neuroprotection and nociception, immune modulation, stress recovery, food intake, energy balance, and metabolic homeostasis [18–20]. Of particular interest is the role of the ECS in appetite and the regulation of energy balance [21], CB_1 receptor blockade in animals and humans producing a reduction in body weight and an improvement of the comorbidities of obesity [22,23].

Obesity is a major public health problem in most developed countries and in the UK, for example, 25 % of adults are now clinically obese with a BMI ≥30. Obesity is characterized by chronic low grade inflammation [24–26] and this has been linked to the development of the associated comorbidities, particularly the components of the metabolic syndrome [27,28]. Adipose tissue is a major site of inflammation in obesity, with the adipocytes secreting a number of cytokines, chemokines, and other inflammation-related factors, including those involved in angiogenesis [26,29]. Several studies have demonstrated the importance of the ECS in inflammatory processes and angiogenic events; for example, in human macrophages [30], mouse obese models [20], mouse cutaneous tissue [17], rat mesenteric arteries, and human cerebral artery endothelial cells [17,30,31]. Little is known, however, of the genes whose expression may be modulated through the endocannabinoid system.

The aim of the present study was to examine the effects of the ECS on the expression of key inflammatory and angiogenic genes in human white adipocytes. The results suggest that CB_1 and TRPV1 receptors participate in the regulation of several important inflammatory and angiogenic factors in human adipocytes and preadipocytes. These key cannabinoid receptors could be a target for the pharmacotherapy of inflammatory and angiogenic events within adipose tissue.

Materials and Methods
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Cell culture
Human subcutaneous preadipocytes and culture media were obtained from Zen-Bio (Durham, NC, USA) and cultured as previously [32]. Cells were from 5 female patients of average age 45.6 years and with a mean BMI of 27.9. The cells were plated at a density of 40 000/cm^2 onto a 24-well plate and maintained in preadipocyte medium (PM) containing DMEM/Ham's F-12 (1:1, v/v), 10 % fetal calf serum (FCS), 15 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B at 37 °C in a humidified atmosphere of 95 % air/5 % CO_2. The cells were induced at confluence by incubation in differentiation medium composed of adipose medium (AM) supplemented with 0.25 mM isobutylmethylxanthine and 10 µM of a PPARγ agonist for 5 days. The cells were then cultured with AM containing DMEM/Ham's F-12 (1:1, v/v), 3 % FCS, 100 nM insulin, 1 µM dexamethasone, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B [33]. The medium was changed every 3 days.

Preadipocytes (fed 14 days after 100 % confluence) were exposed to JTE-907 (10 µM) for 20 h. Fully differentiated cells at day 14 post-induction were exposed to JTE-907 (1 µM or 10 µM) for 4 or 20 h, and to AM251 (100 nM), A. S. (arachidonyl serotonin, 10 µM) and AM630 (10 µM) (all reagents were from Tocris Bioscience, Bristol, UK). The cells were harvested in 500 ml TRI® Reagent (Sigma-Aldrich, St. Louis, MO, USA). Culture media were also collected. All incubations at each time point were performed in replicates of up to 6 wells.

RNA extraction and RT-PCR
Total RNA was isolated from cells using TRI® reagent and treated with Turbo DNA-free™ (Applied Biosystems/Ambion, Austin, TX, USA) according to the manufacturer's instructions. 1 µg of DNaseI-treated RNA was reverse transcribed to obtain cDNA with Reverse-iT 1st Strand Synthesis kit (Abgene, Epsom, UK) in the presence of anchored oligo dT in a total volume of 20 µl.

Primers were designed using Primer Premier 5 software (Biosoft International, Palo Alto, CA, USA) and synthesized by MWG Biotech (Ebersberg, Germany). Primer sequence, size of the amplified fragments, and optimal conditions of amplification were as follows.

βactin (281 bp): 5′-GTGGCATCCACGAAACTACCTT-3′ (forward), 5′-GGACTCGTCATACTCCTGCTTG-3′ (reverse), 23 cycles and annealing temperature 57 °C;

CB_1 (298 bp): 5′-CAGCGTGGCACCCAGAAGA-3′ (forward), 5′-CGGAAAGCGTGTCGCAGGT-3′ (reverse), 35 cycles and annealing temperature 60.6 °C;

TRPV1 (485 bp): 5′-TGTGCCGTTTCATGTTTGTC-3′ (forward), 5′-GTCGTCCTTGCCATCAGGT-3′ (reverse), 40 cycles and annealing temperature 55.9 °C.

CB_2 (454 bp): 5′-GGCAGCGTGACTATGACCTT-3′ (forward), 5′-AGCACTGGGAACCAACAGAT-3′ (reverse), 45 cycles and annealing temperature 57.3 °C.

Standard-PCR was performed with 1 µl of cDNA and specific primers (0.2 µM forward and 0.2 µM reverse) and 22.5 µl of Ready Mix PCR Master Mix (Abgene) in a total volume of 25 µl on a PCR Express thermal cycler. PCR conditions were as follows: 2 min at 94 °C for denaturation, followed by optimal cycles of 20 s at 94 °C for denaturation, 30 s at the optimal annealing temperature, and 40 s at 72 °C for extension, with a final elongation step of 10 min at 72 °C. Negative controls without templates were performed to exclude the formation of primer dimers. All primer pairs produced a single specific band. PCR products were analyzed by electrophoresis on a 1 % agarose gel with ethidium bromide staining and photographed under UV transillumination. The products were sequenced by MWG Biotech (Ebersberg, Germany) to confirm their identity.

Real-time PCR
Quantitative real-time PCR was carried out in a final volume of 12.5 µl consisting of 12.5–100 ng reverse-transcribed cDNA mixed with optimal concentrations of primers and probe and qPCR Core kit (Eurogentec, Southampton, UK) in 96-well plates on a Stratagene M×3005P detector. The primer and probe sets were designed using Primer Express software (Applied Biosystems) and synthesized commercially (Eurogentec). The sequence and optimal concentrations of primers and probes together with the size of products were as detailed previously for β-actin, IL-6 (interleukin 6), MCP-1 (monocyte chemotactic protein-1), TNFα (tumor necrosis factor-alpha), and ANGPTL4 (FIAF, angiopoietin-

like protein 4) [33, 34]. CB$_1$, TRPV1, VEGF (vascular endothelial growth factor) and IL-1β were as follows.

CB$_1$: 5′-CTCATTAAGACGGTGTTTGCATTC-3′ (forward), 5′-CGT-GTCGCAGGTCCTTACTC-3′ (reverse) and 5′-FAM-TGCTCTGCCT-GCTGAACTCCACCG-TAMRA-3′ (probe);

TRPV1: 5′-TGCTGGCCTATGTAATTCTCACC-3′ (forward), 5′-TCT-TCTCCGTGTCCAGGATGG-3′ (reverse) and 5′-FAM-CATCCTCCT-GCTCAACATGCTCATCGCC-TAMRA-3′ (probe);

VEGF: 5′-TGAGATCGAGTACATCTTCAAGCC-3′ (forward), 5′-GTGAGGTTTGATCCGCATAATCTG-3′ (reverse) and 5′-FAM-CCT-GTGTGCCCCTGATGCGATGCG-TAMRA-3′ (probe);

IL-1β: 5′-TGGCCCTAAACAGATGAAGTGC-3′ (forward), 5′-GTAGT-GGTGGTCGGAGATTCG-3′ (reverse) and 5′-FAM-ACCTGGAC-CTCTGCCCTCTGGATGG-TAMRA-3′ (probe).

Typically, the amplification started with 2 min at 50°C, 10 min at 95°C, and then 40 cycles of the following: 15 s at 95°C and 1 min at 60°C.

Human β-actin was used as an endogenous reference; its expression remained unchanged both in response to the various treatments and during preadipocyte differentiation. Relative quantitation values were expressed using the $2^{-\Delta\Delta Ct}$ method (see User Bulletin 2, ABI PRISM 7700, pp 11–15, Applied Biosystems) as fold changes in the target gene normalized to the reference gene (β-actin) and related to the expression of the untreated controls. The PCR efficiency in all runs was close to 100%, and all samples were analyzed in at least duplicate.

Measurement of adipokines by ELISA

IL-6, MCP-1, TNFα, and IL-1β were measured in cell culture media among 8 other proteins using a commercial ELISA array for autoimmune disease (MEH-005A; SABiosciences Corporation, Frederick, USA). The assays were conducted in 96-well microplates, according to the manufacturer's instructions.

Statistical analysis

The statistical significance of differences between groups was assessed by Student's *t*-test. A p-value of <0.05 was considered to be statistically significant.

Results
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Expression of cannabinoid receptor genes in differentiated adipocytes and preadipocytes

We first investigated whether the human white adipocytes used here express the cannabinoid receptor genes when differentiated in culture. RT-PCR was performed on mRNA from mature adipocytes (14 days post-induction). The results showed that the mRNAs for the genes encoding CB$_1$ and TRPV1 were present in the differentiated adipocytes at day 14 (○ **Fig. 1a** by real time-PCR), but CB$_2$ was not expressed in these cells (data not shown). The pattern of expression was the same for preadipocytes. CB$_1$ and TRPV1 were also present in preadipocytes as shown in ○ **Fig. 4a** (vide infra) by real time-PCR; and CB$_2$ was not expressed in preadipocytes either (data not shown). The primers for CB$_2$ were previously tested with mRNA obtained from a human macrophage-like cell line (U937) as a positive control, attaining a clear signal, which was confirmed by sequencing.

Upregulation of cannabinoid receptors, inflammatory, and angiogenic genes by JTE-907

In pilot experiments, the effects of several CB$_1$ and CB$_2$ agonists and antagonists, at different doses, on the expression of inflammation-related genes in human adipocytes were examined and particularly strong responses were obtained with JTE-907 (results not shown). In subsequent experiments, human preadipocytes were differentiated and exposed to JTE-907 at day 14 post-induction at which time they contained multiple lipid droplets. The mRNA levels of several genes were measured 20 h after exposing the human adipocytes to JTE-907 at 2 different doses (1 and 10 μM) (○ **Fig. 1**). Firstly, 2 cannabinoid receptor genes (CB$_1$ and TRPV1) were analyzed (○ **Fig. 1a**). At 1 μM no effect was found on the mRNA levels of the 2 receptors. At the second dose (10 μM), CB$_1$ mRNA level was not significantly changed and TRPV1 mRNA level was increased 3-fold.

Subsequently, the mRNA levels of inflammatory markers (IL-6, MCP-1, TNFα, and IL-1β) and angiogenic factors (VEGF and ANGPTL4) were quantitated (○ **Fig. 1b**). The lowest dose (1 μM) produced no effect on the mRNA level of any of the genes. The highest dose (10 μM) increased IL-6, MCP-1, IL-1β, VEGF, and ANGPTL4 mRNA levels by 41-, 7-, 6-, 6-, and 3-fold, respectively; however, this dose did not affect TNFα gene expression.
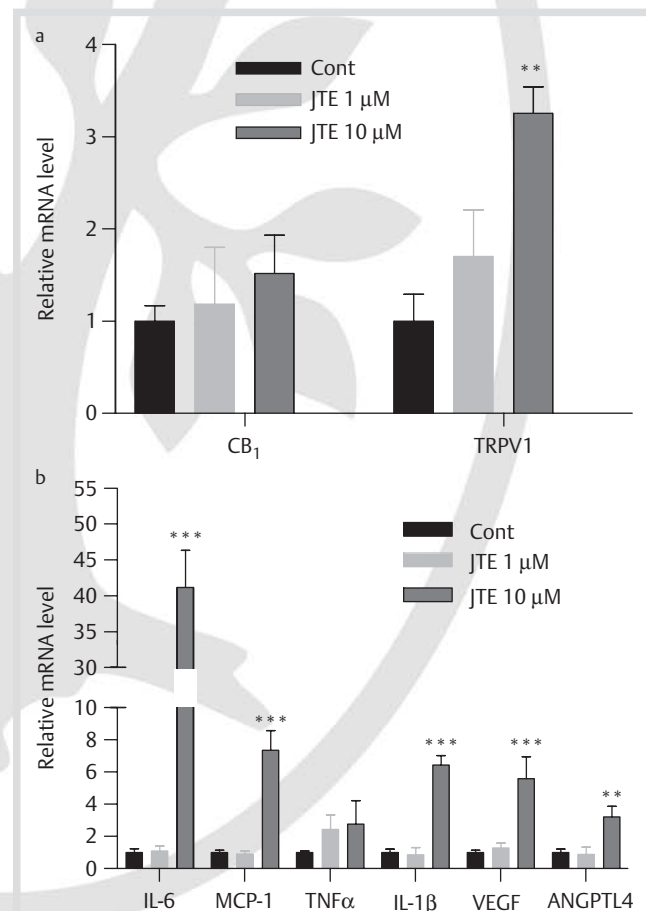

Fig. 1 Effect of JTE-907 on gene expression in human adipocytes. Differentiated human adipocytes (14 days) were exposed for 20 h to JTE-907 (1 and 10 μM). **a**: CB$_1$ and TRPV1 gene expression. **b**: Expression of inflammatory marker genes and angiogenic factors. mRNA levels were normalized to human β-actin and quantitated by real time-PCR. Means ± SE; n = 6. * p < 0.05, ** p < 0.01 and *** p < 0.001 compared with control. JTE: JTE-907.
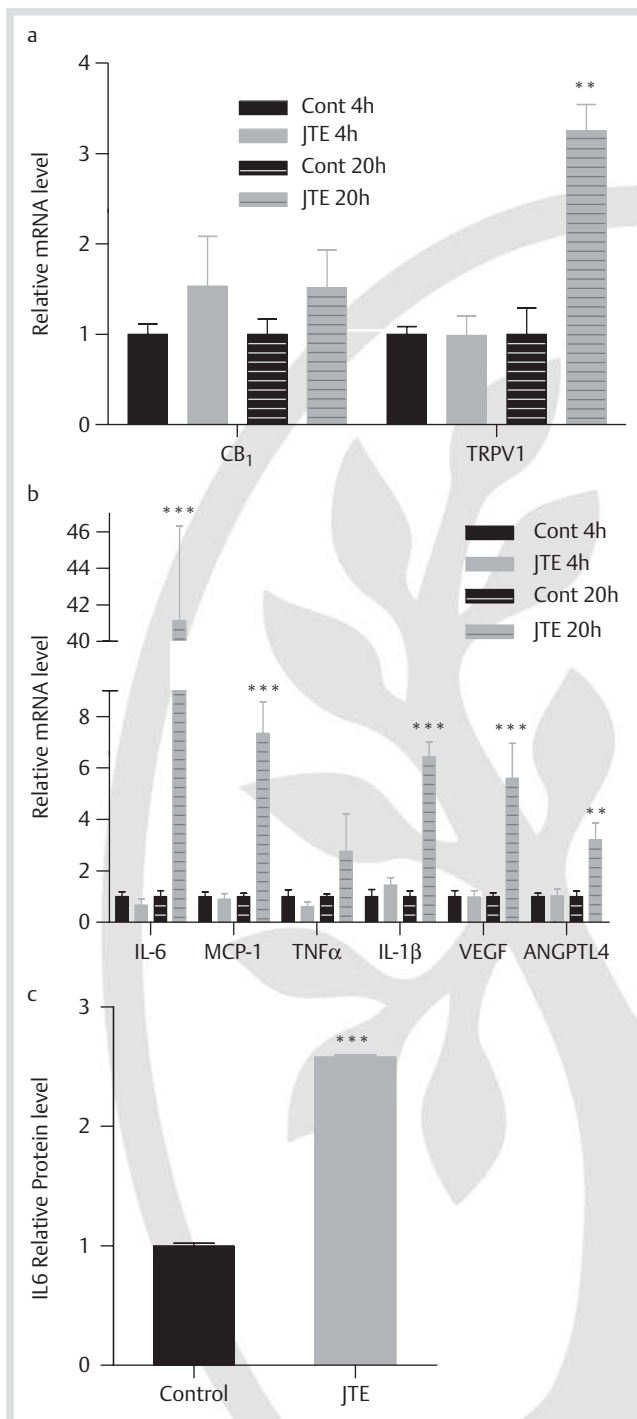
Fig. 2 Effect of JTE-907 on gene expression and protein secretion in human adipocytes. Differentiated human adipocytes (14 days) were exposed to JTE-907 (10 μM) for 4 and 20 h. **a**: CB_1 and TRPV1 gene expression. **b**: Expression of inflammatory marker genes and angiogenic factors. mRNA levels were normalized to human β-actin and quantitated by real time-PCR. Means ± SE, n = 6. ** p < 0.01 and *** p < 0.001 compared with control. **c**: Secretion of IL-6 protein measured by ELISA. Differentiated human adipocytes (14 days) were exposed for 20 h to JTE-907 (10 μM); n = 3. Means ± SE. *** p < 0.001 compared with control.

Doses of 1 and 10 μM of JTE-907 were used in vitro previously by another group [14], without damage to the cells, and a dose of 50 μM in our studies led to similar effects as 10 μM. Exposure of differentiated human adipocytes (14 days) to JTE-907 at 10 μM

for 4 h did not result in any significant change in the level of the mRNA for any of the genes mentioned previously (○ **Fig. 2a, b**). IL-6, MCP-1, TNFα, and IL-1β proteins were measured, in addition to 8 other proteins involved in autoimmune disease, in the medium using a commercial ELISA array in the cells incubated with 10 μM JTE-907 for 20 h. The ELISA array showed a 2.6-fold increase in IL-6 release (○ **Fig. 2c**), which was statistically significant, consistent with the mRNA measurements. The secretion levels of other relevant proteins, such as TNFα and IL-1β, were below the detection sensitivity of the array. There was no significant change in MCP-1 release.

Effect of cannabinoid receptor antagonists on the response to JTE-907

The upregulation of several genes by JTE-907 (10 μM for 20 h) on differentiated human adipocytes (14 days) was inhibited when AM251 (100 nM), a CB_1 receptor antagonist, was added at the same time (○ **Fig. 3a, b**). TRPV1 mRNA level was reduced 4-fold, although no significant effect was found on CB_1 gene expression (○ **Fig. 3a**). The mRNA levels of IL-6, MCP-1, IL-1β, VEGF, and ANGPTL4 decreased 29-fold, 5-fold, 3-fold, 6-fold and 3-fold, respectively; this antagonist did not affect TNFα gene expression (○ **Fig. 3b**).

Arachidonyl serotonin (A.S.), considered to be a TRPV1 and FAAH (degrading enzyme of anandamide) antagonist [35], partially inhibited the effect of JTE-907 on differentiated (14 days) human adipocytes when the 2 agents were added together (both at 10 μM for 20 h) (○ **Fig. 3c, d**). TRPV1 mRNA expression was reduced 3-fold, although no significant effect was found on CB_1 gene expression (○ **Fig. 3c**). The expression of IL-6, MCP-1, VEGF, and ANGPTL4 was significantly lower than with JTE-907 alone, being 10-fold, 6-fold, 5-fold, and 4-fold lower, respectively; TNFα and IL-1β gene expression remained unaltered (○ **Fig. 3d**).

Interestingly, AM630 (a CB_2 antagonist), partially upregulated the effect of JTE-907 alone (○ **Fig. 3e, f**). AM630 and JTE-907 (both 10 μM) were added simultaneously to differentiated human adipocytes and the cells were collected 20 h later. CB_1 and TRPV1 mRNA levels were augmented 1.5-fold and 3-fold, respectively (○ **Fig. 3e**), while MCP-1 and ANGPTL4 expression was increased 2-fold and 4-fold, respectively (○ **Fig. 3f**). There was no effect of AM630 on IL-6, TNFα, IL-1β, and VEGF gene expression (○ **Fig. 3f**).

Differential effects of JTE-907 on the expression of preadipocytes genes

The effect of JTE-907 on the expression in preadipocytes of those genes sensitive to the compound in adipocytes was then examined. Human preadipocytes were fed 14 days after reaching 100 % confluence and exposed to JTE-907 at 10 μM for 20 h (○ **Fig. 4**). Expression of the CB_1 receptor was slightly downregulated (56 % reduction) in preadipocytes with no significant effect in mature adipocytes (○ **Fig. 4a**). The mRNA level of 2 genes involved in inflammation, MCP-1 and IL-1β, was reduced by approximately 60 % in preadipocytes (○ **Fig. 4b**), which was in contrast to increases of more than 700 % and 600 %, respectively, in mature adipocytes (○ **Fig. 1b**).

Discussion and Conclusions
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The high and growing incidence of obesity and its associated disorders now represents a major public health problem [36].
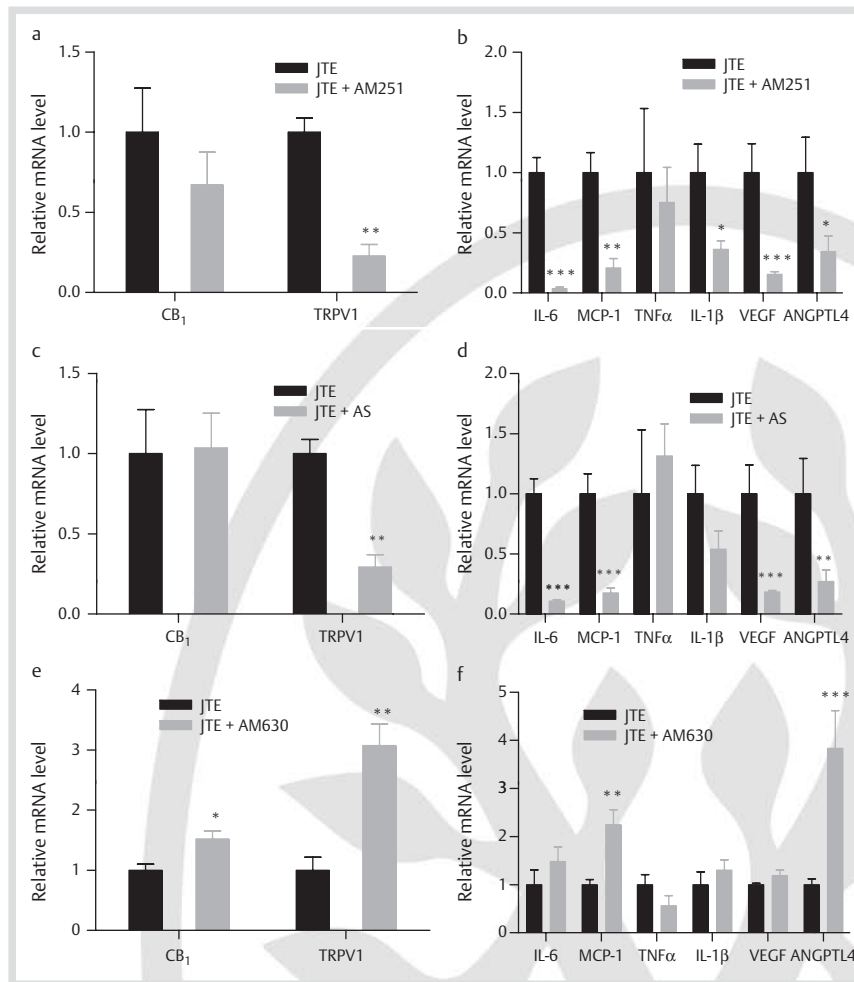
Fig. 3 Inhibition or upregulation of JTE-907 effect on gene expression by antagonists of CB_1, TRPV1 and CB_2. **a** and **b**: Differentiated human adipocytes (14 days) were exposed for 20 h to JTE-907 (10 μM) and JTE-907 (10 μM)+AM251 (100 nM). **a**. CB_1 and TRPV1 gene expression. **b**: Expression of inflammatory marker genes and angiogenic factors **c** and **d**: Differentiated human adipocytes (14 days) were exposed for 20 h to JTE-907 (10 μM) and JTE-907+A.S. (both 10 μM). **c**: CB_1 and TRPV1 gene expression. **d**: Gene expression of inflammatory markers and angiogenic factors **e** and **f**. Differentiated human adipocytes (14 days) were exposed for 20 h to JTE-907 (10 μM) and JTE-907+AM630 (both 10 μM). **e**: CB_1 and TRPV1 gene expression. **f**: Expression of inflammatory marker genes and angiogenic factors. mRNA levels were normalized to human β-actin. Means±SE; n=6. * p<0.05 and ** p<0.01 compared with JTE-907.

The endocannabinoid system and its relation with metabolism, inflammation, and angiogenesis have been considered as one of the key targets in the treatment of obesity and its comorbidities, among other diseases [7,18,37,38]. Cannabinoid receptors are mainly expressed in the central nervous system, but their peripheral location and effects are becoming of increasing interest [30,39]. The present study has examined the effects of JTE-907, a synthetic cannabinoid [14], on the expression of 2 receptors of the ECS (CB_1 and TRPV1) and on the expression of major adipokine genes in preadipocytes and in mature adipocytes.

White adipose tissue secretes a large number of protein signals and protein factors, termed adipokines. The production and circulating level of some of these adipokines is increased in obese patients, for example IL-6, MCP-1, and TNFα [25,40] while their gene expression is reduced in adipose tissue after weight loss, as is the case with IL-1β [41]. The expression of some adipokines, such as leptin, VEGF, ANGPTL4 [32], and RANTES (an immune mediator) [42], is increased under hypoxic conditions, and low oxygen tension has been proposed as a key trigger for the mild chronic inflammatory state associated with obesity [26]. Furthermore, there is a link between these adipokines and the comorbidities associated with obesity [43,44].

Within human white adipose tissue, CB_2 gene and protein expression in preadipocytes and mature adipocytes isolated from omental and subcutaneous tissue has been reported by some authors [9], while others [11] were unable to detect CB_2 gene expression in adipocytes and tissue from the subcutaneous fat depot. Our results are

consistent with the latter, confirming the expression of CB_1 and TRPV1, but not of CB_2 in human adipocytes.

The key findings from the present study are that JTE-907 is able to regulate the expression of receptor genes, which are part of the ECS (CB_1 and TRPV1 receptors), together with a proinflammatory behavior and the upregulation of genes associated with angiogenesis (VEGF and ANGPTL4). Previous studies have shown that JTE-907 has an anti-inflammatory action in mouse cutaneous tissue [14–17], but no effect of JTE-907 has been found in human colon [45]. In these investigations, JTE-907 was believed to be acting through the CB_2 receptor; however, in our study, due to the absence of expression of CB_2 receptors in human adipocytes the actions of this ligand are more likely to occur through CB_1 or TRPV1.

Therefore, the cells were exposed to an antagonist of CB_1 (AM-251), CB_2 (AM-630) and TRPV1 and FAAH (arachidonoyl serotonin) in different experiments, in order to determine the probable route of action of JTE-907 within human white adipocytes. Interestingly, the compound appeared to operate through CB_1 and TRPV1, either acting through both directly or through 1 receptor, which would subsequently activate the second; the last mechanism has been demonstrated by other authors [19,46]. The importance of the TRPV1 receptor in obesity is unclear, with 2 important studies showing contrasting results [47,48]. AM630, an antagonist or inverse agonist for CB_2, which may act as an inverse agonist [49] or weak partial agonist of the CB_1 receptor [50], augmented the effects obtained with JTE-907 alone in the case of genes such as CB_1, TRPV1, MCP-1, and ANGPTL4.
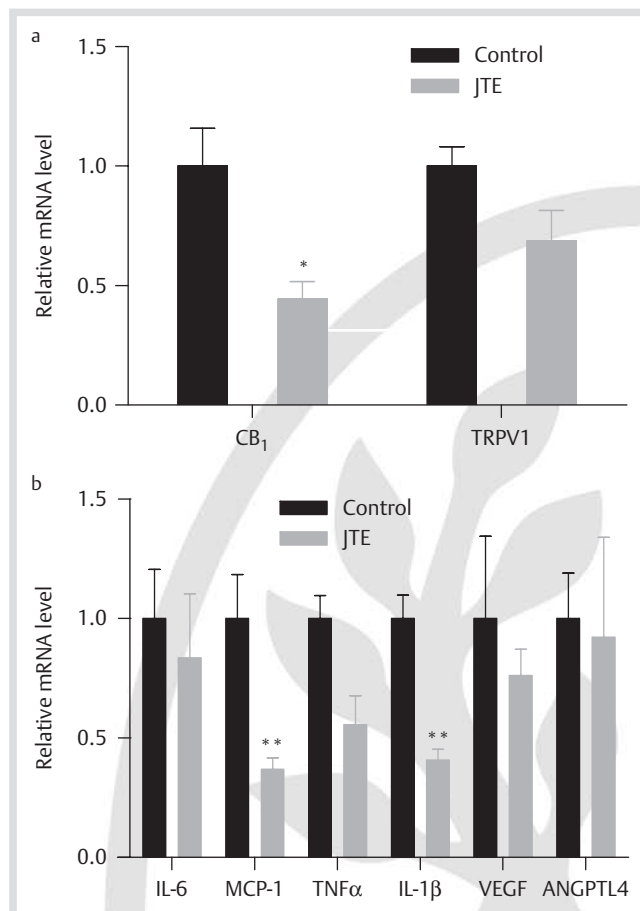
Fig. 4 Downregulation of CB_1, MCP-1, and IL-1β gene expression in human preadipocytes by JTE-907. Human preadipocytes fed 14 days after 100% confluence were exposed for 20 h to JTE-907 (10 µM). **a**: CB_1 and TRPV1 gene expression. **b**: Expression of inflammatory marker genes and angiogenic factors. mRNA levels were normalized to human β-actin. Means ± SE; n = 6. ** p < 0.01 compared with control.

In a recent study, Hoareau et al. [51] reported anti-inflammatory actions of SR141716 (also known as Rimonabant, a CB_1 antagonist) and SR144528 (a CB_2 antagonist) against LPS stimulation in mature adipocytes obtained from human subcutaneous adipose tissue, with stronger effects with the latter. These and other recent results confirming the anti-inflammatory actions of a CB_1 antagonist like Rimonabant [30, 52], may support the idea that AM630 and JTE-907 are exerting their proinflammatory actions as agonists of CB_1. In addition, there is evidence relating some CB_1 agonists with anti-inflammatory actions, such as WIN55,212-2 [53, 54], ACEA [55] or anandamide (CB_1 and CB_2 agonist) [56], or with proinflammatory actions, like anandamide [57]. This confirms the fact that different or even the same, endocannabinoids may work in opposite ways. The increased secretion of IL-6 protein in response to JTE-907 underlines the proinflammatory property of this compound in human adipocytes and indicates that the changes in gene expression are reflected by parallel alterations in the amount of the encoded protein.

The relationship between angiogenesis and JTE-907 has not been described previously. Furthermore, it is the first time this synthetic cannabinoid has been found to upregulate the expression of genes associated with a proinflammatory state and to have a proangiogenic effect. The receptors, CB_1 and TRPV1, have been linked to inflammation and cardiovascular responses

[18, 19]. This would indicate that in adipocytes JTE-907 might be acting as an agonist for these receptors.

There is increasing recognition of the importance of other cells, apart from mature adipocytes, in the function of white adipose tissue, including inflammation. This is particularly evident with respect to macrophages which are recruited during the expansion of adipose mass in obesity, and this is considered to be a key component of the inflammatory response. Preadipocytes are also increasingly recognized to be major players in adipose tissue inflammation [58, 59]. However, JTE-907 did not affect in preadipocytes those same genes that were upregulated in the mature adipocytes. In practise, for those genes in preadipocytes that were influenced by the treatment, there was a downregulation in contrast to the upregulation in the mature adipocytes. Previous studies have shown that the preadipocytes may show a similar, or opposite, behavior to mature adipocytes, depending on the parameter [60, 61].

Inflammation and angiogenesis are key processes in obesity, the metabolic syndrome, and a number of other diseases. The present study suggests that CB_1 and TRPV1 receptors participate in the regulation of the expression of several important inflammatory and angiogenic gene factors in human mature adipocytes. Understanding the mechanism of action of the different cannabinoid receptors within adipose tissue and other peripheral sites may be valuable in the development of pharmacological treatments for obesity-associated diseases.

Acknowledgements
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