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Dysregulation of Cannabinoid CB1 Receptor Expression in Subcutaneous Adipocytes of Obese Individuals

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Abstract: The endocannabinoid system (ECS) plays a key role in the regulation of appetite, body weight and metabolism. We undertook the present study to further clarify the regulation of the cannabinoid CB1 receptor (CB1, CNR1) in human adipose tissue in obesity. CB1 receptor mRNA expression was ~1.6-fold (P<0.004) and 1.9-fold higher (P<0.05) in subcutaneous adipocytes from obese compared to non-obese subjects in microarray and quantitative real-time PCR studies, respectively. Higher CB1 receptor mRNA expression levels in both adipose tissue (~1.2 fold, P<0.05) and adipocytes (~2 fold, P<0.01) were observed in samples from visceral compared to subcutaneous depots collected from 22 obese individuals. Immuno-fluorescence confocal microscopy demonstrated the presence of CB1 receptor on adipocytes and also adipose tissue macrophages. These data indicate that adipocyte CB1 receptor is up-regulated in human obesity and visceral adipose tissue and also suggest a potential role for the ECS in modulating immune/inflammation as well as fat metabolism in adipose tissue.

Key words: adipocyte, adipose tissue, CB1 receptor, endocannabinoid, obesity, real-time PCR

INTRODUCTION

The prevalence of obesity has increased dramatically over the last three decades and now approaches epidemic proportions (Flegal *et al*, 2002). Obesity increases risk for a large number of chronic diseases including insulin resistance, metabolic syndrome, type 2 diabetes mellitus (T2DM) and cardiovascular disease. The mechanisms by which obesity increases risk for these conditions are not fully understood, but it is likely that adipose tissue itself plays a key role. It is now apparent that adipose tissue is not merely a passive storage depot, but is, in fact, a complex, metabolically active tissue that secretes a variety of signaling molecules regulating feeding behavior, energy expenditure and metabolic, endocrine and immune function. In particular, in obese individuals adipocytes and other cells in adipose tissue secrete a large number of pro-inflammatory cytokines which promote a state of chronic, low-grade systemic inflammation that has been associated with many of the comorbidities of obesity (Lee and Pratley, 2005).

A number of novel pathways involved in the regulation of body weight and metabolism have recently been identified. One such pathway is the endogenous cannabinoid or endocannabinoid system (ECS). The ECS consists of two receptors, CB1 and CB2, their endogenous ligands, and multiple enzymes involved in the biosynthesis and degradation of these endocannabinoids (Duncan *et al.*, 2005; Pagotto *et al.*, 2005; Piomelli, 2003). Both receptors belong to the G-protein coupled receptor family and share 48% amino acid sequence homology with each other (Howlett *et al.*, 2002).

There is increasing evidence that the ECS plays a role in the regulation of food intake and appetite. Compared with wild-type littermates, CB1 receptor knockout mice exhibited reduced spontaneous caloric intake, total fat mass and body weight (Cota *et al.*, 2003; Di Marzo *et al.*, 2001). In addition to producing significant decreases in body weight, CB1 receptor blockade with rimonobant also increases

Abbreviations: CB1, cannabinoid CB1 receptor; DAPI, 4',6-Diamidino-2-phenylindole; ECS, endocannabinoid system; QPCR, quantitative real-time PCR; SAC, subcutaneous abdominal adipocytes; SAT, subcutaneous abdominal adipose tissue; SVCs, stromal vascular cells; T2DM, type 2 diabetes mellitus; VAC, visceral adipocyte; VAT, visceral adipose tissue

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adiponectin levels (Bensaid *et al.*, 2003), lowers plasma leptin, insulin and free fatty acid levels (Ravinet Trillou *et al.*, 2003), and improves cardiovascular risk factors such as CRP, HDL-cholesterol, triglycerides, and insulin resistance (Van Gaal *et al.*, 2005). The magnitude of the improvements in these factors is beyond that expected from weight loss alone, suggesting that the ECS, through the CB1 receptor, may play a direct role in regulating metabolism.

Although CB1 receptor is most abundant in neuronal tissue, its distribution is not limited to the brain. Peripheral tissues expressing CB1 receptor include liver (Osei-Hyiaman *et al.*, 2005), cardiac (Batkai *et al.*, 2004), vascular (Ralevic, 2003), gastrointestinal tract (Croci *et al.*, 1998), eye (Porcella *et al.*, 2000), prostate gland (Ruiz-Llorente *et al.*, 2003), placenta (Park *et al.*, 2003), skeletal muscle (Cavuoto *et al.*, 2007) and adipose tissue (Bensaid *et al.*, 2003; Bluher *et al.*, 2006; Cota *et al.*, 2003; Engeli *et al.*, 2005). Previous studies indicated that the ECS has effects on energy expenditure and lipid metabolism in peripheral tissues independent of its centrally mediated effect on food intake. ECS activity and CB1 receptor expression in adipose tissue are increased in obese animals relative to lean controls. A recent paper demonstrated that the expression and activity of endocannabinoid degrading enzymes FAAH and MAGL as well as the synthesizing enzyme NAPE-PLd and DAGL were expressed in adipose tissue and dysregulated in insulin-resistance and in obese rodents (D'Eon *et al.*, 2008). It was suggested that this dysregulation may cause the up-regulation of circulating levels of the endocannabinoids anandamide and 2-AG that is observed in obesity as well as insulin resistance (D'Eon *et al.*, 2008). In human obesity, ECS activity also appears up-regulated as evidenced by circulating levels of the endocannabinoids, anandamide and 2-AG, that were 35 and 52% higher in obese compared with lean postmenopausal women (Engeli *et al.*, 2005). In contrast to results in animals, these authors found CB1 expression in adipose tissue was down-regulated in obesity and did not change with weight loss (Bluher *et al.*, 2006; Engeli *et al.*, 2005). However, CB1 expression levels in isolated human primary adipocytes have not been compared between obese and non-obese individuals.

The purpose of present study was to further characterize the expression of CB1 receptor in adipose tissue cell types and the effects of obesity in humans. To that end, we measured gene expression levels of CB1 receptor in adipose tissue, primary cultured preadipocytes and isolated adipocytes from lean and obese volunteers. In addition, the expression of the CB1 receptor in adipose tissue cell types was confirmed using immunofluorescence confocal microscopy.

Table 1. Characteristics of non-diabetic obese and non-obese subjects (Group 2) for quantitative real-time PCR

	Non-obese	Obese
Female/Male (F/M)	4/6	4/6
Age (years)	30±8	33±7
Height (cm)	164±7	171±10
Weight (kg)	72±11	153±21[a]
Body Mass Index (kg/m^2)	27±3	53±6[a]
Body fat (%)	28±5	39±7[a]
Fasting glucose (mg/dL)	90±10	96±9
2 hr glucose (mg/dL)	120±25	145±37
Fasting insulin (mU/L)	30±5[b]	93±60[a,c]
2 h insulin (mU/L)	143±57[d]	342±183[a,e]

a: $P<0.05$ (compared with non-obese subjects);
b, c, d, and e: Data from 3F/6M, 4F/4M, 2F/6M, and 4F/3M, respectively.

MATERIALS AND METHODS

Subjects

All studies were approved by the Institutional Review Boards of the NIDDK and the University of Vermont and all subjects provided written informed consent prior to participation. The subject selection criteria, metabolic measurements and adipose tissue biopsies for the initial microarray profiling study have been previously described (Lee *et al*, 2005). Nineteen obese subjects [9M/10F, age 29±5 years, BMI 55±8 kg/m^2] and 20 non-obese individuals [10M/10F, 31±6 year, BMI 25±3] participated in a microarray gene expression study using Affymetrix HG-U95 GeneChip microarray sets in isolated subcutaneous abdominal adipocytes (SAC) (Group 1). A second group of obese subjects and non-obese controls participated in studies in which CB1 mRNA expression was measured by quantitative real-time PCR (QPCR) (Group 2, Table 1). In a third study (Nair *et al*, 2005), CB1 mRNA expression levels were measured in preadipocytes/stromal vascular cells (SVCs) cultured from subcutaneous abdominal adipose tissue (SAT) biopsies from 14 obese (7M/7F, age 29±5 years, BMI 55±8) and 14 non-obese (7M/7F, 32±5, BMI 25±4) subjects with Affymetrix HG-U133 GeneChip microarray sets (Group 3). In addition, CB1 mRNA expression levels in cultured subcutaneous preadipocytes/SVCs were measured by QPCR in another independent confirmational subject set of 10 obese (4M/6F, age 33±8 years, BMI 52±5) and 10 non-obese (5M/4F, age 30±9 years, BMI 26±3) subjects (Group 4) (Nair *et al*, 2005). Finally, paired SAT and visceral adipose tissue (VAT) samples were obtained from 22 morbidly obese Caucasian subjects (2M/20F) at the time of gastric bypass surgery

Table 2. Groups of subjects who participated in microarry, confirmational real-time PCR, and immunofluorescence studies

	Group 1	Group 2	Group 3*	Group 4	Group 5
Age	Obese 29±5 Non-ob 31±6	Obese 33±7 Non-ob 30±8	Obese 29±5 Non-ob 32±5	Obese 33±8 Non-ob 30±9	Obese 40±8
M/F	Obese 9/10 Non-ob 10/10	Obese 4/6 Non-ob 4/6	Obese 7/7 Non-ob 7/7	Obese 4/6 Non-ob 5/4	Obese 2/20
BMI	Obese 55±8 Non-ob 25±3	Obese 53±6 Non-ob 27±3	Obese 55±8 Non-ob 25±4	Obese 52±5 Non-ob 26±3	Obese 52±9
Health	healthy	healthy	healthy	healthy	8 were diagnosed with T2DM
Tested cell type	Isolated adipocytes	Isolated adipocytes	Cultured preadipocytes/ SVCs	Cultured preadipocytes/ SVCs	SAT vs VAT, SAC vs VAC
Experimental Method	Affymetrix GeneChip HG-U95 set	Real-time PCR	Affymetrix GeneChip HG-U133 set	Real-time PCR	Real-time PCR Immunofluorescence
Reference	(Lee et al., 2005)		(Nair et al., 2005)	(Nair et al., 2005)	

*All subject groups are independent except that Group 3 is a sub-set of Group 1.

(Group 5). The mean (±SD) age of the patients was 40±8 y (range 26-56) and their BMI was 52±9 kg/m^2 (range 39-63). All subjects in the first 4 groups were selected to be in good health and were on no medications at the time of the study. Among subjects in group 5, eight were diagnosed with type 2 diabetes. Information of subjects is summarized in Table 2.

Adipose tissues sampling and fractionation into adipocytes and SVCs

Subcutaneous abdominal fat biopsies were obtained by needle biopsies under local anesthesia with 1% lidocaine from subjects in groups 1-4 after a 12 hour overnight fast. For subjects in group 5, SAT and VAT samples were collected in the operating room at the time of gastric bypass surgery. The minced adipose tissue was digested as described previously (Permana *et al*, 2004) with some modifications. Briefly, adipose tissue was digested in PBS containing 3 mg/mL collagenase type I, 3% BSA and 5.5 mM glucose for 60 min at 37°C in a water bath. The digested adipose tissue cell mixture was passed through a sterile 230 μm stainless steel tissue sieve (Bellco Glass Inc, Vineland, NJ) and the adipocytes were allowed to float by gravity. The floating cells were collected as the adipocyte fraction and washed with PBS. The stromal vascular cell containing infranatant was placed into another tube and washed with PBS by centrifugation for 10 min at 500 *g*.

Primary cultured preadipocytes

SVCs were isolated from needle biopsies of SAT (Group 3 and 4) and cultured as previously described (Nair *et al*, 2005). At sub-confluency, the cultured cells were trypsinized and plated at a concentration of approximately 1.5×10^6 cells/15-cm dish for RNA extraction, which was carried out approximately 14 days after the biopsy. We have previously demonstrated that after 2 weeks in culture under these conditions, preadipocytes predominate and contamination from other cell types is minimal (Permana *et al*, 2004).

Quantitative real-time PCR

Total RNA was extracted from isolated mature adipocytes and primary cultured preadipocytes/SVCs using an RNeasy Mini Kit from Qiagen (Valencia, CA). An RNeasy Lipid Tissue Mini Kit was used for adipose tissue. During the extraction, RNA was treated with DNase I using the RNase free DNAse Set (Qiagen) according to the manufacturer's instructions. QPCR was carried out as previously described (Lee *et al*, 2003), using the TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA) on a 7300 Real-Time PCR System. The transcript level of CB1 receptor was normalized to that of 18S ribosomal RNA or GAPDH (Applied Biosystems).

Immunofluorescence confocal microscopy

Paraffin sections of adipose tissue sample were mounted on charged glass slides, dewaxed, rehydrated, and antigen retrieved in Target Retrieval Solution (Dako, Carpinteria, CA). Slides were blocked with 1% BSA (Sigma, St.Louis, MO), and immunostained using a method previously described (Tharp *et al*, 2008). Samples were immunostained with an antibody mixture of mouse anti-human CD68 (Dako) and rabbit anti-human CB1 (Cayman Chemical, Ann Arbor, MI) for one hour at 25°C, washed in PBS, incubated with highly adsorbed donkey anti-mouse AlexaFluor-488 and donkey anti-rabbit AlexaFluor-568 conjugated secondary antibodies (Molecular Probes, Eugene, OR). Nuclei were counterstained with 4',6-Diamidino-2-phenylindole (DAPI) (Roche, Indianapolis, IN). Fluorescently stained slides were imaged on a Zeiss LSM 510 META confocal microscope (Carl Zeiss, Thornwood, NY).

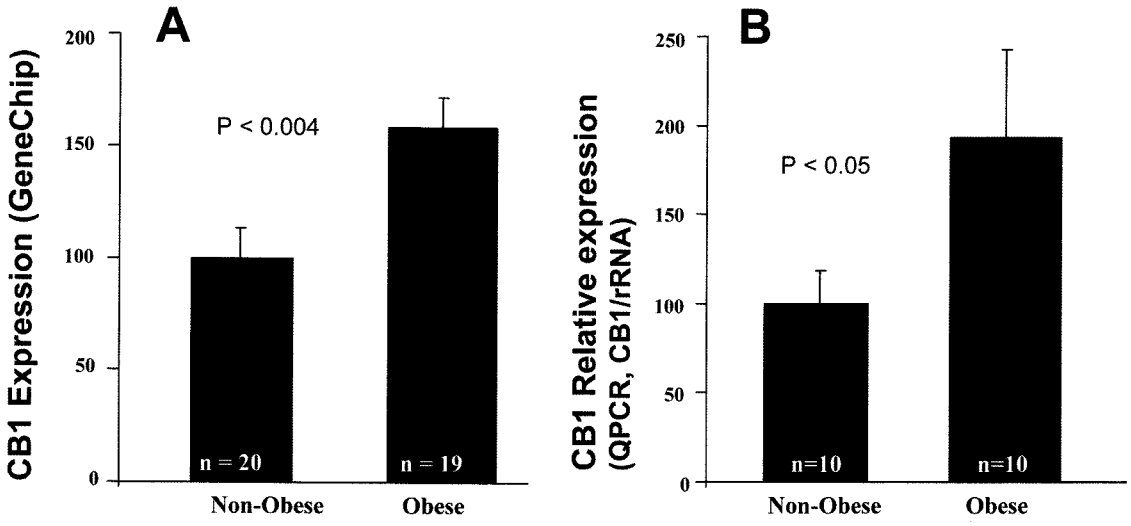

Fig. 1. Higher CB1 expression in adipocytes from obese subjects compared to non-obese controls in Affymetrix genechip analysis using HG-U95 set (A) and in confirmational QPCR of a separate sample set of 10 non-obese vs. 10 obese subjects (B). Mean±SEM

Statistics-data analysis

All results are expressed as means±SD unless otherwise specified. Raw microarray expression data obtained from the Microarray Suite, Version 5.0 software were analyzed using the Mann–Whitney U-test to compare CB1 gene expression levels between non-obese and obese subjects (Group 1). QPCR data for CB1 receptor mRNA were analyzed using a two-tailed Student's t-test. General linear regression models were used to assess the relationships of expression levels between CB1 receptor gene and inflammation-related genes. P values less than 0.05 were considered significant.

RESULTS

Over-expression of CB1 mRNA in human adipocytes from obese compared to lean subjects

We observed up-regulation of CB1 receptor mRNA in SAC from obese subjects compared to non-obese controls in the microarray experiment (Group 1). The Affymetrix HG-U95 set had one probe set for CB1 (36906_at) showing a higher hybridization signal with cRNA samples from SAC of 19 obese subjects compared to 20 non-obese controls (Fig. 1). Expression levels of CB1 were ~1.6-fold higher (P<0.004). Using QPCR, we confirmed the up-regulation of CB1 mRNA in SAC with obesity in an independent sample set of 10 obese and 10 non-obese subjects (Group 2, Table 1). Relative expression levels of CB1 mRNA normalized to that of 18S rRNA were ~1.9 fold higher (193±50 v 100±18 relative units, P<0.05) in obese compared to non-obese subjects (Fig. 1).

CB1 expression is low in primary cultured human preadipocytes

Prior studies have indicated that the expression of CB1 is differentiation dependent, with low levels expressed in preadipocytes. There are three probe sets for CB1 in the Affymetrix HG-U133 set which was used for the microarray study using cultured subcutaneous preadipocyte/SVCs from 14 obese and 14 non-obese subjects (Group 3). None of cRNA samples showed Present calls for two probes sets (207940_x_at and 208243_s_at), while cRNA samples of only 3 obese and 2 non-obese subjects showed a hybridization signal with Present calls for the third probe set (213436_at). Using QPCR we further assessed CB1 expression in cultured preadipocytes/SVCs isolated from 10 lean and 10 obese subjects (Group 4). This analysis showed weak expression of CB1: in most samples (15 out of 20 subjects) expression levels were lower than the lower limit of detection (Ct values higher than 40), while the remaining 5 subjects showed low levels (35<Ct values<40).

Over-expression of CB1 mRNA in adipose cells from visceral compared to subcutaneous depots

Using QPCR, we measured CB1 expression levels in adipose tissue and isolated adipocytes of paired SAT and VAT samples obtained from 22 morbidly obese subjects (Group 5). Relative expression levels of CB1 normalized to that of GAPDH were ~1.2 fold higher (90.7±25 v 74.3±22 relative units, P<0.05) in VAT compared to SAT and ~2 fold higher (29.7±18 v 14.5±11 relative units, P<0.01) in visceral adipocyte (VAC) compared to SAC (Fig. 2).

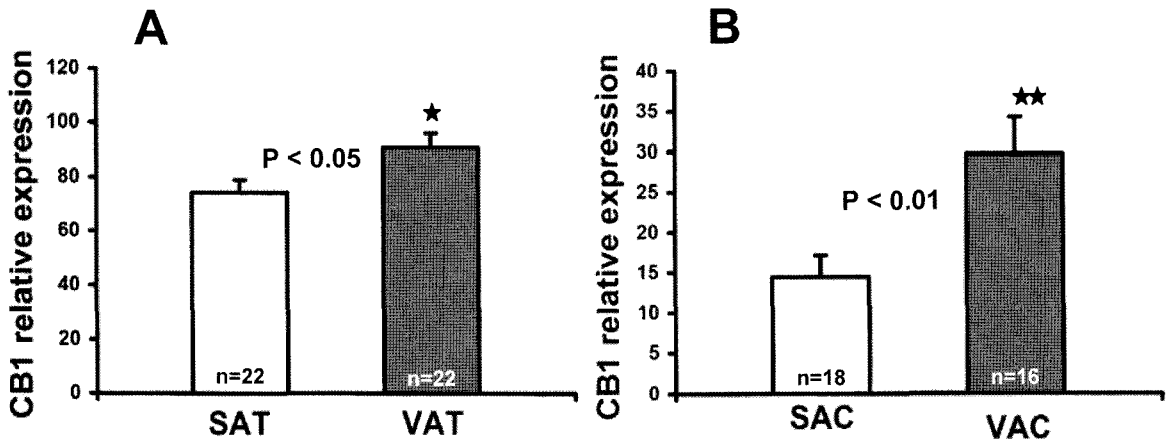

Fig. 2. Over-expression of CB1 in visceral compared to subcutaneous depot in both adipose tissue (A) and isolated mature adipocytes (B) collected 22 morbidly obese Caucasians (Group 5). Mean±SEM

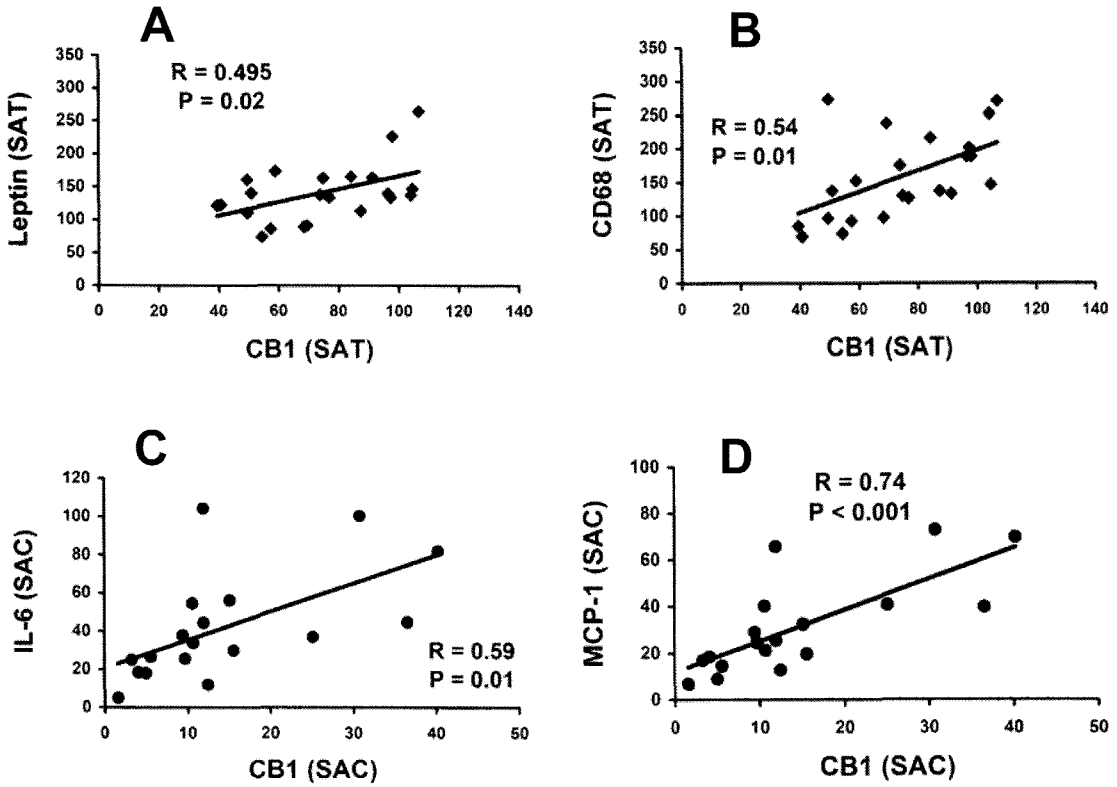

Fig. 3. Correlations between CB1 expression and adipokines (A-C) and macrophage marker CD68 (D) in adipose tissue (A,B) or isolated adipocytes (C,D) (arbitrary unit).

Among subjects in this analysis (Group 5), CB1 expression levels determined by QPCR directly correlated with those of leptin and CD68 in adipose tissue and IL-6 and MCP-1 in isolated adipocytes (R=0.49, R=0.54, R=0.59, and R=0.74, respectively, Fig. 3).

CB1 receptor on adipocytes and adipose tissue macrophages

Confocal imaging of both SAT and VAT immunostained for CB1 displayed marked expression of the receptor on adipocytes in a membrane pattern. Control sections in which one or both of the primary antibodies were omitted confirmed the specificity of staining (Fig. 4). Of note, CB1 was also expressed in SVCs and CD68+ macrophages (Fig. 4). Prior studies have demonstrated that adipose tissue resident macrophages are increased in obesity and can account for up to 40 % of cells in adipose tissue from obese subjects (Weisberg *et al.*, 2003).

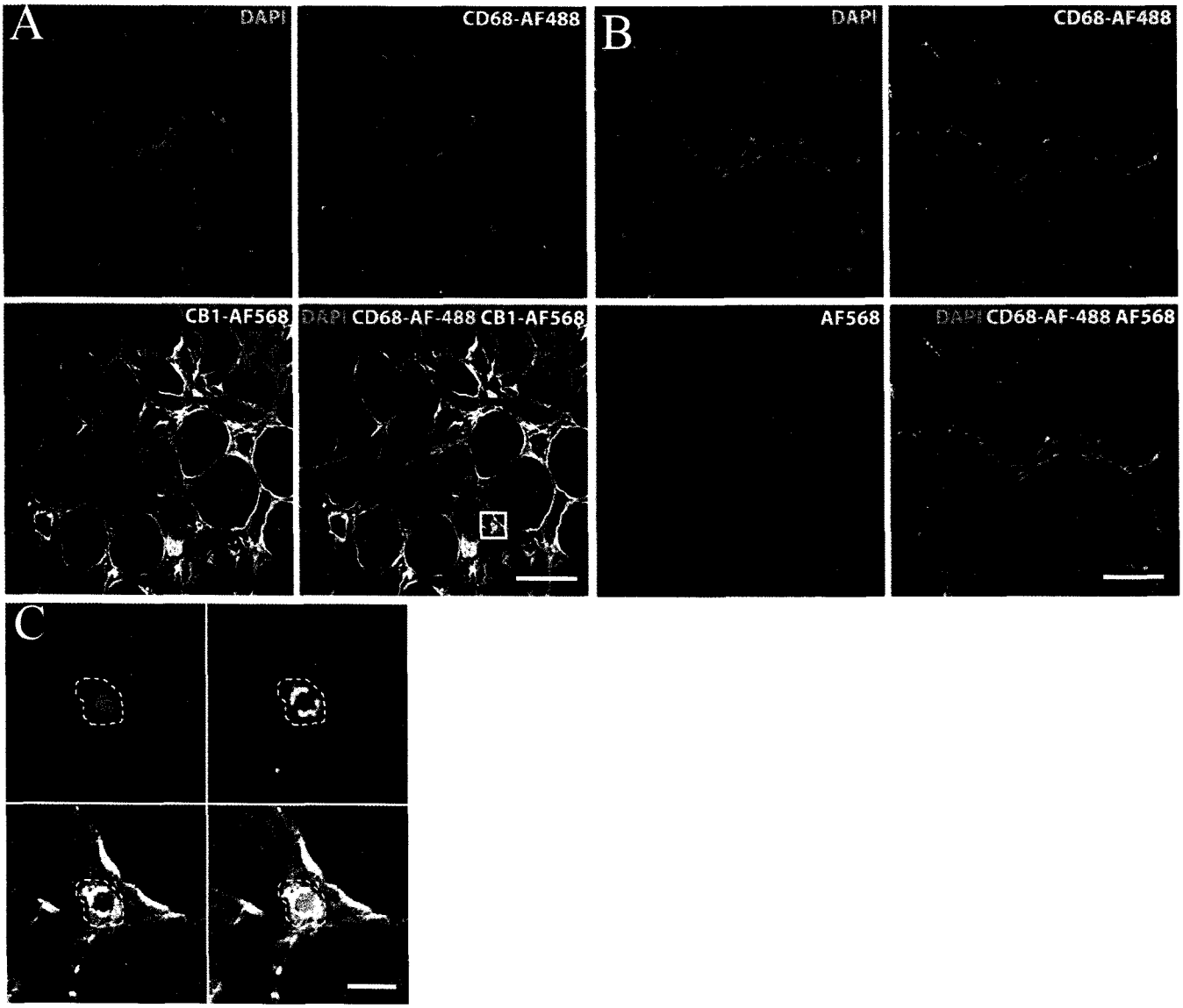

Fig. 4. CB1 receptor expression in adipose tissue. A, Both the adipocyte cell membranes and stromal-vascular cells express CB1 (red), in particular, some CD68 expressing tissue macrophages (green) also express CB1; B, Control tissue incubated without primary antibody to CB1 displays no background staining. Nuclei are counterstained with DAPI (blue); C, detail from lower right of panel A (white box). Macrophage is outlined with dashed white line. Scale bars are 100 mm (A, B) and 10 mm (C).

DISCUSSION

In a recent study, we presented data showing the presence and differential expression of CB1 receptor within the islets suggesting the endocannabinoid system may regulate secretion of the major islet hormones (Tharp *et al.*, 2008). Moreover, in the current study it is demonstrated that CB1 receptor mRNA was up-regulated in SAC from obese compared to non-obese subjects. In addition, higher CB1 expression levels were observed in samples from visceral compared to subcutaneous depots. Immunofluorescence confocal microscopy demonstrated CB1 receptor on adipocytes and also adipose tissue infiltrated macrophages.

Over-expression of CB1 in adipose cells with obesity is consistent with most animal studies (Bensaid *et al.*, 2003) and supports the hypothesis that adipose tissue from obese humans is characterized by increased activity of the ECS. Bensaid et al. (Bensaid *et al.*, 2003) have shown that rat adipose tissue and 3T3 F442A adipocytes express CB1 mRNA and that CB1 mRNA expression was up-regulated (3- to 4-fold) in adipose tissue of obese rats compared with lean controls. Another study showed that mouse epididymal adipocytes expressed CB1 and its activation enhanced lipogenesis in primary adipocyte cultures (Cota *et al.*, 2003) suggesting a role of the ECS as an endogenous regulator of energy homeostasis via peripheral lipogenic mechanisms in addition to the more well described central orexigenic effects (Cota *et al.*, 2003).

Two prior studies have compared the expression of CB1 in adipose tissue from obese and lean individuals. Engeli *et al.* (Engeli *et al.*, 2005) demonstrated that ECS activity was up-regulated, as evidenced by increased circulating levels of endocannabinoids, in obese compared to non-obese post-menopausal women. They also demonstrated that CB1 and FAAH expression in subcutaneous abdominal tissue were decreased in obese subjects and were unchanged with a 13-15 week dietary intervention designed to produce a 5% weight loss (Engeli *et al.*, 2005). A second study in 60 men and women classified into lean, subcutaneously obese and viscerally obese groups (Bluher *et al.*, 2006) demonstrated that circulating 2-AG levels were directly correlated to percent body fat, visceral fat mass and insulin levels, whereas CB1 expression levels in SAT were decreased in obese compared to non-obese subjects. In this study, adipose tissue CB1 expression levels were not different between men and women.

Possible explanations for the differences between these studies and the present results include; 1) different sources for RNA–in the present study expression levels in isolated mature adipocytes were compared, whereas in the latter two studies expression levels in adipose tissue were compared 2) age and menopausal status differences–subjects in the present study were young and pre-menopausal, whereas subjects tended to be older and women were post-menopausal in the latter studies 3) ethnic differences-subjects in Groups 1-3 of the present analysis were Pima Indians while subjects in the latter studies were Caucasians.

CB1 mRNA expression in primary cultured SVCs was very low. We have previously demonstrated that after 2 weeks in culture, preadipocytes predominate and contamination from other cell types is minimal (Permana *et al.*, 2004). Therefore, low expression levels of CB1 in cultured SVCs implicate low CB1 mRNA expression in preadipocytes. These results are similar to prior results in animals and humans. Substantially higher CB1 expression (>5 fold) was observed in differentiated 3T3 F442A adipocytes and isolated human adipocytes from mammary SAT than in undifferentiated adipocytes and isolated preadipocytes, respectively (Bensaid *et al.*, 2003) (Engeli *et al.*, 2005). The differentiation-dependent expression of CB1 in adipose cells is consistent with an important role for CB1 in regulating metabolic functions, including enhancing lipogenesis, in mature adipocytes.

These results, combined with our observations that adipocyte CB1 expression is increased with obesity, suggest that the decreased CB1 expression in adipose tissue with obesity found in other studies (Bluher *et al.*, 2006; Engeli *et al.*, 2005) may be attributed, at least in part, to a mixture of many different types of cells of stromal vascular fraction, such as fibroblasts, endothelial cells, lymphocytes, macrophages, etc.

Higher CB1 expression levels were observed in adipose tissue and adipocytes from visceral compared to subcutaneous depots (Fig. 2). These results are consistent to a previous study, in which CB1 expression levels were significantly higher in visceral compared to SAT in both lean and obese subjects, although the differences were less apparent among those with visceral obesity (Bluher *et al.*, 2006). In addition, the finding that CB1 is overexpressed in VAT is consistent with the observation that VAT is an important predictor for metabolic risk. Further studies comparing CB1 expression levels in visceral adipose cells between obese and non-obese controls are required to investigate whether CB1 is dysregulated in VAT with obesity.

Correlations between CB1 expression and adipokines and macrophage marker CD68 (Fig. 3) suggest that CB1 overexpression may be linked to the pro-inflammatory state (Amaya *et al.*, 2006) and increased infiltration of macrophages in adipose tissue characterizing adipose tissue in obese individuals.

The adipose tissue fractionation process itself can induce the expression of various inflammatory cytokines in adipocytes and SVCs (Ruan *et al.*, 2003). To determine whether CB1 expression was altered by adipose tissue digestion, we compared CB1 expression levels in fresh mouse adipose tissue and in SVCs and adipocytes after collagenase digestion and short term culture. These experiments demonstrated that CB1 expression was not affected by the different procedures used to separate the adipocytes and SVC fractions from adipose tissue (data not shown).

Antagonism of CB1 increases adiponectin mRNA expression in adipocytes, reduces hyperinsulinemia in obese (fa/fa) rats (Bensaid *et al.*, 2003) and contributes to a reduction of the obesity-related inflammatory status (Jbilo *et al.*, 2005). Osei-Hyiaman et al. (Osei-Hyiaman *et al.*, 2005) have shown that CB1 knockout mice were resistant to diet-induced obesity even though their caloric intake was similar to that of wild-type mice, suggesting that endocannabinoids also regulate fat metabolism. In present study, our observation of CB1 expression in various adipose cells and macrophages (Nong *et al.*, 2002) supports the important role of CB1 and the ECS in the regulation of immune/inflammation as well as fat metabolism in adipose tissue.

In summary, the results of the present study demonstrate that CB1 receptor mRNA is expressed in human subcutaneous and visceral adipose tissue, isolated adipocytes, stromal vascular cells and adipose tissue macrophages with higher levels in visceral depots compared to subcutaneous depots. Moreover, our results indicate that CB1 receptor is up-regulated in subcutaneous adipocytes from obese compared to non-obese subjects, consistent with studies in animals

and with studies indicating increased activity of the peripheral endocannabinoid system in adipose tissue in human obesity. Finally, our observation that CB1 receptor is expressed in adipose tissue macrophages suggests a potential role for the endocannabinoid system in modulating adipose tissue inflammation in obesity.

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