Original Article

Activation of the Peripheral Endocannabinoid System in Human Obesity

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Obesity is the main risk factor for the development of type 2 diabetes. Activation of the central endocannabinoid system increases food intake and promotes weight gain. Blockade of the cannabinoid type 1 (CB-1) receptor reduces body weight in animals by central and peripheral actions; the role of the peripheral endocannabinoid system in human obesity is now being extensively investigated. We measured circulating endocannabinoid concentrations and studied the expression of CB-1 and the main degrading enzyme, fatty acid amide hydrolase (FAAH), in adipose tissue of lean ($n = 20$) and obese ($n = 20$) women and after a 5% weight loss in a second group of women ($n = 17$). Circulating levels of anandamide and 1/2-arachidonoylglycerol were increased by 35 and 52% in obese compared with lean women ($P < 0.05$). Adipose tissue mRNA levels were reduced by −34% for *CB-1* and −59% for *FAAH* in obese subjects ($P < 0.05$). A strong negative correlation was found between *FAAH* expression in adipose tissue and circulating endocannabinoids. Circulating endocannabinoids and *CB-1* or *FAAH* expression were not affected by 5% weight loss. The expression of *CB-1* and *FAAH* was increased in mature human adipocytes compared with in preadipocytes and was found in several human tissues. Our findings support the presence of a peripheral endocannabinoid system that is upregulated in human obesity. *Diabetes* 54:2838–2843, 2005

Obesity is one of the main risk factors for the development of type 2 diabetes, and weight loss may be a successful means of reducing the number of patients affected by type 2 diabetes (1–4). Exogenous cannabinoids and endocannabinoids increase food intake and promote weight gain in animals by activating central endocannabinoid receptors (5–8). This phenomenon has been exploited in the treatment of cachexia using tetrahydrocannabinol (9). Endocannabinoids are derived from membrane phospholipids (anandamide [AEA]) or triglycerides (2-arachidonoylglycerol [2-AG]) (10). Endocannabinoids bind to the G-protein–coupled cannabinoid (CB) type 1 and type 2 receptors. In animals, CB-1 is expressed in the brain, gastrointestinal organs, and adipose tissue, whereas CB-2 is predominantly expressed on peripheral immune cells (11). Intracellular degradation by the enzyme fatty acid amide hydrolase (FAAH) limits endocannabinoid action (10).

In genetic animal models of obesity, brain endocannabinoid levels are increased and CB-1 is downregulated (12,13). *CB-1* gene–deficient mice are lean and resistant to diet-induced obesity (14). Similarly, pharmacological CB-1 blockade with SR141716 (rimonabant) reduces food intake and body weight (8,12,15). Central and peripheral mechanisms may contribute to this weight loss (16). Indeed, CB-1 activation in isolated mouse adipocytes increases the activity of the lipogenic enzyme lipoprotein lipase (16). Moreover, CB-1 blockade increases adiponectin gene expression in adipose tissue and elevates circulating adiponectin levels in the obese Zucker rat (17). Recently, the activation of CB-1 receptors in the liver was shown to increase de novo synthesis of fatty acids by activating the transcription factor sterol regulatory element–binding protein 1c (SREBP-1c) in mice (18).

Rimonabant has been tested successfully in phase III trials as an adjunctive obesity treatment (19,20). The role of the endocannabinoid system, especially the balance between central and peripheral effects, for human obesity is now becoming clearer. We studied the peripheral endocannabinoid system, namely CB-1 and FAAH expression, in human tissues, including subcutaneous adipose tissue. *CB-1* and *FAAH* gene expression as well as circulating endocannabinoid levels were compared in lean and obese women. These studies were repeated in obese women after a 5% weight loss.

RESEARCH DESIGN AND METHODS

From a previously described population of Caucasian postmenopausal women, we studied the 40 women who had the lowest and highest BMI for *CB-1* and *FAAH* gene expression in adipose tissue and circulating endocannabinoids in a cross-sectional study (21). In the weight loss study, 30 Caucasian postmenopausal women started a dietary weight reduction protocol and were instructed to reduce energy intake by 600 kcal/day, as previously described (22). Of the 30 women, 17 reached the 5% weight reduction goal after 13–15 weeks and were included in the analysis of adipose tissue *CB-1* and *FAAH* gene expression and circulating endocannabinoids. No participant had type 2 diabetes, renal or liver disease, congestive heart failure, or coronary heart disease. Hormonal replacement therapy was discontinued 4 weeks before the study. All subjects maintained their weight at a constant

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2-AG, 2-arachidonoylglycerol; AEA, anandamide; CB, cannabinoid; FAAH, fatty acid amide hydrolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SREBP-1c, sterol regulatory element–binding protein 1c.

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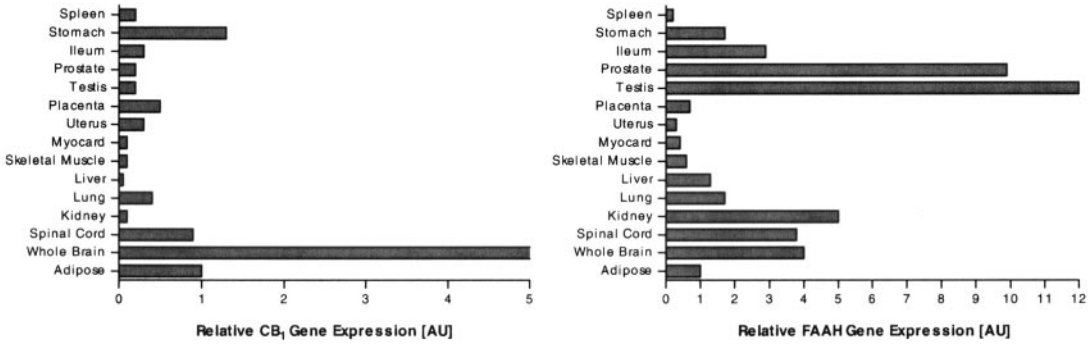

FIG. 1. Expression of *CB-1* and *FAAH* genes in human tissues. Real-time RT-PCR of a multiple tissue human RNA panel, normalized by *18S rRNA* expression. Data are given in arbitrary units (AUs) relative to *CB-1* or *FAAH* gene expression in adipose tissue.

level for at least 3 months before the study. The institutional review board approved all human studies and all subjects gave prior written informed consent.

Anthropometric measurements and blood samples were obtained at 9:00 A.M. after an overnight fast. Periumbilical subcutaneous adipose tissue was obtained by needle biopsy, as previously described. Ambulatory blood pressure and blood lipid measurements were performed by standard procedures (21,22). Mammary subcutaneous adipose tissue was obtained from healthy women (BMI 25–30 kg/m^2; age 40–60 years) by breast reduction surgery for in vitro studies. Preadipocytes and adipocytes were isolated by collagenase digestion and cultured overnight in serum-containing medium, as previously described (23). Paired preadipocyte and adipocyte samples were obtained from each donor after 1 additional day of culture under serum-free conditions.

Analytic methods. Total RNA from preadipocytes, isolated adipocytes, or adipose tissue biopsies was isolated by the Qiagen RNeasy mini kit (including the RNase-free DNase set; Qiagen, Hilden, Germany) and measured with the RNA 6000 Chip and the 2100 Bioanalyzer (Agilent, Waldbronn, Germany). *CB-1* and *FAAH* gene expression in different tissues was measured in the Human Total RNA Panel (BD Biosciences Clontech, Heidelberg, Germany), supplemented with RNA from human adipose tissue from our laboratory. Gene expression was measured with the ABI 5700 Sequence Detection System for real-time PCR (PE Biosystems, Weiterstadt, Germany) using the standard curve method and normalization by endogenous controls (23). Premixed Assays on Demand for human *CB-1*, *FFAH*, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and *18S rRNA* were used (PE Biosystems). Interassay coefficients of variation for *GAPDH* (1.1%), *18S rRNA* (0.9%), *FAAH* (1.6%), and *CB-1* (1.9%) were determined using standardized human adipose tissue cDNA from our laboratory.

Proteins were isolated by incubating a preadipocyte pellet on ice with 50 μl or 1 ml of adipocytes at room temperature with 1 ml radioimmunoprecipitation assay buffer (including pepstatin, phenylmethylsulfonyl fluoride, orthovanadate, aprotinin, and leupeptin as protease inhibitors; all chemicals obtained from Sigma-Aldrich, Seelze, Germany). Total proteins (15 μg) from preadipocytes or adipocytes were separated by SDS-PAGE on 10% SDS gels and blotted on polyvinylidine fluoride membranes. The membrane was blocked with 1% BSA for 1 h; the membranes were then incubated with 0.0025 μg of the first antibody (CB11-A rabbit anti-human; Alpha Diagnostic, San Antonio, TX) for 1.5 h then washed three times in 0.4% Tween 20. Next the membranes were incubated with 0.01 μg of the second antibody (peroxidase-conjugated AffiniPure goat anti-rabbit IgG H+L; Jackson ImmunoResearch, Soham, U.K.) for 1 h and with the Western Lightning Chemiluminescence Reagent for 1 min (PerkinElmer, Boston, MA). Images were developed on CL-X Posure Clearblue X-Ray Film (Pierce Biotechnology, Rockford, IL).

For confocal microscopy, mature isolated adipocytes were fixed on glass slides for 10 min in 4% paraformaldehyde at room temperature, washed twice in PBS, and fixed for 20 min in 80% methanol at −20°C. After being washed twice in PBS, fixed cells were dried on air and then stored at −20°C. Cells were blocked with 1% BSA for 1 h and then incubated with the first antibody (see above) overnight at 4°C. Cells were then washed three times with PBS and then incubated for 1.5 h with the second antibody (Alexa Fluor 546 goat anti-rabbit, 1:1000; Invitrogen, Karlsruhe, Germany) and analyzed with the confocal microscope MCR 1024 (Bio-Rad, Munich, Germany).

Anandamide and 1/2-AG were quantified by liquid chromatography/in-line mass spectrometry, as previously described (24).

Statistics. Data were analyzed by SPSS 11.5.1 (SPSS, Chicago, IL) and are given as means ± SE. Student's *t* test or a paired sample *t* test was used for group comparisons, as appropriate. Pearson's coefficient of correlation and multiple linear regression models with stepwise exclusion of independent variables were used to describe the relation of *FAAH* expression to circulating endocannabinoids. Statistical significance was set at $P < 0.05$.

RESULTS

CB-1 gene expression was surprisingly high in adipose tissue according to a human tissue RNA panel (Fig. 1). Other peripheral tissues with known *CB-1* expression (reproductive and gastrointestinal tract tissue) showed similar or lower expression levels. The *FAAH* gene was more uniformly expressed in peripheral tissues, but similar to *CB-1*, *FAAH* mRNA was present in considerable amounts in adipose tissue (Fig. 1).

These findings led us to further analyze *CB-1* and *FAAH* expression in isolated human adipocytes. RNA expression of both genes as well as CB-1 protein expression were detected in isolated human adipocytes (Fig. 2). The use of specific CB-1 receptor antibodies revealed the expression of a 53-kDa protein by Western blotting, a size related to a low glycosylation form of the CB-1 receptor. Confocal microscopy further demonstrated localization of CB-1 receptors in adipocyte cell membranes (Fig. 2). We also compared paired samples of isolated preadipocytes and mature adipocytes and found increased *CB-1* mRNA and protein levels in mature adipocytes compared with preadipocytes, suggesting a role of CB-1 receptors in the physiology of mature adipocytes. *FAAH* gene expression was also increased in mature adipocytes, but the difference was not as striking as for the *CB-1* receptor gene (Fig. 2).

Subcutaneous adipose tissue biopsies and blood samples were obtained from 20 lean and 20 obese postmenopausal women and, in a second study, from 17 obese postmenopausal women before and after a 5% body weight loss. The clinical data (Table 1) demonstrate that the obese women from both studies were similar for most variables. In the obese group, signs of fasting hyperinsulinemia were the only metabolic changes compared with in the lean control group. The 5% weight loss was associated with an improvement in insulin levels and a decrease in blood pressure. Circulating levels of AEA and 1/2-AG were increased by 35 and 52% in the obese and lean women, respectively. In contrast, adipose tissue mRNA levels were reduced by −34% for *CB-1* and −59% for *FAAH* in obese subjects (Fig. 3). However, these obesity-associated changes in the peripheral endocannabinoid system were not reversed by the weight loss achieved in this study. Neither circulating endocannabinoid levels nor adipose-tissue *CB-1* and *FAAH* mRNA expression were different before and after the 5% weight loss (Fig. 3).

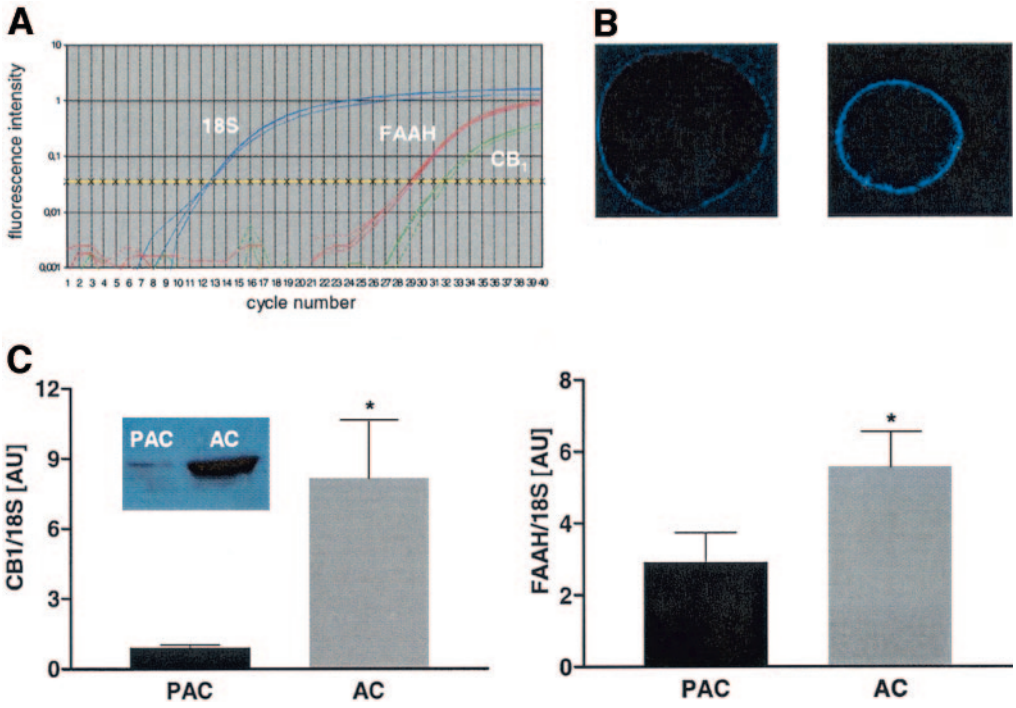

FIG. 2. Expression of *CB-1* and *FAAH* by human adipose cells. *A*: *CB-1* and *FAAH* mRNA was detected by real-time PCR in isolated human adipocytes. *B*: Confocal microscopy revealed localization of the CB-1 receptor in the adipocyte membrane. The negative control (*left*; only second antibody added) confirms the specificity of fluorescence signals seen in the right confocal image. *C*: Increased expression of *CB-1* and *FAAH* genes in isolated mature human adipocytes (AC) compared with isolated human preadipocytes (PAC). *CB-1* gene expression data were confirmed by Western blotting (insert). Data are means ± SE from seven pairs and are given as arbitrary units (AUs), normalized by *18S rRNA* expression. Group comparison by Student's *t* test. **P* < 0.05.

A significant correlation was found between *FAAH* expression in adipose tissue and circulating endocannabinoid levels (Fig. 4). However, this correlation was to some degree dependent on obesity. Stepwise multiple linear regression analysis including *FAAH*, BMI, waist circumference, and body fat as independent variables revealed that the combination of *FAAH* expression and BMI explained most of the variability in AEA levels ($r = -0.73$, $r^2 = 0.53$, $P = 0.001$), whereas waist circumference was the major determinant of 1/2-AG levels ($r = -0.52$, $r^2 = 0.27$, $P = 0.007$).

DISCUSSION

The close link between increased body weight and the risk of developing type 2 diabetes is well established by epidemiological data and the success of sustained weight loss in reducing diabetes risk (1–4). New therapeutic options for obesity are thus highly desired, and blocking the endocannabinoid system appears to be a promising option (15,19,20). Animal studies suggest that blocking CB-1 receptors by rimonabant not only results in central effects such as reducing food intake but also elicits peripheral effects (16–18). In humans, metabolic and obesity-related actions of the endocannabinoid system are now being studied. We found that the *CB-1 receptor* and *FAAH* are markedly upregulated in mature human adipocytes compared with in preadipocytes, suggesting a role of the endocannabinoid system in the physiology of mature human adipocytes. Furthermore, systemic endocannabinoid levels are increased in postmenopausal women with uncomplicated obesity and are associated with decreased *CB-1 receptor* and *FAAH* gene expression in adipose

TABLE 1
Characteristics of patients in the cross-sectional (lean/obese) and weight loss (before/after) studies

	Cross-sectional study		Weight loss study	
	Lean	Obese	Before	After
n	20	20	17	17
Age (years)	57 ± 1	59 ± 1	59 ± 2	59 ± 2
BMI (kg/m^2)	23.5 ± 0.4	38.3 ± 0.7*	33.1 ± 1.1	31.3 ± 1.0†
Waist circumference (cm)	76 ± 1	109 ± 2*	101 ± 3	97 ± 3†
Daytime ambulatory blood pressure (mmHg)				
Systolic	127 ± 4	136 ± 3*	137 ± 3	131 ± 2†
Diastolic	77 ± 2	80 ± 2	82 ± 2	80 ± 1
Mean daily heart rate (bpm)	76 ± 2	82 ± 2*	82 ± 3	80 ± 3
Cholesterol (mmol/l)				
Total	5.4 ± 0.2	5.6 ± 0.2	5.7 ± 0.3	5.5 ± 0.3
HDL	1.5 ± 0.1	1.2 ± 0.1*	1.7 ± 0.1	1.6 ± 0.1
LDL	3.5 ± 0.2	3.8 ± 0.2	3.5 ± 0.2	3.3 ± 0.3
Triglycerides (mmol/l)	1.0 ± 0.1	1.5 ± 0.2*	1.2 ± 0.1	1.3 ± 0.2
Glucose (mmol/l)	4.9 ± 0.1	5.5 ± 0.1*	5.7 ± 0.2	5.7 ± 0.2
Insulin (μU/l)	2.9 ± 0.4	8.2 ± 0.8*	4.8 ± 0.9	3.9 ± 0.7†

Data are means ± SE. Group comparison by Student's *t* test for independent samples (cross-sectional study) or by *t* test for paired samples (weight loss study). **P* < 0.05 vs. lean; †*P* < 0.05 vs. baseline.

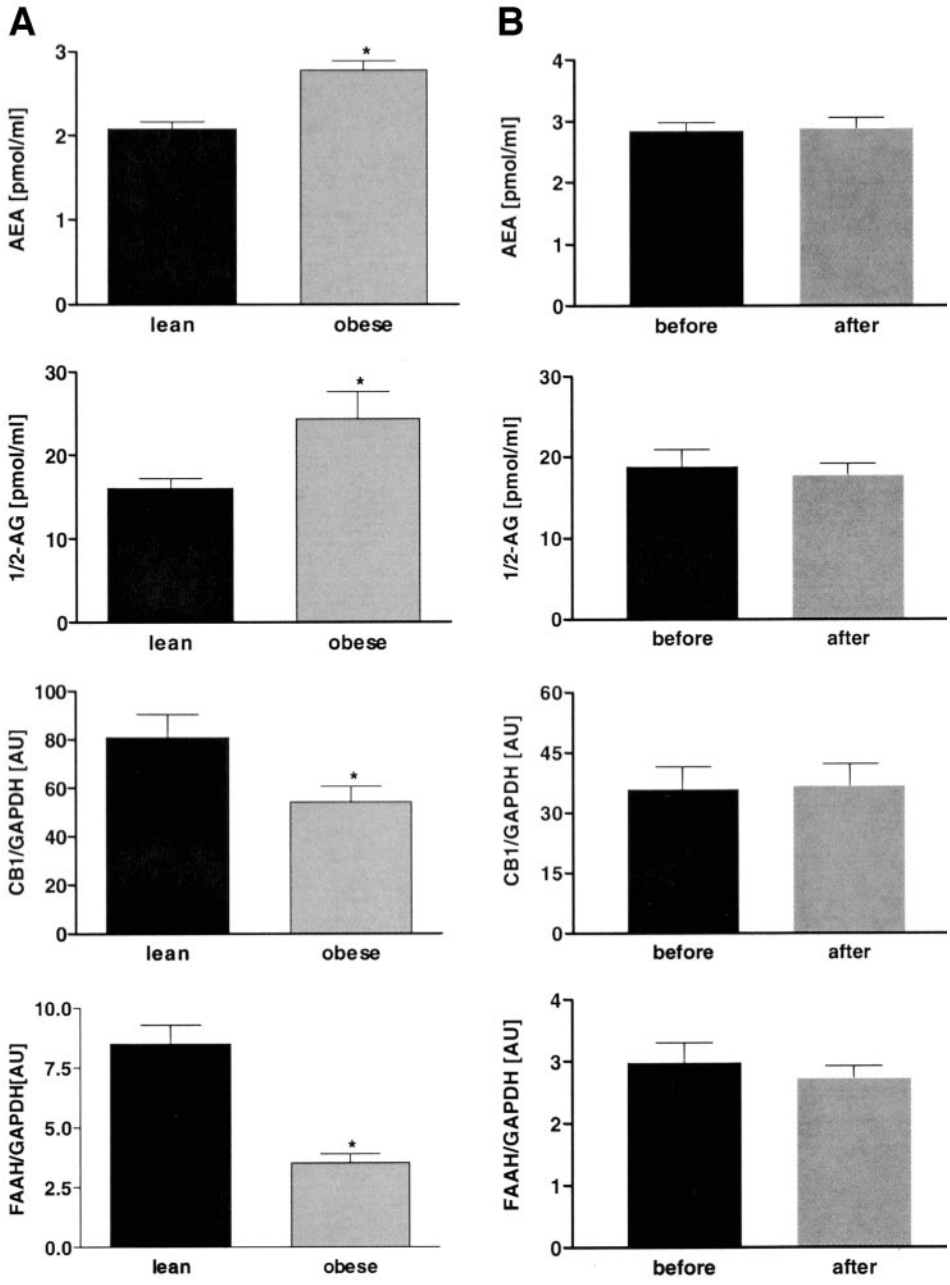

FIG. 3. Influence of obesity and weight loss on the peripheral endocannabinoid system. Subcutaneous adipose tissue biopsies and blood samples were obtained from 20 lean and 20 obese postmenopausal women in a cross-sectional study (*A*) and from 17 obese postmenopausal women before and after a 5% body weight loss by a dietary protocol (*B*). Circulating levels of AEA and AGs (1/2-AG) were increased in the obese women by 35 and 52%, respectively. In contrast, adipose tissue *CB-1* mRNA was decreased by −34% and *FAAH* mRNA by −59% in the obese subjects. Neither circulating levels of AEA and 1/2-AG nor adipose tissue, *CB-1*, or *FAAH* mRNA were influenced by the weight loss protocol. Gene expression is given in arbitrary units (AUs), normalized by *GAPDH* expression. Group comparison was performed with Student's *t* test for independent samples (cross-sectional study) or the *t* test for paired samples (weight loss study). *$P < 0.05$ vs. lean.

tissue. Finally, we showed that the *CB-1 receptor* is expressed in some peripheral human tissues relevant to the pathogenesis of obesity and obesity-associated metabolic disorders.

Both, hypothalamic and uterine levels of endocannabinoids are increased in genetically obese animals with leptin deficiency (*ob/ob* mice) or impaired leptin signaling (*db/db* mice, *fa/fa* rats) (12,25). Recently, high-fat feeding has been shown to increase intrahepatic endocannabinoid levels, even before the onset of obesity (18). We found increased circulating AEA and 1/2-AG levels in obese women. The marked downregulation of *FAAH* gene expression in adipose tissue of obese women suggests that increased endocannabinoid levels may be secondary to decreased enzymatic degradation. Increased endocannabinoid levels in the liver are also accompanied by decreased FAAH activity (18). Other peripheral tissues express more *FAAH* mRNA than adipose tissue does. Nevertheless, adipose tissue may be an important contributor to endocannabinoid inactivation, given the overwhelming mass of adipose tissue compared with other organ tissue. Whether or not interactions with leptin contribute to *FAAH* downregulation in human adipose tissue is unknown (26).

The association of increased circulating endocannabinoids with decreased *CB-1 receptor* gene expression in adipose tissue suggests a negative feedback loop regulation. In one study, diet-induced obesity decreased CB-1 density in extrahypothalamic regions of the rat brain but not in the hypothalamus (13). Central endocannabinoid levels were not measured in that study, thus the existence of a negative feedback loop regulatory mechanism on *CB-1* gene expression is speculative. In contrast, CB-1 receptor density increases in the liver of mice fed a high-fat diet (18), and *CB-1 receptor* gene expression is increased in adipose tissue of obese Zucker rats (*fa/fa*) but unchanged in the uterus of *ob/ob* mice (17,25). The regu-

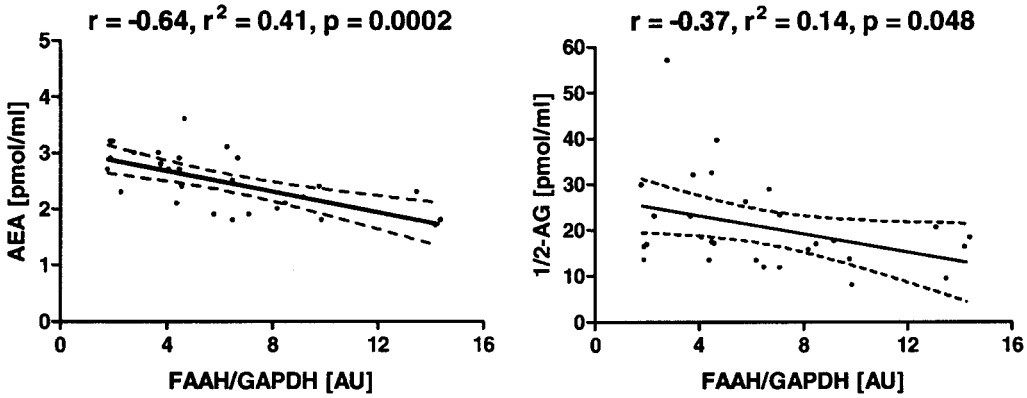

FIG. 4. Relation of *FAAH* expression in adipose tissue and circulating endocannabinoids. Both circulating AEA and Ags (1/2-AG) were negatively correlated with the expression of the *FAAH* gene in 40 human adipose tissue samples. Gene expression is given in arbitrary units (AUs), normalized by *GAPDH* expression.

lation of *CB-1* receptor gene expression in human or rodent adipocytes is unknown.

We hypothesized that increased circulating endocannabinoid levels and associated changes in the adipose endocannabinoid system may be reversible with weight loss. Our expectation was not fulfilled, and there are several possible explanations for these findings. Even after losing 5% body weight, the volunteers were still obese. Dysregulation of the endocannabinoid system may begin early in the development of obesity or possibly even before the development of obesity because of an underlying genetic predisposition. The latter mechanism is suggested by the recent finding of a strong association of a *FAAH* missense mutation with human obesity (27).

Endocannabinoids act upon CB-1 receptors in the brain and peripheral tissues. The role of central CB-1 receptors for the physiology of addiction, locomotion, pain, memory, and satiety have been intensively studied (8,10,11,24,28). Peripheral effects of endocannabinoids include hemodynamic (29–31) and inflammatory (32–34) modulation. The interaction between peripheral and central endocannabinoid mechanisms is illustrated by findings in *CB-1*$^{-/-}$ mice, which are lean and resistant to diet-induced obesity (14). In young animals, the lean phenotype is due to decreased food intake, whereas pair-feeding experiments revealed that food intake is not operative any more at age 20 weeks and older (16). These data suggest that peripheral mechanisms are also involved in the control of body weight by endocannabinoids and led to the discovery of *CB-1 receptor* gene expression in rodent adipocytes (16,17). Other investigators did not find *CB-1* gene expression in rat adipocytes, most likely because they used insensitive methods (35).

We have demonstrated *CB-1* mRNA and protein expression in isolated human adipocytes, thereby confirming the animal data. Our comparison of isolated human preadipocytes and mature adipocytes showed increased *CB-1* mRNA and protein levels in mature human adipocytes. This finding is in accord with data from undifferentiated and differentiated 3T3-F442A mouse clonal preadipocytes (17). Taken together, our findings suggest that CB-1 receptors are important for the function of mature adipocytes but not of preadipocytes. Furthermore, expression of the gene encoding *FAAH* suggests that adipocytes are involved in the control of endocannabinoid availability.

Activation of CB-1 receptors in the gastrointestinal tract may also be relevant for the pathogenesis of obesity. The response of circulating ghrelin to fasting was diminished with rimonabant, suggesting that CB-1 receptors are involved in ghrelin secretion (36). The demonstration of *CB-1* gene expression in the stomach in our study is consistent with this suggestion. The endocannabinoid system and ghrelin may also interact in the brain. The orexigenic effects of centrally administered ghrelin were abolished by rimonabant, suggesting that ghrelin acts at least in part by increasing endocannabinoid production in the hypothalamus (37).

We demonstrated that the CB-1 receptor is expressed in organs relevant to the pathogenesis of obesity in humans, so that results from mechanistic studies in animals may also be applicable to patients. Furthermore, the peripheral endocannabinoid system is activated in human obesity. The observation that endocannabinoid activation is not reversible with a 5% weight loss may suggest that this activation is a cause rather than a consequence of obesity. The physiology and pathophysiology of the peripheral adipose tissue endocannabinoid system warrant further studies.

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