CB1 antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice

Short running title: CB1 antagonism in diet-induced obese mice

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The beneficial effects of the inactivation of endocannabinoid system (ECS) by administration of antagonists of the cannabinoid receptor (CB) 1 on several pathological features associated with obesity is well demonstrated but the relative contribution of central versus peripheral mechanisms is unclear.

**Objective**: We examined the impact of CB1 antagonism on liver and adipose tissue lipid metabolism in a mouse model of diet-induced obesity.

**Research Design and Methods**: Mice were fed either with a chow diet or a high sucrose high fat (HSHF) diet for 19 weeks and then treated with the CB1 specific antagonist SR141716 (10 mg/kg.day⁻¹) for 6 weeks.

**Results**: Treatment with SR141716 reduced fat mass, insulin levels and liver triglycerides primarily increased by HSHF feeding. Serum adiponectin levels were restored after being reduced in HSHF mice. Gene expression of SR-BI and HL was induced by CB1 blockade and associated with an increase in HDL-CE uptake. Concomitantly, the expression of CB1 which was strongly increased in the liver and adipose tissue of HSHF mice, was totally normalized by the treatment. Interestingly, in visceral fat, but not subcutaneous, genes involved in transport, synthesis, oxidation and release of fatty acids were upregulated by HSHF feeding while this effect was counteracted by CB1 antagonism.

**Conclusion**: A reduction in the CB1-mediated ECS activity in the visceral fat is associated with a normalization of adipocyte metabolism which may be a determining factor in the reversion of liver steatosis induced by treatment with SR141716.
Obesity results from an imbalance between energy intake and expenditure and is characterized by increased body weight and abnormal development of adipose tissue with excessive fat storage (1). Recently, evidence has accumulated for the overactivity of endocannabinoid system (ECS) during conditions of unbalanced energy homeostasis (2). The ECS consists of the cannabinoid receptors (CB), their endogenous ligands (the endocannabinoids), and the enzyme proteins catalyzing the endocannabinoid formation and degradation (3). Activation of central CB1 receptors clearly promotes food intake and weight gain (4–6). Accordingly, pharmacological antagonism of CB1 has been shown to improve several pathological features associated with obesity including overweight, hyperinsulinemia, insulin resistance, hyperglycemia and dyslipidemia in obese rodents (7–9) and humans (10; 11).

Even if the reduction in food intake induced by central CB1 blockade may be the main initial cause of body weight loss and associated beneficial effects, several data collected from animal and human studies indicate that peripheral CB1 may also directly control lipid metabolism (12–14). Thus, an activation of ECS has been recently reported in peripheral tissues of animal models of obesity (15; 16) and associated with visceral fat obesity in humans (17; 18). Consequently, it has been proposed that the long-term effects of CB1 antagonism are resolved by stimulation of energy expenditure and by peripheral effects related to adipose tissue, liver, skeletal muscle, and pancreas physiology (19–21).

In the present work, we tested the effects of CB1 antagonism on the regulation of the liver and adipose tissue lipid metabolism in a mouse model of diet-induced obesity. We first examined the global impact of CB1 antagonism on plasma parameters and liver steatosis which were primarily altered by long term feeding of a high sucrose high fat (HSHF) diet. Next, we examined whether CB1 inactivation was associated to biochemical and molecular alterations in the liver and adipose tissue (distinguishing visceral and subcutaneous fat depots) that could account for an improvement of liver lipid metabolism.

RESEARCH DESIGN AND METHODS

Animals and study design. Official French regulations (n° 87848) for the use and care of laboratory animals were followed throughout the experimental period. The experimental protocol was approved by the local ethic committee for animal experimentation (n° BX0622). Four week-old C57BL/6 male mice (Elevage Janvier, Le Genest Saint Isle, France) were housed in individual plastic cages and adapted to a standard diet (AO4, UAR, Epinay-sur-Orge, France) for one week. A series of mice was maintained on the standard diet (CON; n=5) while another series was subjected to a HSHF diet containing casein 20%, corn starch 13%, sucrose 29.3%, cellulose 5%, maltodextrin 2.2%, lard 20%, soya oil 2.5%, mineral 205B SAFE 7%, vitamin 200 SAFE 1% (ref. 235HF SAFE, Augy, France). After 19 weeks, HSHF animals which were not both overweight and hyperinsulinemic were excluded from the study. Selected mice were maintained on high sucrose high fat diet and received orally either 10 mg/kg/day of SR141716 (HSHF+SR series; n=14), or vehicle (HSHF series; n=10). The CB1 specific antagonist SR141716 (Rimonabant) was supplied by Sanofi-Aventis (Paris, France). Animals had free access to fresh food and water throughout the experimental period. Mice were food-deprived 4 h before anesthesia with ketamine / xylazine (7.5 mg / 1 mg per 100 g of body weight) and tissue handling. Epididymal and inguinal fat were surgically removed as
representatives of visceral and subcutaneous fat respectively (22). Tissue samples were frozen in liquid nitrogen pending further analyses.

**Serum and tissue parameters.** Serum parameters were determined using commercial kits: Glucose RTU, TG PAP150 and Cholesterol RTU from BioMérieux (Marcy l’Etoile, France) for glucose, triglycerides (TG) and cholesterol assay respectively, NEFA C from Wako Pure Chemical Industries (Richmond, VA, USA) for free fatty acids (FFA) assay and mouse insulin and adiponectine ELISA kits from AbCys (Paris, France). Liver malonyl-CoA concentration was determined by HPLC as previously described (23). Liver total lipids were extracted according to the method of Folch et al. (24). After mixing thoroughly, 1.0 ml of organic phase was transferred to a clean tube containing 1 ml of 1% Triton X-100 in chloroform and dried using nitrogen. The residue was re-solubilized in 0.25 ml distilled water and used for the determination of TG and cholesterol as in serum. For determination of adiponectin content in adipose tissue, samples were homogenized in 10 volume of PBS. After centrifugation (10 min at 12,000g, 4°C), the supernatants were carefully collected through the fat cake, diluted to 1/40,000 in PBS and used for adiponectine measurements as in serum.

**Fatty acid (FA) oxidation, apoA and apoB secretion.** Freshly removed livers from 5 HSHF and 5 HSHF+SR mice were sliced using a Brendel/Vitron slicer (Tucson, AZ, USA) and thin slices were used to measure [1-14C] palmitic acid oxidation, apoA and apoB secretion as previously described (25).

**[3H]-cholesteryl ether-HDL uptake.** Liver slices prepared as described above were also intended for HDL uptake. First, a HDL fraction was isolated from human plasma by sequential flotation ultracentrifugations (26). HDL were radiolabeled with [3H]-cholesteryl ether (CE) combining [3H]cholesteryl hedacetyl ether with L-a-phosphatidylcholine and butyldihydroxytoluene in a 500:1:6 molar ratio and sonicating to form liposomes. HDL-[3H]CE were obtained by addition of liposomes to the HDL fraction in presence of lipoprotein-free plasma, as a source of cholesteryl ester transfer protein, after an overnight-incubation at 37°C under light agitation. Labeled HDL were separated from remaining liposomes by an other sequential flotation ultracentrifugation and washed twice in KBr (density 1.21). Finally, HDL-[3H]CE were aliquoted and stored at -80°C until used. Measurement of the uptake was carried out at 37°C by incubating 2 liver slices in 1 ml of William’s Medium E containing 40 µg of proteins (0.3 mCi of HDL-[3H]CE), under slight agitation. After 3 hours, slices were removed from medium, washed 3 times, and homogenized in 400 ml of PBS with a mini-beadbeater (BioSpec Products, Inc., Bartlesville, OK). The radioactivity recovered in the homogenate was finally estimated, representing the amount of HDL uptaken by the liver cells.

**Gene expression.** Total mRNA from liver and adipose tissue were extracted with Tri-Reagent (Euromedex, Souffelweyersheim, France) and reverse-transcripted using the Iscript cDNA kit (Bio-Rad, Marnes-La-Coquette, France). Real-time PCR was performed as described previously (27) using a Bio-Rad iCycler iQ. The sequences of forward and reverse primers used for the amplification are presented in the online supplemental Table 1 which can be found in an online appendix at http://diabetes.diabetesjournals.org.

**Statistical analysis.** Results are expressed as means ± SEM. Data were analyzed statistically using the Kruskal-Wallis non-parametric test. Differences were considered significant at P<0.05.

**RESULTS**
Body and organ weights. Baseline body weights of the 3 groups of mice were comparable. After 19 weeks, body weights of HSHF animals were significantly higher than that of control (Fig 1). From week 20 to 26, the body weight of HSHF+SR mice rapidly decreased to become similar to that of CON mice at week 26 (Fig 1). Consistent with that, the masses of both epididymal and inguinal fat pads differed in the order HSHF > HSHF+SR > CON (Table 1). Concomitantly, the liver weight was greater in HSHF and less in HSHF+SR than in CON (Table 1).

Serum and liver parameters. At the end of the experiment, serum glucose concentration of HSHF mice was not different from CON despite an increase in insulin levels indicating that the HSHF mice were in the early stage of developing insulin-resistance (Table 1). Likewise, FFA and total cholesterol levels were higher in HSHF than in CON mice. Surprisingly, HSHF mice had 50% lower plasma TG levels than CON suggesting an increase in TG clearance by the liver and adipose tissue. In parallel with fat mass expansion, serum adiponectin levels were less in mice fed with the HSHF diet than in control. Interestingly, insulin and adiponectin levels in HSHF+SR mice were not different from the control group (Table 1). Serum FFA concentration was less in HSHF+SR than in HSHF, while glucose, TG and cholesterol levels did not differ between these groups. In the liver, administration of HSHF diet induced a steatosis with a 5-fold increase in TG content while total cholesterol content remained unchanged. The malonyl-CoA content, a potent inhibitor of FA β-oxidation, was also markedly increased. Meanwhile, glycogen stores were less in HSHF than in CON mice, reflecting a stimulation of glycogenolysis. Interestingly, TG and malonyl-CoA accumulations were partially reversed and glycogen concentration further decreased by CB1 antagonism (Table 1).

Adiponectin content in visceral and subcutaneous fat. To explore whether the variations of serum adiponectin levels could correspond to a different production of the adipokine by visceral and subcutaneous fats, we determined the adiponectin content in epididymal and inguinal fat respectively. The adiponectin content was less in both fat depots of HSHF than CON mice with a more marked effect in inguinal fat. Remarkably, after treatment with CB1 antagonist, the adiponectin content significantly increased in inguinal fat only (Fig 2).

FA oxidation and parameters related to lipoprotein metabolism. The ability of SR141716 to partially reverse HSHF-induced liver steatosis prompted us to verify whether this effect was related to an increased capacity of hepatocytes to β-oxidize FA or to produce lipids and lipoproteins. Palmitic acid β-oxidation rates measured in liver explants did not differ between HSHF and HSHF+SR mice (Table 2). ApoB secretion was less with HSHF+SR than HSHF explants while ApoA secretion not differed between the two groups. This model was also used to determine whether the blockade of CB1 affected HDL-CE uptake. The recovery of HDL-CE was greater in the liver explants from HSHF+SR than HSHF mice suggesting interesting metabolic adaptations that could affect lipid and lipoprotein metabolism (Table 2).

Liver and adipose tissue gene expression. 1) Liver (Fig 3): We first tested the impact of the diet and of the treatment with SR141716 on CB1 gene expression as an indicator of ECS activity. CB1 mRNA were the greatest in HSHF mice and less in HSHF+SR than in CON mice, reflecting a stimulation of ECS in our mice model of obesity and an effective inhibition of this pathway after CB1 antagonist treatment. Then, mRNA levels of PEPCK and glucose-6-phosphatase (G6P) were measured as an indicator of liver insulin resistance. The
inverse relationship existing between insulin levels and expression levels of this two enzymes suggested that insulin still had the ability to control their transcription. In line with a possible impact of CB1 antagonism on lipoprotein and cholesterol metabolism, we measured the mRNA expression of hepatic lipase (HL) and scavenger receptor class B type I (SR-B1) in the liver. The expression levels of these two genes were the lowest in HSHF mice and did not differ from CON in HSHF+SR mice.

Besides, mRNA levels of the lipogenic enzymes acetyl-CoA carboxylase (ACC) 1 and 2, and FA synthase (FAS) were all markedly less in HSHF than in CON mice. Interestingly, the expression of both ACC isoforms was higher in the liver of HSHF+SR than HSHF mice while antagonism of CB1 had no effect on FAS expression. The expression of stearoyl-CoA desaturase-1 (SCD-1), which converts saturated FA into monounsaturated FA, was higher in HSHF and less in HSHF+SR than in CON mice, suggesting that the inhibition of ECS could have limited the high production of monounsaturated FA primarily induced by HSHF feeding. Besides, neither the diet nor the treatment modified the transcript levels of carnitine palmitoyltransferase I (CPT-I), the rate limiting enzyme of long chain FA β-oxidation.

2) Adipose tissue (Fig 4 A and B): CB1 expression was also induced in both visceral and subcutaneous adipose tissue of HSHF compared to CON mice. In obese mice, SR141716 treatment was efficient in reducing CB1 expression in the two tissues. The expression of tumor necrosis factor-a (TNF-a), an inflammatory cytokine associated with insulin resistance was higher in both visceral and subcutaneous fat of HSHF than CON mice. It is noteworthy that the TNF-a induction was dramatically higher in visceral than in subcutaneous fat (24-fold vs 4.8-fold respectively). Likewise, in HSHF+SR mice, TNF-αmRNA levels were not different from CON in subcutaneous fat and were only half that of CON in visceral fat. In addition, compared to CON, HSHF feeding gave rise to a 4-fold increase in the expression of the gamma 2 isoform of the peroxisome proliferator-activated receptor (PPARg2) in visceral fat while this induction was only 2-fold in HSHF+SR mice (Fig 4A). In subcutaneous adipocytes, PPARg2 expression was surprisingly the highest in HSHF+SR while the two other groups did not differ each other (Fig 4B). Concomitantly, the expression of genes related to uptake (FA translocase, FAT/CD36), lipolysis (hormone-sensitive lipase, HSL), β-oxidation (CPT-I) and lipogenesis (FAS and ACC1) was strongly higher in visceral fat of HSHF than CON mice (Fig 4 A). Conversely, in subcutaneous deposits of HSHF mice, the expression of these genes was either less than CON (FAS and ACC1) or unchanged (FAT/CD36, HSL and CPT-I) suggesting different sensitivity and function of subcutaneous versus visceral adipocytes in conditions of insulin resistance (Fig 4B). Interestingly, in visceral fat, all genes that were upregulated by HSHF feeding were significantly downregulated after treatment with CB1 antagonist except FAT/CD36 (Fig 4A).

DISCUSSION

In this study, the effects of CB1 antagonism were tested on mice previously exposed to a long term-HSHF diet (19 weeks) with a lipid content and FA composition nearly similar to the human western diet. Administration of HSHF diet induced obesity, liver fat accumulation and peripheral insulin resistance as indicated by the elevation of plasma insulin and FFA levels. A significant number of experimental reports describe beneficial effects of CB1 antagonism on insulin resistance and fatty liver in mice and human and these data strongly indicate that the ECS has a major role in the regulation of
lipid metabolism not only at the central but also at the peripheral level (for a review see (28)). From our mouse model of obesity, we provided further evidence that CB1 blockade causes peripheral metabolic and molecular changes in liver and adipose tissue associated with the reversion of fatty liver. We particularly showed that the lipid metabolism of visceral and subcutaneous adipocytes was differently regulated in response to diet-induced obesity and to CB1 antagonism.

**Effects of CB1 antagonism on liver lipid metabolism:** Our findings clearly indicate that the strong upregulation of liver CB1 primarily induced by HSHF diet is fully reversed by the treatment with SR141716 suggesting that the metabolic improvements observed could be mediated by the blockade of these receptors. This concept is supported by others studies using CB1−/− mice, demonstrating that ECS overactivity occurs in the liver of animals fed a high fat diet and that hepatic CB1 are required for the development of diet induced steatosis (14; 29). In line with this, the normalization of liver parameters related to carbohydrate and lipid metabolism such as PEPCK, G6P, ACC and SCD-1 mRNA levels after treatment with CB1 antagonist strongly suggests that these adaptations correspond to a normalization of liver insulin responsiveness as evoked in muscles of rimonabant-treated ob/ob mice (21). The activation of hepatic CB1 receptors has been recently associated with an increase in de novo lipogenesis suggesting that this metabolic pathway participates to steatosis development in conditions of ECS overactivity (30). Unlike, we observed no stimulation of the liver expression of ACC and FAS in HSHF animals which were quite hyperinsulinemic. In the works of Osei-Hyiaman et al. (30), the stimulation of ECS consisted in an acute injection of CB1 agonist to control animals while in our study, ECS activation was induced for a much longer period using HSHF diet. Since the diet contained high proportions of saturated FA, it can be hypothesized that the provision of a diet rich in preformed saturated FA led to the reduced expression of mRNA for lipogenic genes. The inhibitory effect of palmitoyl-CoA on ACC demonstrated by Ogiwara et al. (31) supports this concept. The induction of SCD1 gene and the increase in monounsaturated FA content in the liver of HSHF mice (data not shown) indicate that SFA delivery to the liver was increased. Indeed, in HSHF mice, the liver steatosis appears to be mainly due to an enhanced delivery of FFA to the liver rather to an increase in de novo lipogenesis. Aside from direct effects on the liver, steatosis might also been reduced indirectly by the limitation of the influx of FA originating from adipose tissue. The gene expression profile of visceral adipose tissue is consistent with an hyperactivation of lipid metabolism as suggested by the strong upregulation of genes involved in transport, synthesis, oxidation and release of FA. Altogether, these data suggest that the reversion of liver steatosis induced by the treatment with CB1 antagonist was associated with an improvement of adipose tissue metabolism.

In line with an improvement of cardiovascular risk in type II diabetic patients treated with Rimonabant (11; 32), our findings support the possibility that CB1 antagonism is associated with an alteration of liver HDL catabolism. Previous studies showed that overexpression of SR-BI in the liver, while reducing plasma HDL-C levels, reduced atherosclerosis in mice (33) suggesting that hepatic SR-BI overexpression may promote reverse cholesterol transport. Accordingly, the increase in SR-BI and HL expression induced by CB1 antagonism may be associated with a modification of HDL size and kinetics (34) and thereby explain the increase in HDL-CE uptake observed in our model of liver slices. Additional studies are currently under investigation to clearly
identify the direct effects of CB1 antagonism on liver lipid metabolism.

**Effects of CB1 antagonism on visceral fat:** Recently, evidence has accumulated from animal and human studies that obesity is also associated with overactivation of ECS in visceral fat (17; 35-37). Concordant findings from this study and from literature support the view that CB1 blockade exerts specific effects on visceral fat metabolism that could be associated with the reduction of liver TG content. Hence, the coordinated upregulation of genes acting at different levels of the lipogenic pathway and that of the nuclear activator PPARg strongly suggested that HSHF diet favored TG synthesis and thereby formation of enlarged visceral fat deposits. Adipocyte hypertrophy in obesity is consecutive to a deficit in adipogenesis (38) and the limitation of fat stores would promote ectopic lipid deposition in liver and skeletal muscle, leading to decreased insulin action in these tissues (39). Remarkably, the fact that CB1 antagonism totally or partially normalized the expression levels of lipogenic genes in adipocytes may limit the accumulation of intracellular lipid droplets and give rise to smaller cells and reduction of visceral fat mass as also suggested in (40). The decrease in HSL expression consecutive to CB1 antagonism is of particular importance since excessive HSL-dependent fat lipolysis leads to an increased release of FFA into the circulation which in turn has deleterious effects on insulin sensitivity (41).

It has been suggested recently that obesity-induced inflammation of adipose tissue may directly activate ECS (42). This could result in a protective response against inflammation as described in colon (43). From our research, it appears that ECS activation induced by HSHF diet is also associated with an increase in TNF-a in adipose tissue. This interaction between inflammation and ECS needs to be further explored to determine whether inflammation causes ECS activation or vice versa. However, the concentration-dependent stimulation of lipolysis by TNF-a demonstrated in rodent and human fat cells is considered to be an important pathogenetic factor in the development of insulin resistance and type 2 diabetes (44). Therefore, it is reasonable to suggest that the reduction of TNF-a expression in visceral adipose tissue of HSHF mice treated with CB1 antagonist is linked to the normalization of adipocyte metabolism and to underlying effects on lipid and carbohydrate metabolism.

**Effects of HSHF diet and CB1 antagonism in subcutaneous versus visceral fat:** This study also provides new information regarding the impact of HSHF diet and subsequent CB1 antagonism on the regulation of lipid metabolism in subcutaneous compared to visceral adipose tissue. Taken together, our findings give molecular evidence that 1) HSHF diet causes deleterious effects in visceral adipose tissue that were not observed in subcutaneous fat and 2) CB1 blockade is able to reverse the molecular changes primarily induced by HSHF in visceral adipose tissue and to exert specific effects on subcutaneous adipocytes. These discrepancies in gene regulation between visceral and subcutaneous adipocytes in response to high fat diet and CB1 antagonism are consistent with a different degree of ECS activation in these tissues. This consideration is supported by several recent findings indicating differences in endocannabinoid levels between epididymal and subcutaneous fat (36; 37; 45; 46). In addition, the overexpression of PPARg, FAS and ACC gene in epididymal fat of obese mice is also in favor of the activation of ECS in this tissue since it has been reported that CB1 activation stimulates lipogenesis by increasing PPARg and lipogenic enzyme expression in adipocytes and liver (30; 36; 47). Collectively, data suggest that ECS is more
activated in epididymal than in subcutaneous fat in our mice model of obesity and it can be predicted that antagonism of CB1 was more effective in the tissue presenting elevated levels of endocannabinoids (36; 45).

Interestingly, HSHF diet or CB1 antagonist treatment induced nearly similar effects on the amounts of epididymal and subcutaneous fat suggesting that molecular and metabolic differences observed are not solely related to the modification of the fat depot size. In contrast, the induction of TNF-α expression by HSHF diet was far less important in subcutaneous than in visceral fat and the treatment of obese mice with CB1 antagonist induced the complete normalization of TNF-α expression only in subcutaneous fat whereas inflammation remains high in visceral adipocytes. Concomitantly, the normalization of TNF-α mRNA levels in subcutaneous fat is associated to an increase in adiponectin content in this tissue. An inverse relationship between circulating adiponectin and TNF-α has already been evoked (48) suggesting that adipose tissue inflammation could alter adiponectin production. The increased expression of PPARγ2 induced by CB1 antagonism in subcutaneous fat may also correspond to an activation of adipocyte differentiation (49) and thereby of adiponectin secretion (50). In addition, our findings regarding the adiponectin content in visceral and subcutaneous fat suggest that the normalization of adiponectin plasma levels induced by CB1 antagonism may be exclusively associated with an increased production of this adipokine by subcutaneous adipocytes.

In conclusion, this study indicates that treating obese mice with a CB1 antagonist exerts beneficial effects on liver steatosis and various lipid parameters providing supportive evidence that the hyperactivity of ECS associated with obesity was adjusted by the antagonism of CB1. This notion is further supported by data from an ongoing study indicating that CB1 antagonism exerts no effects on body weight, fat mass and liver triglyceride content in control mice (personal data). Our findings are also consistent with a contribution of peripheral CB1 and suggest different degrees of ECS activity in visceral and subcutaneous fat. In this way the improvement of visceral adipose tissue metabolism appears to be a determining factor for the normalization of plasma parameters and the reversion of liver steatosis. Therefore, future studies should investigate the direct effects of CB1 antagonism on the liver to precise the respective implication of ECS and products secreted by adipose tissue in the regulation of lipid metabolism.

ACKNOWLEDGMENTS

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REFERENCES

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23. Degrase P, Demizieux L, Gresti J, Chardigny JM, Sebedio JL, Clouet P: Hepatic steatosis is not due to impaired fatty acid oxidation capacities in C57BL/6J mice fed the conjugated trans-10,cis-12-isomer of linoleic acid. *J Nutr* 134:861-867, 2004


receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 115:1298-1305, 2005


32. Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J: Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *Jama* 295:761-775, 2006


49. Schoonjans K, Staels B, Auwerx J: The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* 1302:93-109, 1996

Table 1. Effects of CB1 antagonism on body composition, serum and liver parameters.

<table>
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<tr>
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<th>CON (n=5)</th>
<th>HSHF (n=10)</th>
<th>HSHF+SR (n=14)</th>
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<tr>
<td><strong>Organ weight</strong></td>
<td></td>
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<tr>
<td>Liver (g)</td>
<td>1.38 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.49 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Epididymal fat (g)</td>
<td>0.67 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.04 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Inguinal fat (g)</td>
<td>0.31 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Serum</strong></td>
<td></td>
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<tr>
<td>Glucose (mg/mL)</td>
<td>2.79 ± 0.16</td>
<td>2.72 ± 0.21</td>
<td>2.53 ± 0.08</td>
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<td>Insulin (ng/mL)</td>
<td>0.40 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Adiponectin (µg/mL)</td>
<td>63.05 ± 3.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.31 ± 7.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.82 ± 3.95&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Free fatty acids (mmol/L)</td>
<td>0.30 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Triglycerides (mg/mL)</td>
<td>0.74 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.31 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Total cholesterol (mg/mL)</td>
<td>1.09 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
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<td><strong>Liver</strong></td>
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<td>Glycogen (mg/g)</td>
<td>66.7 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.2 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Triglycerides (mg/g)</td>
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<td>46.4 ± 4.9&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Total cholesterol (mg/g)</td>
<td>16.07 ± 0.94</td>
<td>17.98 ± 1.63</td>
<td>15.72 ± 0.7</td>
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<td>MalonylCoA (nmol/g)</td>
<td>1.67 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.43 ± 0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.41 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
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Mice were fed a high sucrose high fat diet for 25 weeks receiving during the last 6 weeks either 10 mg/kg.day<sup>-1</sup> of the CB1 specific antagonist SR141716 (HSHF+SR) or the vehicle (HSHF). In parallel, a series of mice was maintained on a control diet (CON). Mice were food-deprived 4 h before tissue handling. Results are expressed as means ± SEM. Values with different superscript letters (a, b, c) are statistically different at p<0.05.
Table 2. Effects of CB1 antagonism on fatty acid oxidation and parameters related to lipoprotein metabolism in liver explants.

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<tr>
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<th>HSHF</th>
<th>HSHF+SR</th>
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<tr>
<td>Palmitic acid oxidation (nmol/h.g prot⁻¹)</td>
<td>23.0 ± 13</td>
<td>22.0 ± 2.4</td>
</tr>
<tr>
<td>ApoB secretion (µg/h.g prot⁻¹)</td>
<td>155 ± 17ᵃ</td>
<td>123 ± 8ᵇ</td>
</tr>
<tr>
<td>ApoA secretion (µg/h.g prot⁻¹)</td>
<td>141 ± 2</td>
<td>140 ± 3</td>
</tr>
<tr>
<td>HDL-CE uptake ([³H]-cholesteryl ether dpm/h. µg prot⁻¹)</td>
<td>430 ± 119ᵃ</td>
<td>1075 ± 139ᵇ</td>
</tr>
</tbody>
</table>

Thin liver slices (about 200 µm) were obtained from mice fed a high sucrose high fat diet and treated either with 10 mg/kg.day⁻¹ of SR141716 (HSHF+SR; n=5), or vehicle (HSHF; n=5). For fatty acid oxidation and apolipoprotein secretion, slices were incubated at 37 °C in oxygenated William’s Medium E supplemented with L-carnitine (0.5 mmol/L) in the presence of 0.2 mmol/L of [1-¹⁴C] palmitic acid (55.5 GBq/mol) complexed to albumin (FA/BSA molar ratio 2.5/1). After 4 h of incubation, slices were rinsed with cold PBS and immediately submitted to lipid extraction for counting of labelled CO₂ and acid-soluble products while the incubation medium was used for determination of apoB and apoA secreted. Measurement of HDL uptake was carried out at 37°C by incubating liver slices with [³H]-cholesteryl ether-HDL under slight agitation for 3 hours. Then, slices were washed and homogenized in PBS. Radioactivity recovered in the homogenate represented the amount of HDL uptaken by the liver cells. Results are expressed as means ± SEM. Values with different superscript letters (a, b) are statistically different at p<0.05.
Figure legend

Figure 1
Evolution of body weight during the induction period of obesity and during the treatment with CB1 antagonist. C57BL/6J mice were fed a high sucrose high fat diet (42.3% carbohydrates, 22.5% lipids) for 25 weeks receiving orally during the last 6 weeks either 10 mg/kg.day\(^{-1}\) of the CB1 specific antagonist SR141716 (HSHF+SR; n=14) or the vehicle (HSHF; n=10). In parallel, a series of mice was maintained on a control diet (CON; n=5). Results are expressed as means ± SEM. Results of statistical analysis were indicated at weeks 20 and 26, values with different superscript letters (a, b, c) are statistically different at P<0.05.

Figure 2
Effect of CB1 antagonism on adiponectin concentration in visceral (A) and subcutaneous (B) fat. Mice were fed a high sucrose high fat diet for 25 weeks receiving during the last 6 weeks either 10 mg/kg.day\(^{-1}\) of the CB1 specific antagonist SR141716 (HSHF+SR; n=14) or the vehicle (HSHF; n=10). In parallel, a series of mice was maintained on a control diet (CON; n=5). Adiponectin concentration was measured in adipose tissue homogenates prepared as described in material and methods. Results are expressed as means ± SEM. Values with different superscript letters (a, b, c) are statistically different at P<0.05.

Figure 3
Effect of CB1 antagonism on the mRNA expression of CB1 and genes involved in carbohydrate and lipid metabolism in the liver. Mice were fed a high sucrose high fat diet for 25 weeks receiving during the last 6 weeks either 10 mg/kg.day\(^{-1}\) of the CB1 specific antagonist SR141716 (HSHF+SR) or the vehicle (HSHF). In parallel, a series of mice was maintained on a control diet (CON). For each gene, a standard curve was established from four cDNA dilutions (1/10 to 1/10,000) and used for determine the relative gene expression after normalization with a geometric average of 18S and TATA box binding protein expression. Results are expressed as means ± SEM (n=5 per group). Values with different superscript letters (a, b, c) are statistically different at P<0.05. CB1, cannabinoid receptor 1; PEPCK, phosphoenolpyruvate carboxykinase; G6P, glucose-6-phosphatase; HL, Hepatic lipase; SR-BI, scavenger receptor class B type I; CPT-I, carnitine palmitoyltransferase I; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase-1.

Figure 4
Effect of CB1 antagonism treatment on the mRNA expression of CB1 and genes involved in adipocyte metabolism in epididymal (A) and inguinal (B) fat. Mice were fed a high sucrose high fat diet for 25 weeks receiving during the last 6 weeks either 10 mg/kg.day\(^{-1}\) of the CB1 specific antagonist SR141716 (HSHF+SR) or the vehicle (HSHF). In parallel, a series of mice was maintained on a control diet (CON). For each gene, a standard curve was established from four cDNA dilutions (1/10 to 1/10,000) and used for determine the relative gene expression after normalization with a geometric average of 18S and TATA box binding protein expression. Results are expressed as means ± SEM (n=5 per group). Values with different superscript letters (a, b, c) are statistically different at P<0.05. CB1, cannabinoid receptor 1; TNF-α, tumor necrosis factor-α; PPARg2, peroxisome proliferator-activated receptor g2; FAT/CD36, fatty acid translocase; HSL, hormone-sensitive lipase; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; CPT-I, carnitine palmitoyltransferase I.
Figure 3
Figure 4