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# Antiobesity effects of chronic cannabinoid $CB_1$ receptor antagonist treatment in diet-induced obese mice

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**Abstract**

We determined the effect of a cannabinoid CB1 receptor antagonist (AM-251; *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) on food intake, body weight and adipose tissue mass in Western diet-induced obese (DIO) mice using a chronic, interrupted, oral dosing paradigm. The dosing paradigm was 2 weeks on treatment (treatment 1), 2 weeks off-treatment, followed by 2 weeks on treatment (treatment 2). During treatment 1 and treatment 2, food intake and body weight were reduced after a single dose. At 30 mg/kg/day, anorectic efficacy was maintained through 12 days (treatment 1) and 7 days (treatment 2). Body weight of AM-251-treated mice remained less than vehicle-treated mice throughout treatment 1 and treatment 2. Administration of AM-251 reduced inguinal subcutaneous, retroperitoneal and mesenteric adipose tissue mass. Antiobesity effects of AM-251 were lost during the off-treatment period, and hyperphagia was observed in treated animals. With re-initiation of AM-251 treatment, mice again responded to the effects of the compound. These results support the hypothesis that chronic treatment of obese individuals with cannabinoid CB1 receptor antagonists is a viable pharmacologic approach to sustained weight loss.


*Keywords:* Anorectic; Cannabinoid; AM-251; Micro-CT; Obesity

## 1. Introduction

Obesity is increasingly recognized as a global health care problem epidemic in proportion (James et al., 2001). In the United States, where the epidemic is particularly evident, the number of approved drug treatments available to treat the disease has been reduced, rather than increased, over the last 5 years with the withdrawal of dexfenfluramine and fenfluramine. These two drugs were commonly prescribed and were associated with valvular heart disease (Connolly et al., 1997) which lead to their withdrawal. However, although the incidence of valvular heart disease was significantly higher in patients taking these drugs, it may not be as high as initially considered (Derry and Pritchard-Copley, 2002) and indeed in certain cases may spontaneously resolve over time (Vagelos et al., 2002). Nevertheless, current prescribed anti-obesity drug therapy is limited in the United States to orlistat (a gastrointestinal lipase inhibitor) or sibutramine (an anorectic). Given the heterogeneity of the etiology of human obesity and limitations of currently available drugs (precluded concomitant use of sibutramine in hypertensive patients, for example), there is a therapeutic need for very safe and effective compounds to treat obesity (Van der Ploeg, 2000). In this respect, the endocannabinoid system has received significant attention for its potential for pharmacologic manipulation to treat obesity.

The endocannabinoid system comprises endogenous ligands (anandamide, 2-arachidonoyl glycerol, 2-arachidonyl glyceryl ether (noladin ether), virodhamine) and two cannabinoid receptor subtypes ($CB_1$ and $CB_2$) (Hanus et al., 2001; Howlett et al., 2002; Porter et al., 2002). The cannabinoid $CB_1$ receptor is of interest with respect to appetite regulation. The exogenous agonist $\Delta^9$-tetrahydrocannabinol (the principal psychoactive component of marijuana) is hyperphagic in rodents (Williams et al., 1998) and man (Foltin et al., 1988). Experimental studies in rodents have also demonstrated hyperphagic effects of the endogenous cannabinoids anandamide and 2-arachidonoyl glycerol (Williams and Kirkham, 1999). Further evidence that endogenous cannabinoids are

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involved in central nervous system control of appetite is derived from the observations that (i) direct administration of anandamide into the ventromedial hypothalamus (an area rich in cannabinoid $CB_1$ receptors) stimulates food intake (Jamshidi and Taylor, 2001), and (ii) concentrations of 2-arachidonoyl glycerol in the limbic forebrain and hypothalamus are positively correlated with stimulation of food intake in rats (Kirkham et al., 2002). Furthermore, following temporary food restriction, cannabinoid $CB_1$ receptor knockout mice eat less than their wild-type littermates, and cannabinoid $CB_1$ receptor antagonist treatment reduces food intake in wild-type but not knockout mice (Di-Marzo et al., 2001; Van der Ploeg, 2000). Thus, there is rationale to seek a cannabinoid $CB_1$ receptor antagonist as an antiobesity drug.

Discovery of the first selective cannabinoid $CB_1$ receptor antagonist was reported several years ago (Rinaldi-Carmona et al., 1994). This compound, SR141716A (*N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide), has subsequently been shown to have anorectic efficacy in a number of very short-term (acute) food intake studies in rats (Arnone et al., 1997), mice (Di-Marzo et al., 2001) and monkeys (Simiand et al., 1998). These short-term studies investigated anorectic activity after a single dose of the compound. Two longer-term SR141716A efficacy studies in rats (Colombo et al., 1998) and mice (Ravinet et al., 2003) have shown the compound to be transiently anorectic but produce a sustained reduction in body weight compared to a control group.

To further investigate anorectic and weight loss pharmacology of cannabinoid $CB_1$ receptor antagonists, we used a clinically relevant administration route (oral) and a relevant Western diet-induced obese (DIO) mouse model. Furthermore, we investigated the chronic efficacy of a cannabinoid $CB_1$ receptor antagonist, AM-251, in mice using a chronic interrupted dosing paradigm. AM-251 (*N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) is a structural analog to SR141716A differing by the halogen substitution of I for Cl. The interrupted dosing paradigm permitted efficacy assessment after a 2-week treatment period and then again after a 2-week off-treatment period. During the 2-week inter-treatment period, the appetitive behavioral response of mice after abrupt withdrawal of treatment was studied. Efficacy was determined by daily monitoring of food intake, body weight and, after each treatment period, adipose tissue mass. This study represents the first longer duration preclinical assessment of cannabinoid $CB_1$ receptor antagonist in an interrupted dosing paradigm.

## 2. Research methods and procedures

### 2.1. Animal care, handling and compound administration

All in vivo animal work conducted in this study conformed to the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources (National Research Council, 1996). Male C57BL/6 mice were obtained from Charles River (Wilmington, MA) and housed under standard conditions (12-h light/dark cycle, 22 °C). Animals were acclimated to single housing upon arrival, and fed a pelleted high fat diet (45% kcal from fat, D12451 Research Diets, New Brunswick, NJ). Prior to placement in the micro-CT instrument, mice were anesthetized with isoflurane. Anesthesia was maintained during the scanning procedure with 2% isoflurane and 2 l/min oxygen. Mice were continuously monitored during recovery from the anesthesia, before being returned to individual cage housing. For a period of 1 week prior to the baseline micro-CT scan, and before treatment period 1, all mice were acclimated to once daily dosing using 0.5% methylcellulose alone. Mice were administered AM-251 (purchased from Tocris Cookson, Ellisville, MO) via oral gavage at a dose of either 3 or 30 mg/kg/day in a suspension of 0.5% methylcellulose. The suspension was administered via a soft rubber cannula attached to a 1-ml syringe, at a dosing volume of 5 ml/kg. Compound was prepared fresh each day. All mice were administered AM-251 or vehicle alone 60 min before the onset of the dark cycle.

### 2.2. Food intake and body weight

Food intake and body weight were monitored daily. To measure food intake, the pelleted food was weighed and then placed in the cage food container; the food remaining 24 h later was weighed, and the difference represented the daily food intake. Animal weight and food weight were measured using an electronic scale. Unconsumed pelleted high fat food was discarded each day, and fresh, pelleted high fat diet provided to ensure consistent food quality was provided to the mice throughout the study. The high fat food was stored at 4 °C. A schematic of the experimental protocol is shown in Fig. 1.

### 2.3. Micro-CT scanning, image reconstruction and analysis

Images were obtained using a commercially available micro-CT system (MicroCAT®, ImTek, Oak Ridge, TN) with a high-resolution CCD/phosphor screen detector. The scanner consisted of a cylindrical diameter/long field view

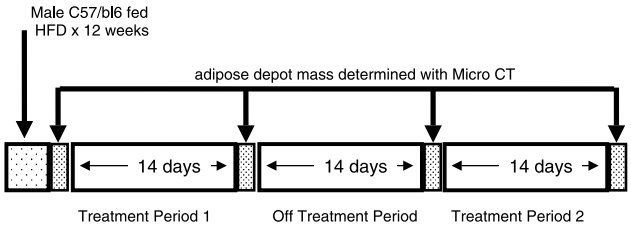


Fig. 1. Schematic representation of the protocol used to determine the effect of the CB1 receptor antagonist AM-251 on food intake, body weight and adipose tissue depot mass in male, diet-induced obese C57BL/6 mice.

of 50 mm/50 mm with a spatial resolution of less than 50 μm. The X-ray source was biased at 40 keV with the anode current set to 400 μA. Anesthetized mice were placed on a radio-transparent mouse bed in a supine position, caudal end closest to the micro-CT with the rostral end held in place against an anesthesia delivery tube. The hind legs were moderately extended and held in place with clear tape to ensure a correct anatomical position (i.e. straight spine) and that the mouse position did not change once the scan procedure was initiated. An initial radiographic image was acquired at 90° to the plane of the mouse bed to allow correct positioning of the mouse by centering the scan acquisition area at the level of the iliac crest of each mouse. Image reconstruction, whereby a micro-CT scan of an individual mouse was manipulated to produce two-dimensional cross-sectional images, was performed using the MicroCAT® Reconstruction, Visualization, and Analysis Software (ImTek). Two sets of reconstructed images per scan were generated for each mouse for the determination of individual fat depot mass. User-defined placement of reconstruction slices were placed relative to defined anatomical sites (i.e. vertebral segments) (Hildebrandt et al., 2002). The first set of reconstructed images, consisting of six slices (intervertebral segments lumbar 6–7 through sacral 4–caudal 1), provided a montage for the analysis of inguinal and epididymal adipose tissue depots. The second reconstruction set, consisting of nine slices (intervertebral and midvertebral landmarks from lumbar 2–3 through lumbar 6–7) was used to define retroperitoneal and mesenteric adipose tissue depot masses. Reconstructed bitmap images were converted to TIFF (Tag Image File Format) images and subsequently analyzed for fat depot mass using Scion Image for Windows (Scion, Frederick MD).

### *2.4. Plasma insulin, leptin, cholesterol, glucose and triglyceride determinations*

Plasma insulin and leptin were analyzed using commercially available ELISA kits designed for murine studies (Crystal Chem, Downers Grove, IL). To assay insulin concentrations, plasma samples were diluted 1:1 with buffer, and assayed according to the standard protocol in Crystal Chem Kit #INSSM021. Plasma leptin concentrations were analyzed in undiluted sample, according to the standard protocol of Crystal Chem Kit #90030. Plasma concentrations of glucose, cholesterol and triglyceride were analyzed using a Hitachi 912 Automatic Analyzer.

### *2.5. Statistical methods*

All data shown are the mean±standard error of the mean (S.E.M.). The effects of time and dose on food intake, body weight or adipose depot mass of the groups studied (vehicle control, low dose AM-251 and high dose AM-251) were determined using a two-way analysis of variance with repeated measures analysis, followed by Newman–Keuls test to determine where differences existed (if any) between the groups. Statistical comparisons of plasma insulin, leptin, cholesterol, glucose and triglyceride were done with a one-way analysis of variance, followed by Newman–Keuls test where appropriate. In all cases, differences were considered statistically different at $P<0.05$.

## 3. Results

The effect of AM-251 on food intake during the first treatment period, the off-treatment period and the second treatment period is shown in Fig. 2(A–C). During the first

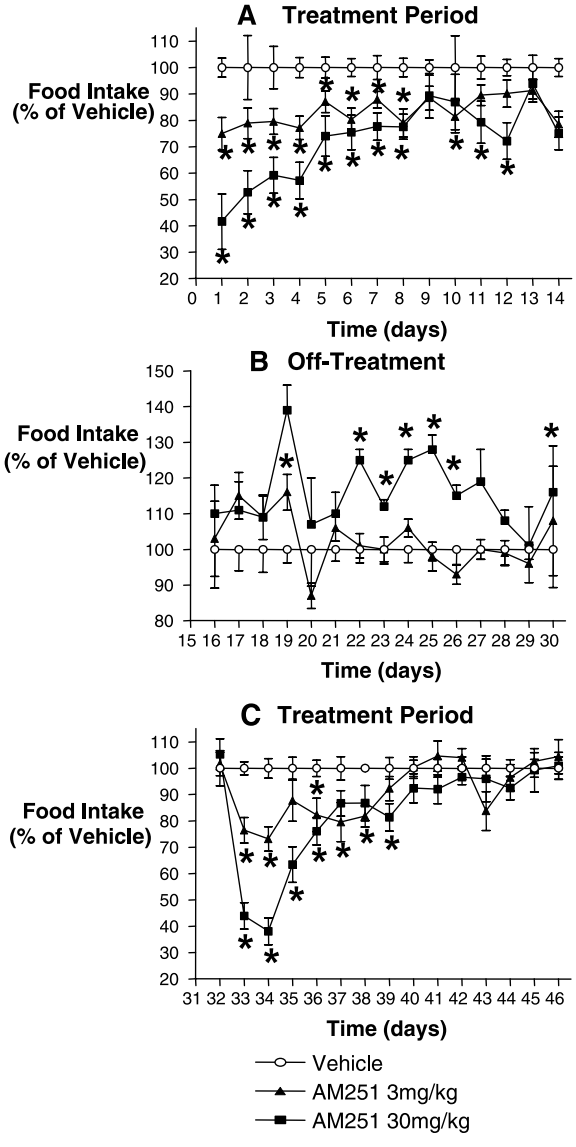


Fig. 2. Effect of AM-251 treatment on food intake (expressed as a percentage of mean vehicle-treated food intake) when administered at a dose of 3 or 30 mg/kg/day, during the three treatment periods studied. (A) Treatment period 1; (B) off-treatment period (no compound administered); (C) treatment period 2 (mice dosed as in treatment period 1). *$P<0.05$ vs. vehicle. Error bars represent the S.E.M., with $n=10$/group.

treatment period (Fig. 2A), there was an immediate and significant dose-dependent decrement in food intake in AM-251-treated male DIO mice. The anorectic effect of AM-251 waned over the 2-week treatment period; however, food intake remained significantly reduced through 8 days at the low dose (3 mg/kg/day) and through 12 days at the high dose (30 mg/kg/day). After withdrawal of either the low or high dose AM-251 treatment, there was a rapid return of food intake. In the high dose AM-251 group, as shown in Fig. 2B, there was a statistically significant hyperphagic response when administration of compound was stopped. The hyperphagia observed in the high dose group was statistically significant from days 7 through 11 of the off-treatment period. To determine if the mice treated with AM-251 remained responsive to the anorectic effect of the compound, treatment was re-initiated after 2 weeks off-treatment for another 2 weeks. As shown in Fig. 2C, there was again an immediate and statistically significant dose-dependent decrement in food intake in AM-251-treated mice. Similar to the dose-dependent responsiveness of the anorexia in the first treatment period, there was a waning of the compound's anorectic efficacy with time, with the low (3 mg/kg/day) dose efficacy waning more rapidly. Anorectic efficacy was observed for 4 and 7 days in the low dose and high dose AM-251-treated groups, respectively.

The results shown in Fig. 3 (panels A–C) indicate the effect of AM-251 treatment on body weight in the male DIO mice. The mean body weights of mice in the three groups were equivalent at the start of the study, as expected from the random assignment of mice to each group. The vehicle-treated control DIO mice continued to gain weight, at a slow rate, during treatment period 1. In contrast, DIO mice in the low dose (3 mg/kg/day) and high dose (30 mg/kg/day) AM-251-treated groups lost weight, in a dose-dependent fashion over the 2-week treatment period. There was a delay of 2–3 days before the weight loss was manifest; however, weight loss was sustained over the 2-week treatment period (Fig. 3A). During the off-treatment period (Fig. 3B), there were no significant increases in body weight of vehicle-treated mice. Not unexpectedly, considering the hyperphagia shown in Fig. 2B, there was a regain of body weight in both AM-251-treated groups during the off-treatment period. However, weight gain only brought the treated animals towards the vehicle-treated control group; hyperphagic mice did not overshoot the body weight of age-matched high fat diet fed controls. When the mice were dosed with AM-251 in treatment period 2 (Fig. 3C), there was again a dose-dependent reduction in weight that was sustained for the 2-week dosing period. The mice clearly remained sensitive to the weight loss effects of AM-251 when dosed at the low or high doses during treatment period 2.

The effect of AM-251 treatment on in situ adipose tissue mass in diet-induced obese mice is shown in Fig. 4A–D. At baseline, the mass of the four depots studied (inguinal subcutaneous, epididymal, retroperitoneal and mesenteric) were not different. There was no effect of the low dose AM-

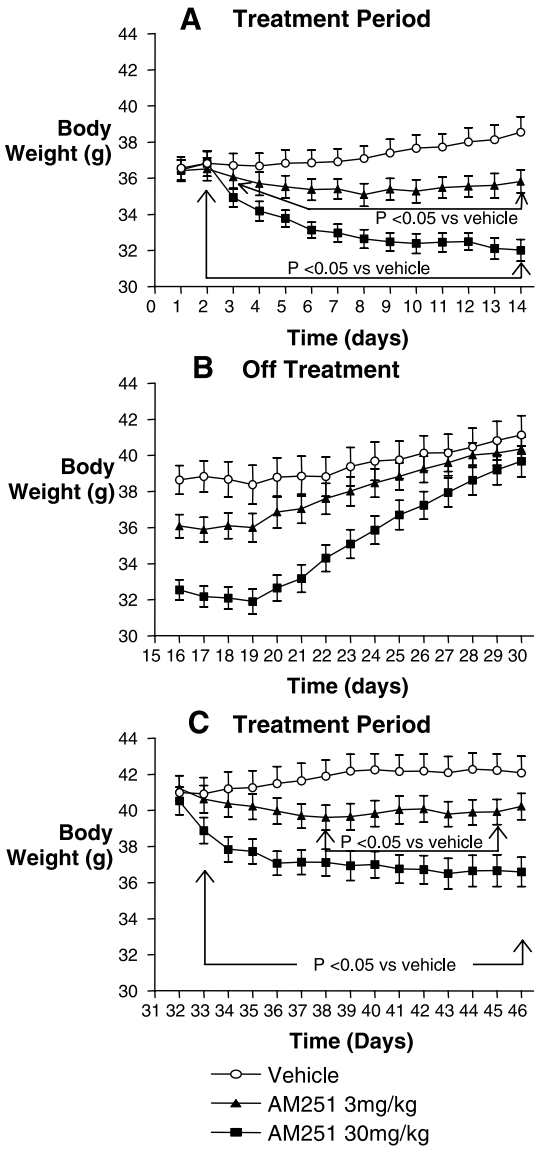


Fig. 3. Effect of AM-251 treatment on body weight when administered at a dose of 3 or 30 mg/kg/day, during the three treatment periods studied. (A) Treatment period 1; (B) off-treatment period (no compound administered); (C) treatment period 2 (mice dosed as in treatment period 1). Error bars represent the S.E.M., with $n$=10/group.

251 treatment (3 mg/kg/day) on the mass of the adipose depots studied. Only the high dose AM-251 treatment (30 mg/kg/day) effected reductions in adipose depot mass. During treatment period 1, at the high dose of AM-251 (30 mg/kg/day), a general reduction in adipose tissue mass was observed. However, as shown in Fig. 4B, the epididymal adipose depot was resistant to AM-251 treatment, since there were no statistically significant effects of AM-251 on this depot after either treatment period 1 or 2. In the other depots (Fig. 4A,C,D), there was a significant reduction in adipose mass after treatment period 1 in high dose AM-251-treated mice (30 mg/kg/day). During the off-treatment period, adipose tissue mass of vehicle-treated mice was not consistently affected (i.e. there were no clear statistically

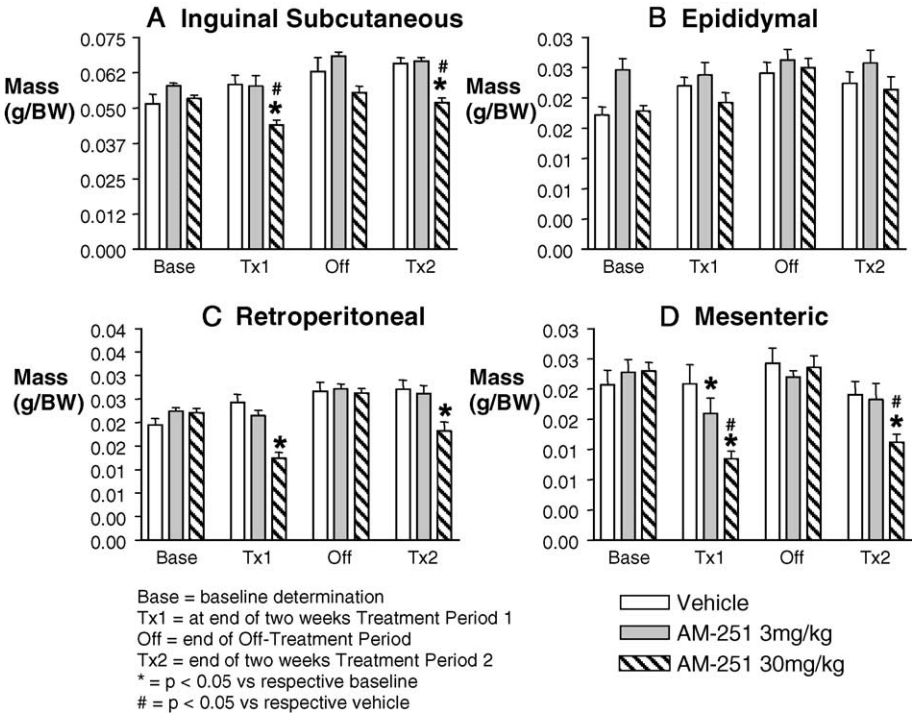


Fig. 4. Effect of AM-251 treatment on inguinal subcutaneous, epididymal, retroperitoneal and mesenteric adipose depot mass. Adipose depot mass was determined in situ with micro-CT technology at baseline (before any treatments initiated), at the end of 2-week treatment with AM-251 (treatment 1), at the end of 2-week off treatment (Off) and again after 2-week treatment with AM-251 (treatment 2). *$P$<0.05 vs. respective baseline determination. $^{\#}P$<0.05 vs. respective vehicle-treated group, i.e. within a treatment period. Error bars represent the S.E.M., with $n$=6/group.

significant effects). However, there was a general trend in the vehicle-treated animals for adipose tissue to become greater with time. These effects are most likely related to the continued exposure and consumption of the mice to the highly palatable high fat diet. The adipose depot mass of mice that had been treated with high dose AM-251 increased to the level of the vehicle-treated group's during the "off-treatment" period. However, after treatment period 2, the same pattern of AM-251 treatment mediated reductions in adipose tissue mass were again evident (hatched bars of Fig. 4A,C,D).

The experiment terminated after the treatment period 2 adipose tissue micro-CT scans, and at that time, we obtained a plasma sample from the mice in each of the three groups studied and measured insulin, leptin, cholesterol, triglyceride and glucose concentrations. We did not obtain baseline, post-treatment period 1 or post-"off-treatment" samples since this may have compromised the status of the animals before starting the experiment, or midway through the experiment. As shown by the data in Table 1, there was no effect of 2 weeks daily treatment with AM-251 at a dose of 3 mg/kg/day on any of the parameters measured. At a dose of 30 mg/kg/day for 2 weeks, there was no effect of AM-251 treatment on plasma glucose or triglycerides. There was a 55% reduction in plasma insulin levels with high dose AM-251 treatment; however, the variability precluded this trend from being statistically significant from vehicle-treated mice. However, as shown in Table 1, 2 weeks of AM-251 treatment at a dose of 30 mg/kg/day lead to a significant reduction in plasma cholesterol (22% decrease) and leptin (29% decrease) concentrations.

Table 1
Plasma analyses after treatment period 2

| | Leptin (pg/ml) | Insulin (pg/ml) | Glucose (mg/dl) | Cholesterol (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|---|---|---|
| Vehicle | 19.4±1.1 | 5.1±1.6 | 290±23 | 200±5 | 93±13 |
| AM-251 (3 mg/kg) | 19.7±1.7 | 4.5±1.5 | 315±31 | 192±4 | 98±15 |
| AM-251 (30 mg/kg) | 13.7±1.0* | 2.3±1.1 | 315±13 | 157±3* | 128±19 |

Values shown are the mean±S.E.M. ($n$=9–10/group).

* Indicates a significant ( $P$<0.05) difference between vehicle- and AM-251-treated (30 mg/kg/day) group.

## 4. Discussion

The anorectic efficacy of the cannabinoid $CB_1$ receptor antagonist SR141716A is established (Arnone et al., 1997; Colombo et al., 1998; Di-Marzo et al., 2001; Ravinet et al., 2003; Simiand et al., 1998); however, anorectic effects of AM-251 have not been previously reported. Our current study sought to determine if AM-251 (different from SR141716A by virtue of a single halogen switch, I for Cl)

(Lan et al., 1999) demonstrated antiobesity efficacy in a chronic, interrupted dosing paradigm. AM-251 is a potent (a $K_i$ value of 7.49 nM at cannabinoid $CB_1$ receptors) and selective (306-fold selective over $CB_2$) cannabinoid $CB_1$ receptor antagonist (Gatley et al., 1996, 1997). In vivo cannabinoid $CB_1$ receptor antagonist effects of AM-251 have been reported (Gardiner et al., 2002); however, this is the first report describing anorectic and weight loss effects of AM-251. Considering the in vitro pharmacologic profile of the compound, its structural similarity to SR141716A and the known pharmacology of that compound, efficacy was anticipated.

The principal novel aspect of this study was the chronic "on–off–on" treatment paradigm used to explore the anorectic/weight loss efficacy of the cannabinoid $CB_1$ receptor antagonist AM-251. Prior to this report, two studies reported on the anorectic efficacy of "chronic" cannabinoid $CB_1$ receptor antagonist treatment. The first study, of 2 weeks duration, demonstrated that in male non-obese Wistar rats SR141716A (10 mg/kg/day intraperitoneal administration) transiently inhibited food intake, with anorectic efficacy lost after 4 days treatment (Colombo et al., 1998). In the second study in male diet-induced obese mice, SR141716A (10 mg/kg/day administered orally) reduced energy intake by 48% after 1-week treatment. This anorectic effect waned over the subsequent 4-week treatment to a 12% decrease compared to vehicle-treated mice (Ravinet et al., 2003). However, the reduction in energy intake remained significant. Thus, these studies indicated that there was desensitization to the anorectic efficacy of cannabinoid $CB_1$ receptor antagonists, or at least SR141716A as it was the only compound studied, with "chronic" administration. In our study, also using a diet-induced obese male mouse model and using oral dosing, cannabinoid $CB_1$ receptor antagonist anorectic efficacy lasted for a period of 8 and 12 days during the first treatment period at 3 and 30 mg/kg/day, respectively. Our data and the data of Ravinet et al. (2003) show that under conditions of high fat diet-induced obesity, where energy intake is in excess of energy consumption, cannabinoid $CB_1$ receptor antagonists have a more sustained anorectic effect than in normal chow fed, growing rats. This may be of importance in consideration of the respective human population in which anorectic drug efficacy is, ultimately, sought.

A second major finding of this study was the weight loss efficacy of the cannabinoid $CB_1$ receptor antagonist AM-251. Similar to the anorectic efficacy discussed above, literature comparisons are restricted to the aforementioned normal, growing Wistar rat and diet-induced obese mouse studies (Colombo et al., 1998; Ravinet et al., 2003). In non-obese male Wistar rats, cannabinoid $CB_1$ receptor antagonist treatment significantly attenuated the rate of weight gain over a 2-week period (Colombo et al., 1998). In the 5-week diet-induced mouse study, weight loss efficacy was observed with SR141716A for a period of 5 weeks. The current study supports the weight loss efficacy of these two previous studies; the high dose (30 mg/kg/day) and low dose (3 mg/kg/day) of AM-251 treatment lead to sustained reductions in body weight of male diet-induced obese mice. The significant reduction in body weight was observed both during treatment period 1, without prior exposure to the compound, and also during treatment period 2, after the mice had regained much of the weight previously lost during treatment period 1. It was interesting to note that although anorectic efficacy waned over time, during both treatment periods, the reduction in body weight was sustained. Therefore despite a waning of anorectic efficacy, cannabinoid $CB_1$ receptor antagonist treatment lead to a significant and sustained reduction in body weight. This is the primary goal of any therapeutic approach used to treat human obesity.

In designing this study, it was of particular interest to study not only the relative desensitization to anorectic effects that may occur with continued use of a cannabinoid $CB_1$ receptor antagonist in an obese mouse model, but also the effect of withdrawing the compound and then re-instituting treatment after a 2-week period. This approach has not been reported previously. It was interesting to observe that during the off-treatment period, in both low and high dose AM-251-treated groups, there was a return of treated animals' body weight to the vehicle-treated body weight range. During the 2-week off-treatment period, the high dose group of AM-251-treated mice exhibited hyperphagia. Although the mice in the high dose group consumed more food than vehicle controls during the 2-week off-treatment period, body weight did not exceed the vehicle group. This gain of body-weight was also similar to that observed in Wistar rats, although that study tracked food intake/body weight only for 1 week after treatment was terminated (Colombo et al., 1998). The implications of the observed hyperphagia remain speculative but in the mouse may include a physiological response to return body weight to a genetically determined "set-point" (Levin et al., 1997). It will be important to characterize any such response in humans treated with a cannabinoid CB1 receptor antagonist. Possibly, tapering the drug administration over time may attenuate the hyperphagic response.

In this study we measured in situ, and longitudinally, the effect of cannabinoid $CB_1$ receptor antagonist treatment on adipose tissue depot mass using micro-CT technology. This technology affords the opportunity to measure adipose tissue mass in the same animal over time, and, in this experiment, in response to a drug treatment. The technology has recently been validated for the measurement of adipose tissue in mice (Hildebrandt et al., 2002). Administration of the high dose of AM-251 (30 mg/kg/day) reduced the mass of inguinal subcutaneous, retroperitoneal and mesenteric adipose tissue depots in DIO mice. The observed reductions in adipose tissue are in agreement with those previously observed with SR141716A treatment of diet-induced obese mice (Ravinet et al., 2003). Interestingly, epididymal adipose tissue was not responsive to cannabinoid $CB_1$ receptor

antagonist treatment; epididymal adipose depot mass did not change in response to AM-251 treatment either during treatment period 1 or treatment period 2. It was also observed in our studies that the high dose of AM-251 significantly reduced plasma leptin concentrations. The reduction in plasma leptin concentrations along with significant reductions in epididymal, inguinal subcutaneous and retroperitoneal adipose depot mass is in agreement with previous reports of the relationship between plasma leptin and the extent of adiposity in rodents and man (Maffei et al., 1995). It was also observed that here was a reduction in plasma total cholesterol in the high dose AM-251-treated group. Although this observation is of interest, as hypercholesterolemia is an independent risk factor for cardiovascular disease, the mechanism for this response remains to be determined.

There is an increasing body of evidence that the endocannabinoid system plays an important role in the regulation of appetitive behavior and, from a pharmacological point of view, that inhibition of the endocannabinoid system through antagonism of the cannabinoid $CB_1$ receptor may be an effective therapeutic approach to treating human obesity. Current preclinical evidence supports anorectic and weight loss efficacy through a 5-week period, and the current study extends our understanding in that efficacy was again clearly achievable after a 2-week ''off-treatment'' period; these data indicate tolerance or desensitization to the pharmacologic efficacy of cannabinoid $CB_1$ receptor antagonists may not limit their repetitive use in man. However, it is also apparent from these studies that during the ''off-treatment'' period, mice, which had the greater response to AM-251, also had a greater hyperphagia following cessation of treatment. This observed ''rebound'' of energy consumed may also be of relevance to the treatment of humans with this class of compounds. Whilst there is a growing body of preclinical evidence to support a role of the endocannabinoid system in control of appetitive behavior, and that cannabinoid $CB_1$ receptor antagonists may be an effective approach, ultimately data from clinical trials with novel cannabinoid $CB_1$ receptor antagonists will provide the key information as to the potential for this class of compounds to help treat the very serious epidemic of obesity.

## References


Arnone, M., Maruani, J., Chaperon, F., Thiebot, M.H., Poncelet, M., Soubrie, P., Le Fur, G., 1997. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology (Berl.) 132, 104–106.

Colombo, G., Agabio, R., Diaz, G., Lobina, C., Reali, R., Gessa, G.L., 1998. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. Life Sci. 63, L113–L117.

Connolly, H.M., Crary, J.L., McGoon, M.D., Hensrud, D.D., Edwards, B.S., Edwards, W.D., Schaff, H.V., 1997. Valvular heart disease associated with fenfluramine–phentermine. N. Engl. J. Med. 337, 581–588.

Derry, S., Pritchard-Copley, A., 2002. Appetite suppressants and valvular heart disease—a systematic review. BMC Clin. Pharmacol. 2, 6.

Di-Marzo, V., Goparaju, S.K., Wang, L., Liu, J., Batkai, S., Jarai, Z., Fezza, F., Miura, G.I., Palmiter, R.D., Sugiura, T., Kunos, G., 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature 410, 822–825.

Foltin, R.W., Fischman, M.W., Byrne, M.F., 1988. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. Appetite 11, 1–14.

Gardiner, S.M., March, J.E., Kemp, P.A., Bennett, T., 2002. Complex regional haemodynamic effects of anandamide in conscious rats. Br. J. Pharmacol. 135, 1889–1896.

Gatley, S.J., Gifford, A.N., Volkow, N.D., Lan, R., Makriyannis, A., 1996. 123I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. Eur. J. Pharmacol. 307, 331–338.

Gatley, S.J., Lan, R., Pyatt, B., Gifford, A.N., Volkow, N.D., Makriyannis, A., 1997. Binding of the non-classical cannabinoid CP 55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. Life Sci. 61, L-7.

Hanus, L., Abu-Lafi, S., Fride, E., Breuer, A., Vogel, Z., Shalev, D.E., Kustanovich, I., Mechoulam, R., 2001. 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 3662–3665.

Hildebrandt, A.L., Kelly-Sullivan, D.M., Black, S.C., 2002. Validation of a high-resolution X-ray computed tomography system to measure murine adipose tissue depot mass in situ and longitudinally. J. Pharmacol. Toxicol. Methods 47, 99–106.

Howlett, A.C., Barth, F., Bonner, T.I., Cabral, G., Casellas, P., Devane, W.A., Felder, C.C., Herkenham, M., Mackie, K., Martin, B.R., Mechoulam, R., Pertwee, R.G., 2002. International Union of Pharmacology: XXVII. Classification of cannabinoid receptors. Pharmacol. Rev. 54, 161–202.

James, P.T., Leach, R., Kalamara, E., Shayeghi, M., 2001. The worldwide obesity epidemic. Obes. Res. 9 (Suppl. 4), 228S–233S.

Jamshidi, N., Taylor, D.A., 2001. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. Br. J. Pharmacol. 134, 1151–1154.

Kirkham, T.C., Williams, C.M., Fezza, F., Di, M.V., 2002. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br. J. Pharmacol. 136, 550–557.

Lan, R., Liu, Q., Fan, P., Lin, S., Fernando, S.R., McCallion, D., Pertwee, R., Makriyannis, A., 1999. Structure–activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. J. Med. Chem. 42, 769–776.

Levin, B.E., Dunn-Meynell, A.A., Balkan, B., Keesey, R.E., 1997. Selective breeding for diet-induced obesity and resistance in Sprague–Dawley rats. Am. J. Physiol. 273, R725–R730.

Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganathan, S., 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat. Med. 1, 1155–1161.

Porter, A.C., Sauer, J.M., Knierman, M.D., Becker, G.W., Berna, M.J., Bao, J., Nomikos, G.G., Carter, P., Bymaster, F.P., Leese, A.B., Felder, C.C., 2002. Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. J. Pharmacol. Exp. Ther. 301, 1020–1024.

Ravinet, T.C., Arnone, M., Delgorge, C., Gonalons, N., Keane, P., Maffrand, J.P., Soubrie, P., 2003. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. Am. J. Physiol., Regul. Integr. Comp. Physiol. 284, R345–R353.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Simiand, J., Keane, M., Keane, P.E., Soubrie, P., 1998. SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. Behav. Pharmacol. 9, 179–181.

Vagelos, R., Jacobs, M., Popp, R.L., Liang, D., 2002. Reversal of Phen–Fen associated valvular regurgitation documented by serial echocardiography. J. Am. Soc. Echocardiogr. 15, 653–657.

Van der Ploeg, L.H., 2000. Obesity: an epidemic in need of therapeutics. Curr. Opin. Chem. Biol. 4, 452–460.

Williams, C.M., Kirkham, T.C., 1999. Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. Psychopharmacology (Berl.) 143, 315–317.

Williams, C.M., Rogers, P.J., Kirkham, T.C., 1998. Hyperphagia in pre-fed rats following oral delta9-THC. Physiol. Behav. 65, 343–346.