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Cannabinoid-1 receptor inhibition prevents the reduction of 24-hour energy expenditure with weight loss

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ABSTRACT

Pharmacologic inhibition of the cannabinoid-1 receptor (CB1R) in rodent models leads to weight loss and time-dependent changes in energy balance. This study evaluated the effects of CB1R inhibition on weight loss, energy expenditure (EE), and food intake (FI) in an obese canine model following 4 weeks of treatment. Eighteen maintenance-fed obese beagles were evenly and randomly allocated to a CB1R inverse agonist (AM251) (2 mg/kg), a 70% food-restricted (FR) diet, or a control group (C). Evaluations included body weight and composition (dual-energy x-ray absorptiometry scan), EE (doubly labeled water), and FI. Change in body mass at week 4 was significantly greater ($P < .050$) in the AM251 (–1476.7 g) and FR groups (–1100.0 g) than in the C group (–228.3 g). Food intake was decreased from week 2 onward in the FR and AM251 groups ($P < .05$). Absolute and lean mass-adjusted EEs were decreased only in the FR group ($P < .01$); EE in the AM251 group was greater ($P < .05$) than that in the FR group. Pharmacologic inhibition of CB1R in a canine model led to sustained effects on FI and EE. Weight loss was greater with AM251 than could be accounted for by food restriction (~25%), an effect likely mediated by the EE response to CB1R inhibition.

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1. Introduction

Studies evaluating the endogenous cannabinoids and the cannabinoid-1 receptor (CB1R) led to the recognition that the endocannabinoid-CB1R system plays an important role in regulating whole-body energy balance [1]. The actions of endocannabinoids are mediated primarily by CB1R expressed in brain tissue [2], with detectable levels of CB1R mRNA in some peripheral tissues playing a lesser role [3]. The endocannabinoid system interacts with several well-established central nervous system energy homeostatic mechanisms that include the leptin [4], pro-opiomelanocortin [5], neuropeptide Y [6], ghrelin [7], and melanin-concentrating hormone pathways [8].

The specific mechanisms through which the endocannabinoid-CB1R system influences energy balance remain unclear. Peripheral administration or microinjection of endocannabinoids into sites located in the hypothalamus or limbic

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forebrain [9-11] has been shown to stimulate feeding behavior in rodents. Endocannabinoid levels in the hypothalamus and limbic forebrain are also increased in the fasting state [11]. The acute administration of rimonabant or taranabant, both CB1R inverse agonists, in rodents leads to dose-dependent reductions in food intake (FI) [12,13]. Administration of rimonabant over 6 weeks to candy-fed Wistar rats led to an initial reduction in FI and weight loss over the first week of treatment; however, restoration of FI and gradual weight gain were observed from week 2 onward [14].

Inhibition of the CB1R also appears to increase energy expenditure (EE) in other acute and multiple-day rodent studies and in acute human studies [15-19]. The EE effects of CB1R inhibition likely account for the greater weight loss observed over 6 weeks in actively treated rodents compared with pair-fed controls [14]. Recent studies in rodents suggest that some of the metabolic effects of CB1R inhibition may be mediated by brown adipose tissue (BAT) [19].

In overweight or obese humans, long-term treatment with rimonabant or taranabant in conjunction with a hypocaloric diet leads to gradual weight loss over 6 to 9 months followed by maintenance of weight loss for up to 1 or 2 years [20,21].The weight loss pattern seen in humans following long-term treatment with rimonabant and taranabant differs from that observed in prior rodent studies evaluating the effects of long-term pharmacologic CB1R inhibition [14].

The current study was conducted to evaluate the effects of 4-week CB1R inhibition on body weight, energy balance, EE, and FI in a large nonhuman animal species, specifically in an obese canine animal model. As the dog has a metabolism more reflective of human than rodents [22], we expect this model to be more correlative with human efficacy than the previously used rodent models. The aim of this study was to determine whether 4-week CB1R inhibition in obese dogs leads to sustained effects on energy balance and body weight effects similar to the pattern seen in human studies. The CB1R inverse agonist AM251 used in the present study is a close analog of rimonabant differing in one atom. Like rimonabant, AM251 is brain penetrant [12]. In both in vitro and rodent in vivo pharmacological studies, only minor differences in their potency and duration of action were reported [12,23-25].

2. Materials and methods

2.1. Experimental design

This study evaluated the CB1R inverse agonist AM251 in obese, spayed female beagles older than 2 years. The dogs were previously rendered obese by allowing them to eat puppy chow ad libitum. Before and during the study, the dogs were individually housed and fed their standard diet (Purina Laboratory Canine Diet 5006; Purina Mills, St Louis, MO); water was provided ad libitum. All dogs were maintained at a stable body weight by adjusting food consumption until a steady body weight was achieved on a consistent amount of daily food eaten before participation in the study. Animal testing protocols were reviewed and approved by the Merck Research Laboratories' Institutional Animals Care and Use Committee.

A total of 18 dogs were randomly assigned in equal proportions to 3 groups (ie, 6 dogs in each group). Dogs in the control (C) group were allowed free access to 100% maintenance food and received vehicle (100% PEG400) alone by oral gavage. *One hundred percent maintenance food* is defined as the amount of food with which a dog remains weight stable when fed that amount on a daily basis, as determined before study enrollment. Dogs in the food-restricted (FR) group were allowed free access to 70% maintenance food and received vehicle (100% PEG400) alone by oral gavage. Dogs in the active treatment group (AM251) were allowed free access to 100% maintenance food and received the CB1R inverse agonist AM251 at 2 mg/kg in vehicle at 10 mg/mL by oral gavage.

The total study duration was 6 weeks, including a 2-week vehicle run-in and a 4-week treatment period. Dosing ceased the day before a final intravenous glucose tolerance test (IVGTT)/dual-energy x-ray absorptiometry (DXA) scan for randomly selected dogs in each treatment group. Each dog was observed at least once daily by qualified animal care personnel. All abnormal clinical health observations were recorded. Dogs also were observed following treatment for signs of behavioral changes and/or adverse events.

2.2. EE measurements

On days –4 and 24, blood samples were collected from every dog (2 mL in heparinized blood tubes). Each dog was subsequently catheterized (via cephalic vein) and infused with an intravenous dose of $D_2^{18}0$ (0.2 g/kg). The $D_2^{18}0$ was a mixture of 2 parts 97% $^{18}0$-enriched water and 1 part 99.9% deuterium-enriched water made isotonic with sodium chloride. Subsequent 2-mL heparinized blood samples were collected 6 hours and 4 days after the $D_2^{18}0$ injection. The syringe used for $D_2^{18}0$ injection was weighed immediately before and after the injection for accurate quantification of dose volume. The 2-pool model was used to calculate EE [26].

Analyses were performed by Metabolic Solutions (Nashua, NH). Rates of CO_2 production were calculated using the following equation: CO_2 production (mol/h) = $(N/2.196) \times [(1.007 \times k^{18}O) - (1.041 \times k^{2}H)]$, where N represents the water pool size (in mol) and was estimated from the average of the dilution of both tracers and where the factors 1.007 and 1.041 account for the slight overestimates of body water determined by the respective tracers.

Rates of total EE (TEE) were calculated using the following equation: TEE (kcal/h) = $[(3.944/RQ) + 1.104] \times CO_2$ production, where RQ represents the respiratory quotient (determined from the dietary composition and equal to 0.86) and CO_2 production is expressed in liters per hour.

2.3. Dual-energy x-ray absorptiometry

Body composition was evaluated using a Lunar Prodigy (GE Lunar, Madison, WI) DXA scanner[27,28] with Lunar Dpx-IQ software using the human pediatric calculations on medium setting. The DXA evaluations partitioned body mass into 3 components: fat mass, lean soft tissue (LST) mass, and bone mineral content. The DXA studies were conducted over a 3-day period at baseline and at the follow-up evaluation. Dogs were anesthetized during the DXA scan protocol. Dual-energy x-ray

absorptiometry also provides a measure of total body mass, and the weights corresponding to the animal's body composition are thus presented separately from scale weights.

Changes in body fat (~9 kcal/g) and LST mass (~1 kcal/g) provide a measure of energy balance [29]. The DXA-measured LST compartment also provides an estimate of "metabolically active" body mass; LST-adjusted EE measurements were calculated as EE/LST for descriptive purposes in the subgroup of dogs with body composition measurements.

2.4. Body weight

Dogs were individually weighed on a calibrated scale, accurate to 0.1 kg, once weekly.

2.5. Metabolic monitoring

Intravenous glucose tolerance test and DXA scans were performed daily on 6 randomly selected dogs (ie, 2 dogs per treatment group) for 3 consecutive days immediately before day –13 and following the conclusion of dosing (end of week 4). The day before the glucose challenge, dogs were fed their daily food ration at 8:00 AM; and food was removed at noon. All dogs received 2 g glucose per kilogram (500 g/L dextrose solution) infused via the cephalic vein catheter over 3 minutes. The end of the infusion was counted as IVGTT time 0. Blood samples (4 mL, EDTA) were obtained from each dog at –30, –15, 0 (immediately before glucose challenge), 5, 30, 45, 60, and 120 minutes relative to glucose infusion. An aliquot of each whole blood sample was immediately tested for glucose concentration using a glucometer. The remaining aliquots of whole blood were kept on ice and centrifuged as soon as possible, and plasma was collected into duplicate 96-well plates and stored at –70°C until the enzyme-linked immunosorbent assay insulin analysis could be performed.

On days 0 and 28, blood samples were collected for analysis of plasma hormones and metabolic markers. The plasma from heparinized blood samples (6 mL) was retained frozen in a 96-well format (ie, 3 aliquots from each blood sample) and later analyzed for triiodothyronine, cortisol, triglycerides, cholesterol, and blood urea nitrogen concentrations. An additional blood sample (2 mL, EDTA) was collected for immediate determination of hemoglobin A_{1c}.

2.6. Statistical methods

Statistics were run on CMG StatServer (Version 2.93, in-house software) using 2-tailed *t* tests with a *P* value of .05 as the threshold for statistical significance; *P* values greater than .05 were considered nonsignificant. Results are expressed as mean ± standard error of the mean in the text and figures.

3. Results

Animals were scored for loose stools, diarrhea, and emesis as well as behavioral effects. There was no change in the end points or overall health status of any dog, and activity patterns were similar across the 3 treatment groups throughout the entire 4-week treatment period. No behavioral changes were observed in any of the dogs in this study. Previous unpublished observations from our group indicate that gastrointestinal events occur at doses higher than were used in this study.

3.1. Body composition

Mean baseline body mass ± standard errors were similar across the 3 treatment groups (Table 1). Data in Fig. 1 are presented as the absolute change (grams) from baseline. Change from baseline at week 4 was significantly greater in the AM251 group (–1476.7 g, $P < .050$) and in the FR group (–1100.0 g, $P < .050$) than in the control group (-228.3 g).

The treatment groups were generally well balanced at baseline with respect to the percentage of total body mass comprised as fat measured by DXA (ie, 51.0% ± 5.2%, 51.2% ± 2.0%, and 52.9% ± 1.9% for the C, FR, and AM251 treatment groups, respectively; Table 1). Dogs with more than 27.5% body fat are generally considered obese [30]. When absolute changes at week 4 were examined between the groups (Fig. 1), there were a significant decrease in total body weight and fat mass in both the FR and AM251 groups and no change in lean mass for the control group. Loss of fat mass in dogs treated with AM251 was significantly greater than that in the FR group ($P < .05$).

At week 4, the AM251 group showed a significant mean percentage reduction from baseline in total body mass (–11.0% ± 2.0%, $P < .01$ vs baseline). This effect was primarily driven by a significant reduction in fat mass (–9.0% ± 1.8%, $P < .01$ vs baseline) with a nonsignificant ($P > .10$) reduction in LST mass (–2.0% ± 1.0%). The AM251 group had a significantly greater decrease in body fat than the FR group. A similar trend was observed in the decrease of body mass, but the difference between these treatments did not reach significance. The FR group showed a significant mean percentage reduction in total body mass (–6.3% ± 0.6%, $P < .05$ vs baseline) with nonsignificant percentage reductions in fat mass (–4.9% ± 0.5%) and LST mass (–1.4% ± 0.9%). No significant changes from baseline in total body mass (–3.3% ± 0.9%), fat mass (–3.7% ± 0.4%), or LST mass (0.4% ± 1.0%) were observed at week 4 for the C group.

3.2. Body weight by scale

Weight data in Fig. 2 are presented as the percentage change from baseline to allow comparison with cannabinoid inverse

Table 1 – Baseline body composition of dogs evaluated to study the effects of long-term CB1R inhibition on body weight, feed intake, and energy parameters

Diet/treat	Body weight (kg)	Fat mass (kg)	Lean mass (kg)	Bone mineral composition (kg)
Maintenance diet/control	18.6 ± 0.5	9.26 ± 0.59	9.21 ± 0.40	0.40 ± 0.02
Food restricted	18.6 ± 0.6	9.41 ± 0.39	8.94 ± 0.22	0.39 ± 0.01
Maintenance diet/AM251	18.7 ± 0.5	9.71 ± 0.34	8.63 ± 0.31	0.41 ± 0.02

Data are shown as mean ± standard error.

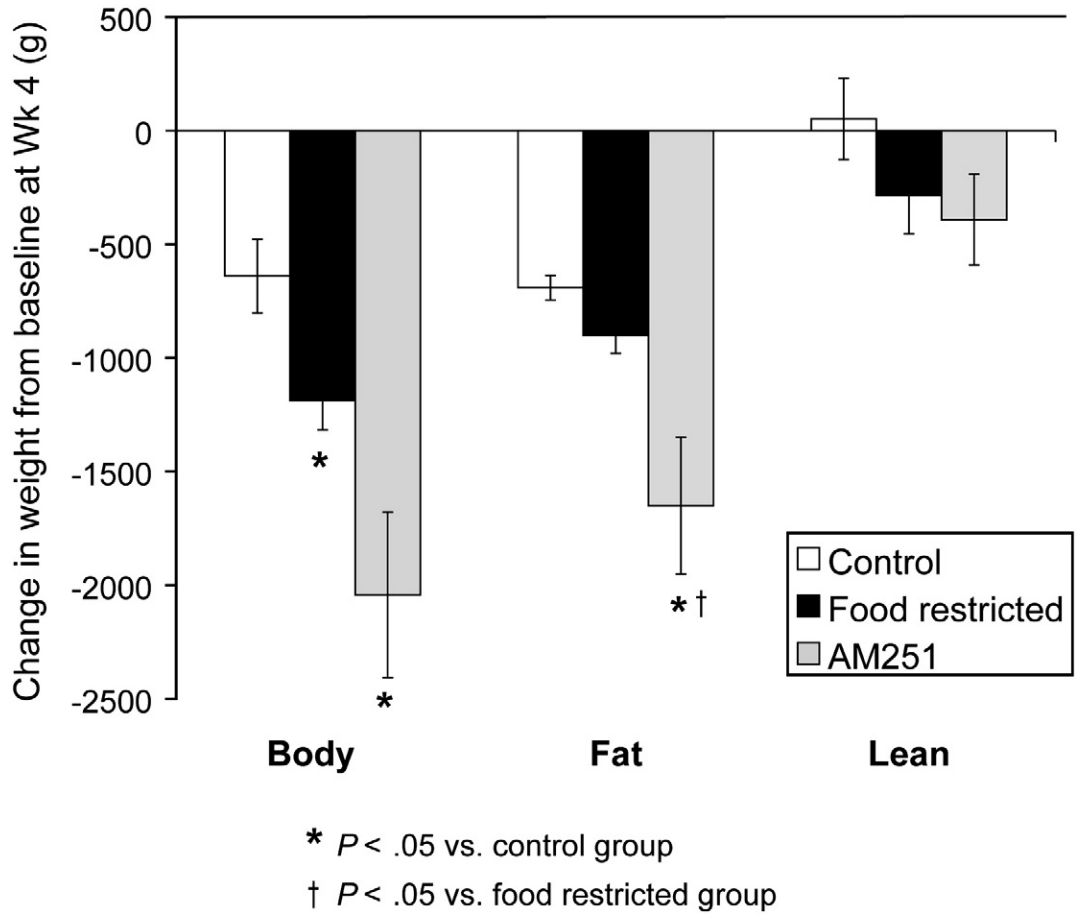


Fig. 1 – Change from baseline in total body mass, fat mass, and lean mass at week 4 by treatment group. Results are expressed as mean change (grams) ± standard error of the mean. Statistical comparisons are for the difference between groups observed at week 4.

agonist effects across species. Small percentage reductions from baseline body weight (0.7%-1.3%) were observed in the C group throughout the 4-week treatment period (Fig. 2). In contrast, the FR and AM251 treatment groups showed similar progressive reductions from baseline in body weight throughout the duration of the study (2.0% to 5.9% for FR and 2.2% to 7.9% for the AM251 group). For both of these groups, the reductions from baseline body weight reached statistical significance at weeks 3 and 4 ($P \leq .050$ vs baseline for both treatment groups). At week 4, the AM251 group demonstrated the largest reduction from baseline body weight (7.9% ± 1.9%, $P < .001$) followed by the FR (5.9% ± 1.1%, $P < .001$) and the C groups (1.3% ± 0.5%).

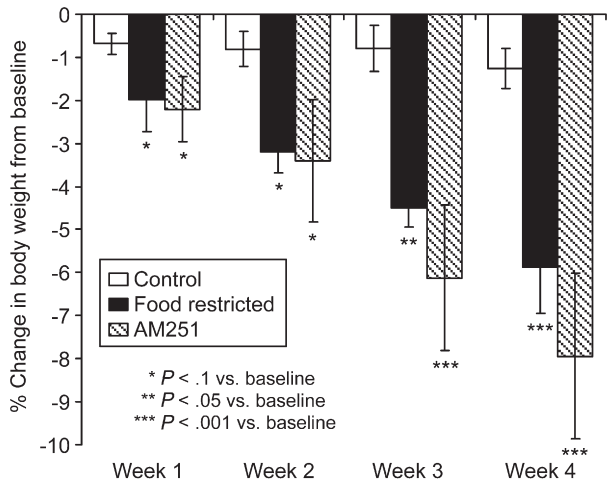


Fig. 2 – Change from baseline in body weight determined by calibrated scales each week, expressed as a percentage of initial body weight, presented by treatment group. Results are expressed as mean percent ± standard error of the mean. Statistical comparisons are within group vs baseline.

3.3. *Food intake*

To allow comparison of results with other species and because each dog was maintained on a different amount of food that was specific to that dog's need for weight maintenance, FI changes are presented as percentages (Fig. 3). At week 1, a significant reduction in FI was observed in the FR group (ie, 70% maintenance group; $P < .050$ vs baseline), whereas smaller, nonsignificant reductions were seen in the AM251-treated dogs ($P > .10$ vs baseline and FR group). Significant reductions from baseline in FI were observed for both the FR and AM251 treatment groups beginning at week 2 and at each week thereafter ($P \leq .050$ vs baseline for both treatment groups

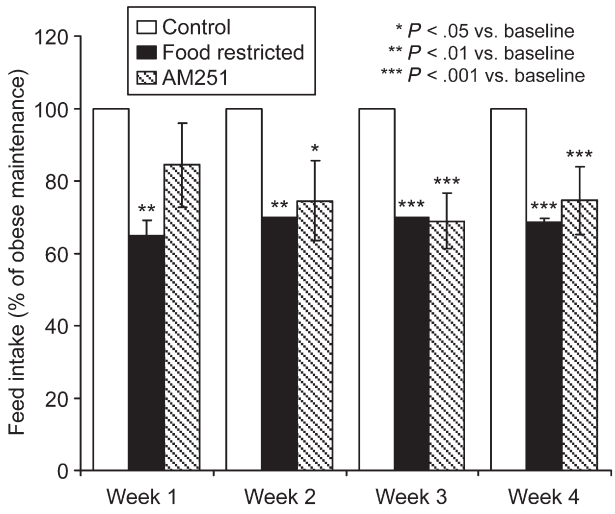
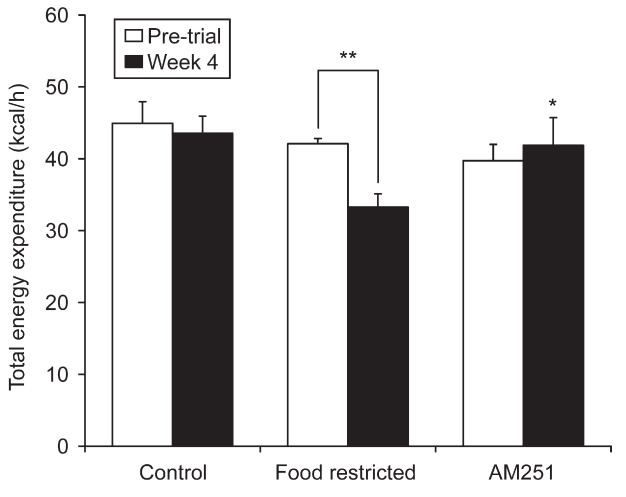


Fig. 3 – Percentage change from baseline in FI at each week presented by treatment group. Results are expressed as mean percentage change ± standard error of the mean. Statistical comparisons are within group vs baseline.



*Change from predose energy expenditure significantly different vs. food restricted group ($P < .05$)

** Week 4 energy expenditure significantly different vs. Pre-trial ($P < .01$)

Fig. 4 – Total EE at baseline (ie, week 0) and week 4, expressed in kilocalories per hour, presented by treatment group. Results are expressed as absolute mean TEE ± standard error of the mean.

at weeks 2-4; P = not significant for FR vs AM251 group) (Fig. 3). The magnitudes of the reductions in FI for these 2 groups averaged approximately 70% relative to baseline (ie, pretrial 100% maintenance feeding) and were stable over time. No significant changes from baseline in FI were observed for the C group (ie, 100% maintenance group; $P > .10$ vs baseline at weeks 1-4).

3.4. *Energy expenditure*

Total body water for all groups at the start and finish of the study is shown in Table 2. No changes were observed except for the FR group. After 4 weeks of dosing, the FR group has both decreased fractional turnover and pool size. When calculating TEE, the C group reduced EE from a baseline of 44.9 ± 3.0 kcal/h to 43.6 ± 2.4 kcal/h at week 4, resulting in a nonsignificant mean percentage reduction from baseline of 2.9% (Fig. 4). Similarly, the C group showed a numerical reduction in EE relative to LST from 4.9 ± 0.4 kcal/kg at baseline to 4.7 ± 0.2 kcal/kg at week 4, resulting in a nonsignificant mean percentage reduction from baseline of 4.1%.

The FR group showed a significant reduction in EE of 20.9% at week 4 ($P < .01$ vs baseline), calculated from a mean value of 42.1 ± 0.7 kcal/h at baseline and 33.3 ± 1.8 kcal/h at week 4 (Fig. 4). A significant reduction in EE relative to LST (4.9 ± 0.2 at baseline to 3.9 ± 0.2 kcal/kg LST per hour at week 4, –20.4%; $P < .01$) also was seen in this group.

By contrast, a numerical increase in EE of 5.5% (39.7 ± 2.3 at baseline to 41.9 ± 3.9 kcal/h at week 4) was observed in the AM251 group at week 4 (Fig. 4). Energy expenditure also increased in this group relative to LST by 6.3% (4.8 ± 0.3 to 5.1 ± 0.6 kcal/kg LST per hour). Absolute or lean mass-adjusted EE was significantly greater ($P < .05$) in the AM251 group at week 4 compared with the FR group.

Table 2 – EE parameters

Group	K_O (%/h)	K_H (%/h)	TBW		
			N_O (mol)	N_H (mol)	Mean (mol)
Predose					
Control	0.770 ± 0.042	0.570 ± 0.045	423.2 ± 14.4	430.1 ± 13.3	426.7 ± 13.8
Food restricted	0.680 ± 0.039	0.488 ± 0.034	409.0 ± 7.1	415.2 ± 8.1	412.1 ± 7.6
AM251	0.675 ± 0.048	0.491 ± 0.039	403.3 ± 6.1	410.4 ± 4.6	406.9 ± 5.1
Postdose					
Control	0.728 ± 0.032	0.535 ± 0.032	417.1 ± 15.5	431.5 ± 15.7	424.3 ± 15.5
Food restricted	0.598 ± 0.017 *	0.435 ± 0.018 *	375.4 ± 7.1 *	390.9 ± 8.5 *	383.1 ± 7.8 *
AM251	0.727 ± 0.084	0.525 ± 0.065	383.0 ± 5.0	398.3 ± 4.2	390.7 ± 4.4

Data are shown as mean ± standard error. K_H indicates elimination constant of ^2H water; K_O, elimination constant of ^{18}O; N_H, pools of water as estimated from the initial dilution of the ^2H water; N_O, pools of water as estimated from the initial dilution of the ^{18}O; TBW, the total body water, pool size estimated from the average dilution observed for ^{18}O and ^2H water.

* $P < .05$ from control group within dosing period.

3.5. *Circulating metabolic end points*

At week 4, plasma levels of triiodothyronine (nanograms per milliliter) were unchanged from baseline across the 3 treatment groups (baseline and week 4 values, respectively, were 1.06 ± 0.07 and 1.22 ± 0.09 for the C group, 1.06 ± 0.06 and 1.09 ± 0.03 for the FR group, and 1.18 ± 0.07 and 1.13 ± 0.06 for the AM251 group). In addition, no significant within-group changes in cortisol, total cholesterol, triglycerides, fasting glucose, insulin, hemoglobin A_{1c}, and IVGTT parameters (ie, glucose, insulin $AUC_{0\text{-}120\ min}$, and insulin sensitivity) were observed at week 4 (data not shown). Significant reductions from baseline in blood urea nitrogen ($P < .05$) were observed for both the FR and AM251 groups ($P < .050$ vs baseline for both treatment groups), whereas no significant within-group change was seen in the C group at week 4.

4. Discussion

In the current study, pharmacologic inhibition of CB1R using AM251 in obese dogs led to gradual weight loss and a decrease in FI over 4 weeks. Whereas AM251-treated and control dogs showed no change from baseline in EE, a significant reduction from baseline in EE was observed in the FR group. Furthermore, a significant between-group reduction in EE was observed for dogs treated with AM251 vs control dogs fed approximately the same amount of chow, even after adjusting for lean mass, demonstrating the role of CB1R not only in suppression of FI but also in driving EE.

Because AM251-treated dogs had FI values similar to those for dogs in the FR group, the observed difference in adiposity between these groups at week 4 was likely due to differences in EE brought about by inhibition of CB1R. Diet restriction alone led to a total weight loss of approximately 6 kg compared with a weight loss of approximately 8 kg in the AM251 group. Although the difference in mean body weight loss was not significantly different between these 2 groups, given that the loss in adiposity of the AM251 group was larger than that of the FR group and the different responses of EE between the 2 groups, we anticipate that continued dosing would further augment the body weight difference between these 2 groups. Thus, given that there were no noted differences in physical activity, the effect of CB1R inhibition on EE accounted for roughly 25% of the total weight loss observed in dogs treated with AM251.

Previously published studies support the observation that inhibition of CB1R elicits increases in the facultative component of metabolic rate in both animal models and clinical trials. Liu et al [18] reported increased levels of hypothalamic endocannabinoids in genetically obese rodents. These authors investigated the effects of 7 days of rimonabant treatment (10 mg/kg by intraperitoneal injection) on EE in 8- to 10-week-old Lep^{ob}/Lep^{Ob} mice. Compared with vehicle-treated control animals, treatment with rimonabant significantly reduced FI and body weight on days 5 and 6 of dosing; however, no significant between-group difference in FI was observed on day 7. Relative to baseline, rimonabant increased oxygen consumption at approximately 30 minutes compared with vehicle-treated mice over a 180-minute evaluation period, although no significant between-group difference was observed. By contrast, at day 7, treatment with rimonabant significantly increased mean oxygen consumption compared with that seen in vehicle-treated control mice over a 90-minute evaluation interval. Based on an earlier report of an in vivo microdialysis study showing that rimonabant increases hypothalamic noradrenaline outflow in a dose-dependent manner [31], Liu et al [18] hypothesized that CB1R inhibition increases central efferent outflow to an unspecified target organ or tissue.

In 2007, Herling et al [16] reported the acute effects of AVE1625, a CB1R antagonist, on energy balance in male Wistar rats. AVE1625 (30 mg/kg) or vehicle was administered to postprandial rats by oral gavage at the beginning of the light phase, which resulted in a marked reduction in FI without effects on locomotion. The effect of CB1R inhibition on FI was tested by repeating the study in rats that were restricted from eating for 6 hours following treatment with AVE1625 or vehicle. Marked increases in both EE and lipid oxidation were seen in the active treatment group.

In a follow up report in 2008, Herling et al [14] compared the effects of treatment with rimonabant (10 mg/kg) over 6 weeks in candy-fed female Wistar rats and pair-fed controls. Rimonabant-treated animals lost more weight compared with pair-fed controls, and the magnitude of the between-group weight loss was maintained over the course of the study; weight loss with active treatment exceeded that of pair-fed controls after about 2 weeks. Food intake was initially reduced in the rimonabant group, most notably among rats fed a candy diet; but over time, FI approximated that seen in control animals fed ad libitum. Total EE and lipid oxidation rates increased during the first few days of active treatment, and these metabolic effects were maintained across the 6-week treatment period. By contrast, pair-fed rats showed an adaptive lowering from baseline in the EE rate. The findings reported in our article extend the observations of Herling et al [14] to obese dogs. Although treatment with AM251 had an effective increase of facultative metabolic rate in obese dogs relative to comparably food-restricted dogs, FI in actively treated dogs remained suppressed; and weight loss continued out to the 4-week time point. The gradual, continuous decrease in body weight observed over time in AM251-treated obese dogs is more like the pattern of weight loss seen in humans treated long term with rimonabant or taranabant [20,21] relative to that observed in rodent animals following CB1R inhibition.

The acute effects of rimonabant on EE in lean male Sprague-Dawley rats and $CB1R^{-/-}$ and $CB1R^{+/+}$ mice were reported by Kunz et al [17] in 2008. Comparing wild-type mice with controls, a single oral dose of drug (10 mg/kg) in the postabsorptive state significantly increased oxygen consumption ($P < .0005$ vs control) and physical activity ($P < .005$ vs control) at 3 hours. A second rimonabant dose administered to fasting rats after 9 hours had no effect on either oxygen consumption or physical activity. The changes in physical activity with active drug treatment could not fully explain the observed changes in oxygen consumption. Corresponding studies showed similar effects of active treatment on oxygen consumption and physical

activity in wild-type mice but not in CB1R null mice. The observations of Kunz et al [17] implicate CB1R as the underlying mechanism responsible for mediating rimonabant thermic effects.

Dogs are a species that better reflect basal metabolic rate and metabolism of humans than do rodents [22]; and thus, studies in dogs appear to provide a better prediction of human efficacy than those in rodents. Richey et al [32] in 2009 examined body weight, FI, and resting metabolic rate over 16 weeks during high-calorie feeding of adult male mongrel dogs as part of a study exploring the effects of low-dose rimonabant on visceral and subcutaneous fat. A significant reduction in FI was observed in actively treated animals by week 2, with a return of FI to control levels thereafter. Body weight loss paralleled the reduction in FI at week 2, and weight loss was then maintained up to 16 weeks. Dogs in the placebo group gained weight (6.2% vs −2.5% at week 16 with active treatment). Resting metabolic rate remained unchanged across the 16 weeks in both groups of dogs, although there was no control group for comparison that had weight loss in the absence of CB1R inhibition. By contrast, in the current study, we observed a longer duration of FI and a greater body weight loss as well as a metabolic rate effect. Dogs in the current study were not overfed during the evaluation period and were treated with AM251, not rimonabant. As we observed continued FI suppression as well as a sustained effect in metabolic rate, the dose of AM251 used in our study provides greater efficacy than that of rimonabant. This is most likely due to the differences in feeding regimens in the experimental paradigms of our study vs that of Richey et al [32].

The most recent study, reported by Verty et al [19] in 2009, examined rimonabant (10 mg/kg intraperitoneally) over 21 days in rats surgically implanted with a biotelemetry system that measured interscapular BAT temperature as a biomarker of BAT thermogenesis. Three-week rimonabant administration significantly reduced body weight over the entire treatment period even though there was only a transient decrease in FI. The BAT temperature increased markedly with active treatment throughout the duration of the study along with a corresponding increase in uncoupling protein (UCP1) activity. Selective sympathetic denervation attenuated rimonabant-induced BAT temperature elevation and the magnitude of weight loss, suggesting a key role of CNS endocannabinoids in mediating peripheral thermogenic effects. Weight loss with BAT and UCP1 induction also has been reported in beagles treated with a $\beta 3$ adrenergic receptor agonist [33], and the presence of BAT is now recognized in adult humans [34]. Other sympathetically mediated thermogenic effects may also be relevant in humans [35].

Studies with the CB1R inverse agonist taranabant also demonstrate significant reductions in FI (22% over 24 hours, $P < .001$ vs placebo) and increases in EE (6% over 2-5 hours after dosing, $P = .011$ vs placebo) following the administration of a single 12-mg dose in humans. The postdose increase in EE was accompanied by a lowering in the respiratory quotient ($P = .023$) and thus a rise in the rate of lipid oxidation, an effect comparable to that reported for rimonabant in the rodent studies of Herling et al [14,16].

5. Conclusions

In the present study, 4-week treatment of obese dogs with AM251 led to weight loss through sustained effects on both FI and EE. Drug-treated animals demonstrated greater weight loss than could be accounted for by the restriction of FI alone, likely partially accounted for by CB1R-mediated maintenance of facultative metabolic rate in the face of decreased energy intake, a circumstance that is normally associated with a reflexive decrease in EE. This study extends the observation of CB1R's regulation of EE from rodents to dogs. Caution to avoid behavioral effects known to exist in rodents at high doses when working in a higher species and the obvious limitation of studies with large animals kept us to the current dose selection. This is probably the reason we did not observe changes in insulin, glucose, or insulin sensitivity that have been observed in rodents. AM251, a close analog of rimonabant, will not provide greater advantages as a drug therapeutic than rimonabant itself; yet as this article shows, it serves as a valuable tool for furthering our understanding of the metabolic pathways to which CB1R contributes. Although it is unlikely that central CB1R inhibition will advance as a target for human weight control due to mechanism-related psychological effects [20,36] in animal models, greater elucidation of the CB1R mechanism and methodologies reported herein may yield new insights into the control of energy balance.

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Conflict of Interest

Authors Alison Strack, Susan Nicolich, Joana Achanfuo-Yeboah, Paul K. Cunningham, Donald Hora Jr, Donald Thompson, Gerry Hickey, Terry Faidley, Amy O. Johnson-Levonas, Tung M. Fong, and Steven B. Heymsfield are or were employees of Merck & Co, Inc, and may own stock or have stock options in this company.

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