The association among body fat accumulation, insulin resistance, and other metabolic disorders, including type 2 diabetes, is well documented (3, 13, 19, 26). Obesity is a known risk factor for cardiovascular diseases, cancer, insulin resistance, and the development of type 2 diabetes, this latter being the fifth leading cause of death in the United States (American Diabetes Association) and a growing problem worldwide (46). Of recent interest in this regard is the cannabinoid system and its role in the regulation of metabolism, body weight, and energy expenditure (8, 33).

One of the compounds that can affect obesity is rimonabant, a selective type 1 cannabinoid receptor (CB1R) antagonist (7, 34, 39). Rimonabant has been reported to decrease appetite and body weight, although the former effect seems to be transient and might not explain the sustained body weight loss (9, 11, 35, 36).

Studies conducted in rodents have shown that rimonabant decreases adiposity in different regions of the body. Obese mice treated with rimonabant decreased epididymal, perirenal, and lumbar fat depots (36) after 40 days of treatment. Similarly, rats treated with rimonabant for 10–14 days showed a decrease in total body fat (9) and subcutaneous, retroperitoneal, mesenteric, and epididymal fat (11). A recent report has demonstrated a significant reduction in visceral fat in mice treated with rimonabant for 6 wk concomitant with a candy diet (16). However, what constitutes true visceral fat in the mouse model remains to be clearly identified.

To date, human studies testing the effect of rimonabant on obesity have demonstrated a significant reduction in waist circumference (7, 34, 43), which serves as a surrogate of visceral adiposity. Increased fat accumulation in the abdomen, particularly visceral adiposity, has been associated with a variety of metabolic abnormalities, including insulin resistance and cardiovascular diseases (4, 14, 30, 42, 44). Interestingly, dogs, unlike rodents, have the typical abdominal fat compartments seen in humans, i.e., clearly defined as visceral and subcutaneous fat depots. These compartments are enhanced in dogs after a prolonged high-fat diet (24, 25) and are associated with the canine metabolic syndrome. Thus, it is of interest to study the effects of a cannabinoid antagonist in an animal model with fat compartments similar to humans to gain insight into possible therapies for fat deposition.

In this study, we monitored the effect of rimonabant treatment on fat distribution in the abdominal trunk of dogs maintained on a high-fat diet. In addition, because obesity results from an imbalance between caloric intake and energy expenditure, and resting metabolic rate is the major component of energy expenditure, we examined whether resting metabolic rate contributes to the body weight loss induced by rimonabant.

**MATERIALS AND METHODS**

**Animals.** A total of 20 male adult mongrel dogs were included in this study. Animals were housed in kennels at the vivarium of the Keck School of Medicine at the University of Southern California. This study was approved by the ethics committee of the University of Southern California.

**Induction of fat accumulation.** Dogs were initially maintained on a standard diet for a 3-wk acclimation period (3,885 kcal/day, 39.4% carbohydrates, 33.2% fat, 27.4% proteins). Food was presented from 0900 to 1200 daily. To induce fat accumulation, we switched from the standard diet to a hypercaloric high-fat diet (HHFD) that consisted of

---

Rimonabant prevents additional accumulation of visceral and subcutaneous fat during high-fat feeding in dogs

Joyce M. Richey,* Orison O. Woolcott,* Darko Stefanovski, L. Nicole Harrison, Dan Zheng, Maya Lottati, Isabel R. Hsu, Stella P. Kim, Morvarid Kabir, Karyn J. Catalano, Jenny D. Chiu, Viorica Ionut, Cathryn Kolka, Vahe Mooradian, and Richard N. Bergman

Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California

Submitted 8 December 2008; accepted in final form 10 April 2009


First published April 14, 2009; doi:10.1152/ajpendo.90972.2008.—We investigated whether rimonabant, a type 1 cannabinoid receptor antagonist, reduces visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) in dogs maintained on a hypercaloric high-fat diet (HHFD). To determine whether energy expenditure contributed to body weight changes, we also calculated resting metabolic rate. Twenty male dogs received either rimonabant (1.25 mg·kg⁻¹·day⁻¹, orally; n = 11) or placebo (n = 9) for 16 wk, concomitant with a HHFD. VAT, SAT, and nonfat tissue were measured by magnetic resonance imaging. Resting metabolic rate was assessed by indirect calorimetry. By week 16 of treatment, rimonabant dogs lost 2.5% of their body weight (P = 0.029), whereas in placebo dogs body weight increased by 6.2% (P < 0.001). Rimonabant reduced food intake (P = 0.027), concomitant with a reduction of SAT by 19.5% (P < 0.001). In contrast with the VAT increase with placebo (P < 0.01), VAT did not change with rimonabant. Nonfat tissue remained unchanged in both groups. Body weight loss was not associated with either resting metabolic rate (r² = 0.24; P = 0.154) or food intake (r² = 0.24; P = 0.166). In conclusion, rimonabant reduced body weight together with a reduction in abdominal fat, mainly because of SAT loss. Body weight changes were not associated with either resting metabolic rate or food intake. The findings provide evidence of a peripheral effect of rimonabant to reduce adiposity and body weight, possibly through a direct effect on adipose tissue.

adipose tissue; body weight; energy expenditure; magnetic resonance; resting metabolic rate

---

The association among body fat accumulation, insulin resistance, and other metabolic disorders, including type 2 diabetes, is well documented (3, 13, 19, 26). Obesity is a known risk factor for cardiovascular diseases, cancer, insulin resistance, and the development of type 2 diabetes, this latter being the fifth leading cause of death in the United States (American Diabetes Association) and a growing problem worldwide (46). Of recent interest in this regard is the cannabinoid system and its role in the regulation of metabolism, body weight, and energy expenditure (8, 33). One of the compounds that can affect obesity is rimonabant, a selective type 1 cannabinoid receptor (CB1R) antagonist (7, 34, 39). Rimonabant has been reported to decrease appetite and body weight, although the former effect seems to be transient and might not explain the sustained body weight loss (9, 11, 35, 36).

Studies conducted in rodents have shown that rimonabant decreases adiposity in different regions of the body. Obese mice treated with rimonabant decreased epididymal, perirenal, and lumbar fat depots (36) after 40 days of treatment. Similarly, rats treated with rimonabant for 10–14 days showed a decrease in total body fat (9) and subcutaneous, retroperitoneal, mesenteric, and epididymal fat (11). A recent report has demonstrated a significant reduction in visceral fat in mice treated with rimonabant for 6 wk concomitant with a candy diet (16). However, what constitutes true visceral fat in the mouse model remains to be clearly identified.

To date, human studies testing the effect of rimonabant on obesity have demonstrated a significant reduction in waist circumference (7, 34, 43), which serves as a surrogate of visceral adiposity. Increased fat accumulation in the abdomen, particularly visceral adiposity, has been associated with a variety of metabolic abnormalities, including insulin resistance and cardiovascular diseases (4, 14, 30, 42, 44). Interestingly, dogs, unlike rodents, have the typical abdominal fat compartments seen in humans, i.e., clearly defined as visceral and subcutaneous fat depots. These compartments are enhanced in dogs after a prolonged high-fat diet (24, 25) and are associated with the canine metabolic syndrome. Thus, it is of interest to study the effects of a cannabinoid antagonist in an animal model with fat compartments similar to humans to gain insight into possible therapies for fat deposition.

In this study, we monitored the effect of rimonabant treatment on fat distribution in the abdominal trunk of dogs maintained on a high-fat diet. In addition, because obesity results from an imbalance between caloric intake and energy expenditure, and resting metabolic rate is the major component of energy expenditure, we examined whether resting metabolic rate contributes to the body weight loss induced by rimonabant.

**MATERIALS AND METHODS**

**Animals.** A total of 20 male adult mongrel dogs were included in this study. Animals were housed in kennels at the vivarium of the Keck School of Medicine at the University of Southern California. This study was approved by the ethics committee of the University of Southern California.

**Induction of fat accumulation.** Dogs were initially maintained on a standard diet for a 3-wk acclimation period (3,885 kcal/day, 39.4% carbohydrates, 33.2% fat, 27.4% proteins). Food was presented from 0900 to 1200 daily. To induce fat accumulation, we switched from the standard diet to a hypercaloric high-fat diet (HHFD) that consisted of...
Rimonabant treatment. After the initial fat feeding period of rimonabant (n = 11) or placebo (n = 9), animals were randomly segregated into two groups. Animals were matched for body weight (31.7 ± 1.3 and 31.8 ± 1.5 kg; rimonabant vs. placebo group, respectively). Rimonabant (Sanofi-aventis, Paris, France) was encapsulated (AMC and 31.8/H11006 (area of 226/H11003 and 31.8/H11006 (area of 226/H11003 in supine position with the front legs flexed and the hind legs extended. The scanning consisted of 30 contiguous slices 1-cm thick (longitudinal relaxation time T1-weighted, repetition time = 500 ms, echo time = 14.0 ms), placing the first slice at the level of the junction between the inferior limb and the trunk. In an attempt to get comparable repeated measurements along the study, the left renal hilum was chosen as a landmark for all sessions. The analysis of the data was based on 11 consecutive slices, 5 upper and 5 below the landmark and the slice at the level of the landmark. Body composition in the abdominal trunk was divided in the following five categories: visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), total fat (VAT + SAT), nonfat tissue, and total tissue (total fat + nonfat tissue). Images were retrieved to a personal computer with eFILM Lite software (versions 2.0.1 and 2.1.2; Merge Healthcare, Milwaukee, WI). Every image was analyzed using the software Scion Image for Windows (Alpha 4.0.3.2.; Scion, Frederick, MD) to differentiate fat from nonfat tissue based on pixel intensity as previously described (25). VAT was defined as the fat located in the intra- and retroperitoneal region, and this region was separated from the SAT by manual trace. For each slice, the area was calculated in square centimeters (area of 226 × 256 pixels equivalent to 34.9 × 34.9 cm²) and multiplied by the thickness of the slice to obtain the volume. The total volume of each component of the trunk was obtained by summing the partial volumes from each slice.

Resting metabolic rate. In a subset of dogs (rimonabant, n = 7; placebo, n = 5), we assessed resting metabolic rate by indirect calorimetry. The calorimetric chamber used consisted of a standard dog kennel adapted for the experiments. All dogs were acclimated to the chamber 3 wk before the initiation of the protocol. Dogs were fasted for at least 12 h before each experiment. We assessed resting metabolic rate at weeks 0, 2, 6, 12, and 16. For each weekly session, the recordings were obtained between 0630 and 1030 over a period of 2 days, randomly assigning six dogs to each day. The gas analyzer module VacuMed Vista MX (VacuMed, Ventura, CA) was calibrated before each experiment, which consisted of 25 min, with recordings every 30 s. Data were analyzed using TurboFit software (VacuMed), and the resting metabolic rate was calculated using the modified Weir’s equation: resting metabolic rate = 1.44(3.9 × VO₂ + 1.1 × VCO₂), where VO₂ is the oxygen consumed and VCO₂ the CO₂ exhaled (45). For a given animal, we considered as an accurate value for resting metabolic rate when six consecutive measurements of the levels of CO₂ in the chamber and the resting metabolic rate were within 3% difference of each other.

Statistical analysis. All data were expressed as means ± SE. Comparison of means within groups were performed using the repeated-measures one-way ANOVA test, with Tukey’s test as a post hoc analysis. Nonpaired t-test was used to compare means between groups. General linear model was used to determine multiple correlation (r) between variables measured repeatedly throughout the study. All of the analyses were done using Statistics (StatSoft, Tulsa, OK). Differences were assumed to be significant if the P value was <0.05.

RESULTS

Initial induction of fat accumulation (weeks −6 to 0). The initial 6 wk of HHFD resulted in a 7.1% increase in body weight (n = 20) from 29.6 ± 0.8 to 31.8 ± 1.0 kg (P < 0.001). These changes were accompanied by a 35.8% increase in total fat in the abdominal trunk (733.3 ± 58.5 to 995.5 ± 90.8 cm³, P < 0.001) with no changes in nonfat tissue (P = 0.166). There were increases in both VAT (28.1%, P < 0.001) and SAT (66.8%, P < 0.001), with a predominance of SAT accumulation in absolute numbers (145.7 ± 32.0 vs. 116.6 ± 21.0 cm³, SAT and VAT, respectively) (Fig. 1). The fat percentage of the total tissue increased from 24.9 to 31.5% (P < 0.001).

Effect of rimonabant on body weight and food intake (weeks 0 to 16). By week 16 of treatment and continued HHFD, rimonabant treated dogs lost 2.8% of their body weight,
whereas placebo dogs increased their body weight by 6.5%. However, body weight was not different between groups \((P = 0.156)\). In the rimonabant group, we observed transient decreases in food intake, at week 2 \((P < 0.001)\) and at week 16 \((P = 0.027)\). Placebo group showed no changes in food intake throughout the study. When we normalized food intake to body weight, we found a significant difference between groups only at week 2 \((65.1 \pm 4.1 \text{ vs. } 81.2 \pm 3.0 \text{ kcal-kg}^{-1}\text{-day}^{-1})\), rimonabant vs. placebo, respectively; \(P = 0.007\) (Table 1).

**Effect of rimonabant on the abdominal fat composition.** Fat changes in the abdominal trunk from representative dogs in each group are shown in Fig. 2. The body weight loss seen in the rimonabant group was consistent throughout the study, and occurred concomitant with a loss of total fat in the abdominal trunk. Interestingly, VAT remained unchanged in the rimonabant group, whereas SAT decreased consistently from week 6 to the end of the study. Conversely, the placebo group showed a progressive increase in total fat, including VAT and SAT, reaching significant changes in the last 4 wk of the study (Table 1). Trends of total fat, VAT, and SAT changes in both groups throughout the study are shown in Fig. 3.

At the end of the study, total fat, VAT, and SAT, but not nonfat tissue, expressed as percent changes of the baseline (\(\Delta\)), were significantly different between groups (rimonabant vs. placebo, \(\Delta\) total fat: \(-14.0 \text{ vs. } 26.2\%, P < 0.001\); \(\Delta\) VAT: \(-9.8\% \text{ vs. } 26.7\%, P = 0.004\); \(\Delta\) SAT: \(-19.5\% \text{ vs. } 28.8\%, P < 0.001\); \(\Delta\) nonfat tissue: \(5.9\% \text{ vs. } 2.6\%, P = 0.303\)). Although absolute values of VAT and SAT seemed to be higher at week 6 in the rimonabant group, compared with placebo (Fig. 3), they were not statistically different \((P = 0.101 \text{ and } 0.381\), for VAT and SAT, respectively). Importantly, body weight changes were associated with fat tissue and nonfat tissue changes in the rimonabant group \((\text{vs. total fat: } r^2 = 0.43, P = 0.001; \text{vs. VAT: } r^2 = 0.37, P = 0.001; \text{vs. SAT: } r^2 = 0.39, P < 0.001; \text{vs. nonfat tissue: } r^2 = 0.22, P = 0.041)\) and in the placebo group \((\text{vs. total fat: } r^2 = 0.8, P < 0.001; \text{vs. VAT: } r^2 = 0.70, P < 0.001; \text{vs. SAT: } r^2 = 0.72, P < 0.001; \text{vs. nonfat tissue: } r^2 = 0.47, P < 0.001)\). Hence, total fat and nonfat tissue were predictors of body weight changes in the rimonabant group.

**Body weight changes are not associated with either resting metabolic rate or food intake changes.** In a subset of dogs \((n = 12)\), we evaluated the contribution of food intake and resting metabolic rate to body weight changes induced by rimonabant. The dogs treated with rimonabant \((n = 7)\) lost 4.3% of their body weight \((P < 0.001)\), whereas the placebo dogs \((n = 5)\) increased their body weight by 5.9% \((P = 0.003)\). However, the difference in body weight between the groups was not significant (Fig. 4A). Rimonabant reduced food intake only during the first 2 wk of the study \((\text{baseline: } 2,570.9 \pm 185.5 \text{ kcal/day; week 2: } 1,808.4 \pm 152.3 \text{ kcal/day; } P < 0.001\)\). The placebo group showed no changes on food intake at any week. When normalized to body weight, food intake was significantly less in the rimonabant group compared with the placebo group only at week 2 \((60.8 \pm 5.6 \text{ vs. } 81.4 \pm 4.4 \text{ kcal-kg}^{-1}\text{-day}^{-1}, P = 0.022)\) (Fig. 4B).

At the end of the study, rimonabant treatment did not result in changes in resting metabolic rate \((\text{week 0: } 1,618.8 \pm 75.9; \text{ week 16: } 1,414.1 \pm 85.5 \text{ kcal/day; } P = 0.264; \text{repeated-measures ANOVA})\). Likewise, resting metabolic rate remained unchanged in the placebo group \((\text{week 0: } 1,632.9 \pm 63.7; \text{ week 16: } 1,628.5 \pm 139.9 \text{ kcal/day; } P = 0.93)\). When normalized to body weight, resting metabolic rate decreased by 10.2\% \(P = 0.001\).
Moreover, there was no difference in resting metabolic rate between groups at any week (Fig. 4C). We found that body weight changes in the rimonabant group were not associated with either resting metabolic rate ($r^2 = 0.24; P = 0.154$) or food intake ($r^2 = 0.24; P = 0.166$) changes. Furthermore, in the rimonabant group, total fat, VAT, and SAT changes were not associated with resting metabolic rate (vs. total fat: $r^2 = 0.10, P = 0.660$; vs. VAT: $r^2 = 0.08, P = 0.756$; vs. SAT: $r^2 = 0.15, P = 0.420$), but were associated with food intake changes (vs. total fat: $r^2 = 0.41, P = 0.013$; vs. VAT: $r^2 = 0.34, P = 0.044$; vs. SAT: $r^2 = 0.38, P = 0.024$).
DISCUSSION

Excessive abdominal fat is a high risk factor for the development of insulin resistance and type 2 diabetes. Clinical trials have shown a reduction in waist circumference in subjects treated with rimonabant. However, to date, direct evidence showing the effects of rimonabant on visceral and SAT in the abdominal region has not been reported. In the present study, we induced abdominal fat accumulation in lean dogs (an animal model similar to human obesity) using a high-fat hypercaloric diet. We examined the effect of rimonabant treatment on visceral and subcutaneous fat in the abdomen. We also examined the contribution of food intake and energy expenditure to body weight changes induced by rimonabant. The present study confirms previous findings, demonstrating an effect of rimonabant to reduce adiposity and body weight with only a transient reduction in food intake. In addition, we clearly demonstrate that rimonabant prevents additional fat accumulation in the VAT depot while significantly reducing SAT content during the maintenance of a HHFD. These changes in fat depots occurred in the absence of any changes in nonfat tissue. Thus CB1R antagonism has a profound effect to reduce the amount of stored fat in an animal model reminiscent of human obesity.

The decrease in body weight induced by rimonabant was significant after 2 wk of treatment until the end of the study (Fig. 4A). However, the dogs exhibited only a transient decrease in food intake at week 2 (Fig. 4B). This transience in the effect of rimonabant on food intake has been consistently
noted that, in the present study, fat feeding was maintained throughout the treatment period. We have recently hypothesized that fat accumulates initially in the VAT depot and then spills over into the SAT depot, known as the overflow hypothesis (2). Hence, in the presence of continued fat feeding, rimonabant may act to prevent further fat accumulation in the VAT while reducing SAT. These actions of rimonabant on adipose tissue are indicative of direct peripheral effects of rimonabant. Several in vitro studies support a direct effect of rimonabant on adipose tissue. The expression of CB1R has been found in both rodent (1, 6) and human (10, 32, 38) adipocytes. Interestingly, rimonabant has been shown to block the rate of lipogenesis induced by the CB1R agonist WIN-55,212 in primary adipocytes (6) and stop the accumulation of lipid droplets in cultured clonal preadipocytes (12). Whether CB1R expression is found in canine fat tissue is largely unknown. However, we have recently shown that rimonabant treatment prevents the accumulation of large adipocytes in subcutaneous and omental fat in dogs fed a HHFD (17).

It has been reported that increased fat accumulation in adipocytes leads to an increase in lipolysis (47). Although we found an increase in the number of large adipocytes (>75 μm) with fat feeding, we did not observe any differences in basal lipolysis measured in vitro (data not shown). These observations lead us to speculate that there is a threshold in which lipid accumulation in adipocytes must reach to observe changes in lipolytic activity. Consistent with this notion is a recent report in rats that demonstrate no change in basal lipolysis in high-fat-fed rodents at a time when mesenteric and retropitoneal fat pads are significantly increased (5). However, after prolonged periods of fat feeding and consequently increased fat accumulation, differences in lipolytic activity were noted. Moreover, some studies suggest that the antiobesity effects of rimonabant are due to increased lipolysis, yet we observed no differences between the two groups. The molecular mechanism as to how rimonabant prevents visceral fat accumulation and reduces subcutaneous fat content remains to be elucidated. Clearly, the roles of lipogenesis, lipoprotein lipase activity, and reesterification rates must be taken into account. A potential mechanism may be gleaned from mouse studies that demonstrate inhibition of lipid accumulation in adipocytes by blocking preadipocyte proliferation (12).

An alternative mechanism for the reduction in body adiposity and weight loss may be attributed to changes in energy expenditure. Energy expenditure has been shown to be enhanced with rimonabant treatment. Because resting metabolic rate represents the largest component of total energy expenditure (29, 37), we examined whether body weight loss induced by rimonabant was dependent on resting metabolic rate changes. In a subset of dogs, we conducted a study to determine whether increases in energy expenditure could explain the body weight changes. Surprisingly, we found no significant changes on resting metabolic rate during the study (Fig. 4). Moreover, there was no association between resting metabolic rate and body weight changes. These results suggest that other components of total energy expenditure might explain the body weight loss (29) (i.e., physical activity and adaptive thermogenesis). It has been reported that rimonabant increases grooming (18) and scratching activity (18, 28) in rodents. In addition, it has been shown that acute (28) and chronic (15) administration of rimonabant increases total energy expenditure in rats.
Physical activity was not monitored in this study. However, weekly core body temperatures (data not shown) in the dogs remained invariable throughout the study, indirectly suggesting that the body heat production was not altered. Thus the reduction of body weight may be due to changes in spontaneous physical activity.

Clinical studies aimed to determine the effect of other anti-obesity drugs in combination with a low-calorie diet on abdominal fat distribution, either by computed tomography or MRI, demonstrate a decrease in both VAT and SAT compartments. Orlistat, an inhibitor of pancreatic lipase (40), and sibutramine, a blocker of serotonin and norepinephrine re-uptake, (20, 21, 48) were able to significantly reduce abdominal fat content in obese subjects. However, unlike our study, the aforementioned studies are treatment therapies in combination with a low-calorie diet. In the face of continued fat feeding, it may be difficult to see a true reduction in visceral fat, particularly if fat is preferentially deposited in this depot.

It has been reported that overexpression of adiponectin in mice resulted in expansion of SAT and was associated with an improvement of the metabolic profile (22). In addition, it has recently been reported that transplantation of SAT in the VAT depot improved insulin sensitivity in mice (41). Apparently, these results might be in conflict with the fact that the effect of rimonabant was more pronounced in SAT. Although the findings from these studies are interesting, the extrapolation of those results to human obesity is unknown, given the differences in fat distribution and composition between humans and rodents. In fact, if SAT were beneficial, liposuction would significantly impair the metabolic profile in humans that undergo this procedure. For example, some studies have not shown changes in metabolic parameters after 10–12 wk of follow-up (27) or within 1–4 yr of a significant sustained reduction of SAT (31). Together, these human studies suggest that SAT does not have a beneficial effect on metabolic disease.

In conclusion, this study provides the first direct evidence of the effect of rimonabant on fat distribution in the abdominal region in a canine model which parallels fat distribution in humans. Chronic treatment with rimonabant resulted in a reduction of body weight and fat content in the abdominal trunk with no changes in nonfat tissue, suggesting a direct effect of rimonabant on adipocytes. In contrast, with the significant increases of both SAT and VAT in placebo, rimonabant prevented additional VAT accumulation and decreased SAT. The reduction in overall body weight was not associated with either resting metabolic rate or food intake changes. However, an increase in spontaneous physical activity cannot be excluded as a possible mechanism to induce weight loss. These findings provide evidence of a peripheral effect of rimonabant to reduce adiposity and body weight, possibly through a direct effect on adipose tissue.

ACKNOWLEDGMENTS

We are very grateful to radiologist Linda Needham for help with the MRI scans and to Rita Thomas for excellent assistance with the MRI images. We also thank our lead laboratory animal technician Edward Zhuliga and assistant Edgardo Paredes. We give special recognition to Sophia Yae and Justin Dittmann for assistance with experiments. Additionally, we express our extreme gratitude to Dr. Erlinda Kirkman for excellent veterinarian expertise in facilitating these studies.

Parts of this study have previously been presented at the 43rd and 44th Annual Meetings of the European Association for the Study of Diabetes (Amsterdam, 2007 and Rome, 2008; respectively) and at the 67th and 68th Scientific Sessions of the American Diabetes Association (Chicago, 2007 and San Francisco, 2008; respectively).

GRANTS

This work was supported by Sanofi-aventis.

REFERENCES