A Selective Cannabinoid-1 Receptor Antagonist, PF-95453, Reduces Body Weight and Body Fat to a Greater Extent than Pair-Fed Controls in Obese Monkeys


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Abbreviations: CB1, cannabinoid-1 receptor; HDLC, high-density lipoprotein cholesterol; GTT, glucose tolerance test; CRP, C-reactive protein; MCP-1, monocyte chemoattractant protein 1; SEM, standard error of the mean; ELISA, enzyme-linked immunosorbant assay; DIO, diet-induced obese; AUC, area under the curve

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Abstract

Cannabinoid-1 (CB₁) receptor antagonists exhibit pharmacological properties favorable to treatment of obesity, due to both centrally-mediated effects on appetite and peripherally-mediated effects on energy metabolism. However, the relative contribution of these effects to the weight loss produced by CB₁ receptor antagonists remains unclear. Here we compare food intake-related and independent effects of the CB₁-selective antagonist, PF-95453, in obese cynomolgus monkeys. Monkeys were divided into three study groups (n=10 each) and treated once daily for eight weeks with either vehicle or PF-95453 as follows: [1] fed ad libitum and dosed orally with vehicle, [2] fed ad libitum and dosed orally with PF-95453 (0.5mg/kg weeks 1-3, 1.0mg/kg weeks 4-8), [3] fed an amount equal to the amount consumed by the drug-treated group and dosed orally with vehicle (pair-fed). PF-95453 treatment significantly reduced food consumption by 23%, body weight by 10%, body fat by 39%, and leptin by 34% while increasing adiponectin by 78% relative to vehicle-treated controls. Pair-fed animals did not exhibit reductions in body weight or leptin but did show significantly reduced body fat (11%) and increased adiponectin (15%) relative to vehicle-treated controls but were markedly less than after PF-95453 treatment. Indeed, significant differences were noted between the drug-treated and pair-fed groups with respect to body weight reduction, body fat reduction, increased adiponectin, and leptin reduction. Similar to humans, monkeys treated with the CB₁ receptor antagonist exhibited decreased body weight and body fat, a substantial portion of which appeared to be independent of effects on food intake.
Introduction

The mammalian endocannabinoid system has been extensively characterized over the last twenty years and is now known to play a key role in energy homeostasis by modulating both food intake and peripheral energy metabolism (Pagotto et al., 2006; Nogueiras et al. 2008; Osei-Hyiaman et al., 2008; Nogueiras et al. 2009; Cota et al., 2009; Quarta et al. 2010). In general, stimulation of the endocannabinoid system influences metabolic pathways that lead to weight gain, lipogenesis, and impaired glycemic control (Hao et al., 2000; Bensaid et al., 2003; Engeli et al., 2005; Jbilo et al., 2005; Poirier et al., 2005; Matias et al., 2006).

The endocannabinoid system is comprised of transmembrane endocannabinoid receptors, their endogenous ligands (endocannabinoids), the proteins involved in endocannabinoid synthesis and activation, and the intracellular signaling pathways affected by receptor activation (De Petrocellis et al., 2004). To date, two cannabinoid receptors, type-1 (CB₁) and type-2 (CB₂), have been identified (Howlett et al., 2002). Both are members of the G protein-coupled receptor superfamily that modulate down-stream signaling pathways including inhibition of intracellular cyclic AMP accumulation, stimulation of MAP kinase activity, and modulation of potassium and calcium channel activities (Howlett et al., 2002). The CB₁ receptor is highly expressed in the CNS with high densities found in the cerebral cortex, hippocampus, and hypothalamus, but can also be found at much lower yet functionally relevant levels in many peripheral tissues that are involved in energy metabolism (Herkenham et al., 1991; Arnold et al., 2001, Kunos et al., 2008). CB₂ receptors are found primarily in immune and hematopoietic cells (Gallegos et al., 1995, Kunos et al., 2008).

As components of the endocannabinoid system have been identified, pharmacological opportunities to modulate the system and effect therapeutic change have been increasingly explored. The observation that CB₁ receptor antagonists reduce food intake and may be useful as anorectic drugs for the management of obesity and metabolic disease was first made in 1997.
when Arnone and colleagues reported that the CB₁ receptor antagonist, SR141716A (rimonabant) (Rinaldi-Carmona et al., 1994), selectively inhibited sucrose consumption relative to normal chow consumption in male rats (Arnone et al., 1997). Since this observation, rimonabant (DeKloet and Woods 2009; Kunos et al., 2008; Pagotto et al., 2006) and other CB₁ receptor antagonists, such as taranabant (Lin et al., 2006), CP-945598 (Griffith et al., 2009), PF-514273 (Dow et al., 2009) and CE-178253 (Cao et al., 2007), have been used extensively in preclinical studies, primarily in rodents, and in clinical settings to define the role of the endocannabinoid system in appetite behaviors, and more broadly to understand the role of the endocannabinoid system in regulation of energy balance.

However, to date, the relative importance of the effects of these agents on eating behaviors relative to modulation of energy metabolism has not been adequately evaluated. This has been due, in part, to differences in the relative contribution of central versus peripheral CB₁ receptor-mediated mechanisms in old versus young rodents, lean versus obese rodents, and rodents with various defects in the mechanisms controlling energy intake and metabolism (Pagotto et al., 2006), suggesting a need to utilize animals in steady state to adequately conduct the comparison. Such evaluations in rodents have been further complicated by the observation that toleration to the anorectic activity of these agents begins after the first few days of treatment, whereas body weight loss is sustained throughout treatment, suggesting a time-dependent variation in the relative contribution of central versus peripheral actions of CB₁ receptor blockade during prolonged treatment in rodents (Pagotto et al., 2006).

While a comparison of the relative contribution of central versus peripheral actions of CB₁ receptor blockade might be more instructive in humans, the difficulties associated with inter-human variability in the mechanisms controlling eating behaviors together with the inherent difficulties in conducting adequately controlled pair-feeding studies in humans would make interpretation of the results of such studies difficult.
We therefore chose to evaluate the relative contribution of anorectic and food intake-independent effects of a potent and highly selective CB₁ receptor antagonist, PF-95453, in weight-stable, diet-standardized obese cynomolgus monkeys, using a protocol that employed both a vehicle-treated control group and a pair-fed control group to optimize the ability to differentiate differences related to anorectic behaviors from those related to food intake-independent actions of the compound. To our knowledge, this is the first report of a pair-fed, controlled CB₁ receptor antagonist weight loss study in nonhuman primates. In addition, the results of this study confirm the food-intake independent contributions to improvements in cardiometabolic endpoints by CB₁-receptor antagonism in a non-rodent study.
Methods

In vitro and in vivo pharmacology assessments - In vitro competition binding studies, using \[^3\text{H}]\text{SR141716A}\) as radioligand, and in vitro functional assays measuring \[^{35}\text{S}]\text{GTP} \gamma \text{S}\) binding at \(\text{CB}_1\) receptors were conducted in membranes isolated from human embryonic kidney 293 cells or Chinese hamster ovary cells stably transfected with either human \(\text{CB}_1\) or \(\text{CB}_2\) receptors as previously described (Cao et al., 2007). Acute overnight fast-induced refeeding food intake studies were conducted in male Sprague Dawley rats as previously described (Carpino et al., 2006). Indirect calorimetry studies in male Sprague Dawley rats, using an Oxymax indirect calorimeter (Columbus Instruments, Columbus, OH) were conducted as previously described (Hanwood et al., 2003). Weight loss studies were conducted in diet-induced obese Sprague Dawley rats (DIO-rats) obtained from Charles River laboratories (Willmington, DE) that had been fed a high-fat diet for 15 weeks and were a minimum 600 g body weight at study initiation, and in diet-induced obese C57Bl/6J mice (DIO-mice) that had been fed a high-fat diet (45% calories from fat) for 10 weeks prior to use. DIO-rats and DIO-mice were treated once daily with either 0.5% methylcellulose (vehicle) or vehicle containing various concentrations of PF-95453 for four days with individual body weights recorded daily.

Primate protocol - Thirty cynomolgus monkeys (Macaca fascicularis) that were initially identified by their propensity to gain weight on a standard monkey chow diet (Purina LabDiet®, Richmond, IN), were maintained for approximately four years on a semi-synthetic diet to achieve weight-stable obesity prior to study start. This diet was prepared on site and stored at 4°C until use. The diet contained 60% carbohydrate (20% sucrose, 20% fructose, 20% starch), 20% fat, and 20% protein as calories supplied (Wagner et al., 2006). Monkeys were divided equally into three treatment groups (vehicle control group, pair-fed control group, PF-95453 treatment...
group) based on sex (4 females and 6 males per group), body weight, and plasma TG concentrations, as determined from blood samples collected 8 weeks prior to dosing.

The study consisted of two sets of baseline measurements, collected 3 and 6 weeks prior to initiation of dosing, followed by eight weeks of daily dosing with either vehicle (0.5% methylcellulose; vehicle control and pair-fed control groups) or with the CB₁ receptor antagonist, PF-95453, initially at a dose of 0.5 mg/kg/day for the first three weeks of the study and then at a dose of 1.0 mg/kg/day for the remaining five weeks of the study (PF-95453 treatment group). At the conclusion of dosing, all animals were administered vehicle that lacked PF-95453 addition for an additional four weeks to allow drug washout.

All treatment animals were dosed orally with either vehicle or PF-95453 once a day with their afternoon feeding. PF-95453 dosing solution was prepared on a twice weekly basis as follows: PF95453 was weighed, wetted with a minimum amount of the vehicle, and the mixture ground with a mortar and pestle to form a thick paste. PF-95453 was then gradually brought to final concentration by slowly adding vehicle while stirring to ensure a homogeneous suspension. All dosing solutions were stored at 4°C and stirred continuously using magnetic stir plates. The final concentrations of PF-95453 dosing suspensions for the 0.5 mg/kg and 1.0 mg/kg treatments were 10 mg/mL (0.05 mL/kg), 20 mg/mL (0.05 mL/kg), respectively. Each animal in the vehicle control and pair-fed control groups received 0.05 mL/kg of vehicle daily. Vehicle or drug was delivered to each animal by allowing the dose to be absorbed into a piece of fruit, or other palatable treat. All treats were approximately 10-30 kcal.

Daily food allowance for each monkey was calculated as 120 kilocalories per kilogram of body weight. An additional 10% was added to the prescribed amount to compensate for waste. This total amount provided exceeds the daily requirement for an adult animal (80 kcal per kg) in order to allow weight gain. The monkeys were fed twice a day with 1/3 of the total amount provided in the morning between 7-8 a.m. and the remainder fed in the afternoon between 1-2
The larger ration was provided in the afternoon as monkeys generally eat more during the late afternoon and evening hours when personnel are not in the rooms.

Quantitative food assessment was performed every Tuesday through Friday for three weeks prior to dosing in order to establish baseline consumption averages, and then every other week on Tuesday through Friday throughout the remainder of the study (e.g. after 2, 4, and 6 weeks of treatment and after 2 and 4 weeks of washout). Pair-housed monkeys were separated prior to providing afternoon rations and were kept separated through the following day’s morning feeding and diet count. Approximately 1 hour after the morning feeding, any remaining food was removed and weighed. Diet consumption was averaged over the three weeks of baseline. At baseline, all animals consumed approximately 60% of the food that was made available to them (Table 1). Weekly average consumption was calculated for each monkey during treatment and washout periods. During all normal feedings (e.g. when diet consumption was not being determined), monkeys were pair-housed to allow social enrichment.

During the treatment phase of the study, the food made available to the pair-fed group was adjusted after the food consumption assessment in the treatment group. This amount reflected the mean percentage of ration that had been eaten by the drug treatment group during the prior week. Therefore, chronologically, the pair-fed group trailed the vehicle-treated and drug-treated groups by one week.

Monkeys were sedated with ketamine (10-15 mg/kg IM), followed by supplemental maintenance doses (3-5 mg/kg IM) as necessary, at 3 and 6 weeks prior to the start of dosing (baseline), after 3, 5, and 8 weeks of dosing, and then 2 and 4 weeks after cessation of dosing (washout), in order to record body weight and collect blood samples for measurement of clinical chemistries. Whole blood samples in EDTA-treated tubes were placed on ice immediately after collection, centrifuged and the resulting plasma removed and stored at -80°C. For all procedures requiring sedation, monkeys were fed the afternoon meal ~12pm the day before
sedation. They were allowed 2-3 hours to eat then fasted for ~18 hour before sedation. The animals were not dosed on the days of any sample collection.

Glucose tolerance tests (GTTs) were performed six weeks prior to dosing initiation (baseline) and after five weeks of dosing, as described previously (Wagner et al., 2006, 2010). Glucose tolerance testing was coordinated with body weight measurement and blood sample collection to minimize anesthetization procedures. Monkeys were sedated with 10-15 mg/kg ketamine hydrochloride IM and any maintenance dose was 3-5 mg/kg IM. After baseline samples (-5 minute and 0 minutes) were collected, the glucose challenge (750 mg/kg) was given as intravenous 50% dextrose. Blood samples were collected at 5, 10, 20, 30, and 60 minutes post-injection. Samples were processed as described above. Areas under the curve (AUC) for glucose and insulin were determined as were K values for glucose disappearance.

Body composition and percentage body fat were determined in all animals three weeks prior to dosing initiation (baseline) and after eight weeks of dosing, using dual-energy x-ray absorptiometry (DEXA) whole-body scans (Norland XR-46 densitometer, Fort Atkinson, WI). Under ketamine sedation and after collection of all blood samples (see above) animals received atropine 0.04 mg/kg IM and were then intubated, and administered isoflurane anesthesia. DEXA scanning was then performed and analyzed to assess lean and fat body mass.

All experimental procedures involving animals in this study were approved by, and complied with the guidelines of the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences.

**Clinical chemistry analyses** – Total plasma cholesterol, triglyceride and high density lipoprotein cholesterol (HDLC) concentrations were measured by enzymatic techniques as described previously (Wagner et al., 2010). All lipid analyses were performed on an ACE Alera Clinical Chemistry System (Alfa Wasserman, Inc., West Caldwell NJ). Free fatty acid
concentrations were determined by using an enzymatic colorimetric assay (Wako NEFA C kit, Wako Chemicals Inc., Richmond, VA).

Plasma glucose was assayed by enzymatic colorimetric methods using the new glucose UV kit (Roche Diagnostic Systems, Indianapolis, IN). The inter-assay and intra-assay coefficients of variation was <5%. Plasma insulin was determined using Mercodia (Uppsala, Sweden) ELISA kits, with <10% inter-assay and intra-assay coefficients of variation. Plasma C-reactive protein (CRP) and leptin were measured using ELISA kits from ALPCO Diagnostics (Salem, NH), with <10% inter-assay and intra-assay coefficients of variation. Plasma adiponectin was determined using Mercodia (Uppsala, Sweden) ELISA kits, with <10% inter-assay and intra-assay coefficients of variation. Plasma monocyte chemoattractant protein 1 (MCP-1) was measured using ELISA kits from R & D Systems (Minneapolis, MN), with <6% inter-assay and intra-assay coefficients of variation.

**Pharmacokinetic assessments** - Blood collection for pharmacokinetic (PK) analyses were conducted after administration of the initial 0.5 mg/kg dose of PF-95453 on the first day of the drug treatment phase of the study and after administration of the first dose of the 1.0 mg/kg dose-escalation phase of the study (during the fourth week of treatment). Three animals from the PF-95453 treatment group participated in the PK analyses. All animals evaluated received their dose between 8:00-9:00 am the day of the PK analysis. Blood was collected from awake animals via tail-sticks at 2, 4, 6, and 24 hours post-dose. Approximately 200 µl of blood was collected from the tail tip into a capillary tube treated with EDTA. Plasma drug levels were determined by liquid chromatography/tandem mass spectrometry using a PE Sciex API 3000 spectrometer. Plasma free fractions for PF-95453 were determined by equilibrium dialysis, as previously described (Maurer et al., 2005).
Statistical analysis - All results are reported as mean values ± SEM. Statistical analyses were performed using Statistica 6 (StatSoft, Tulsa, OK). Log transformation of variables was performed when normality assumptions were not met (glucose, insulin, leptin, adiponectin, triglycerides, fat mass). Baseline values for all parameters evaluated are the average of two separate measurements 3 weeks and 6 weeks prior to dosing. Intergroup comparisons at baseline were performed using one-way ANOVA. Comparisons of all variables at subsequent time-points were made using repeated measures analysis of variance. GTT parameters at baseline and five weeks of treatment were made by analysis of covariance (ANCOVA), with baseline values serving as covariate. Post hoc tests were performed to identify specific intra- or intergroup differences at each time point when ANOVA for treatment by group interaction variable was p<0.05.
Results

In vitro pharmacology of PF-95453 – PF-95453, 1-[9-(4-chlorophenyl)-8-(2-chlorophenyl)-9H-purin-6-yl]-4-methylaminopiperidine-4-carboxylic acid amide hydrochloride, whose chemical structure is shown in Fig. 1, is a selective, high affinity CB₁ receptor antagonist that inhibits both basal and cannabinoid agonist-mediated CB₁ receptor signaling in vitro and in vivo. PF-95453 exhibits sub-nanomolar potency at human CB₁ receptors in both receptor binding assays (Kᵢ = 0.5 ± 0.06 nM, n = 22) and in assays of functional antagonism (Kᵢ = 0.18 ± 0.04 nM, n = 5). PF-95453 exhibits a >20,000-fold specificity for human CB₁ receptors relative to human CB₂ receptor (Kᵢ > 10,000 nM) in radioligand binding assays. The high specificity of PF-95453 is further underscored by the absence of any significant or meaningful activity against a panel of receptors, enzymes, and ion channels at concentrations of up to 1000 nM.

Preliminary rodent studies - PF-95453 was evaluated in overnight fast-induced re-feeding acute food intake studies in male Sprague-Dawley rats. In this model, PF-95453 exhibited time, dose, and concentration-dependent anorectic activity with an ED₅₀ of 0.9 mg/kg. Based on the concentration effect relationship determined between food intake reduction and unbound plasma drug concentrations, the EC₅₀ for overnight fast-induced re-feeding (0.3 nM) is in close agreement with in vitro Kᵢ values obtained for CB₁ receptor binding and functional antagonism (see above). Indirect calorimetry studies were also performed with the close-in, equi-efficacious N-ethyl analog of PF-95453, CE-178253: 1-[9-(4-chlorophenyl)-8-(2-chlorophenyl)-9H-purin-6-yl]-4-ethylaminopiperidine-4-carboxylic acid amide hydrochloride. CE-178253 acutely stimulated energy expenditure in rats by 29% at 1.0 mg/kg and 39% at 3.0 mg/kg and decreased the respiratory quotient from 0.85 to 0.75 at both doses, indicating a metabolic shift from carbohydrate to fat oxidation.
As a result of modifications in both eating behaviors and energy metabolism, representatives of this series of CB1 receptor antagonists also promoted weight loss in DIO-rats and in DIO-mice. For example, DIO-rats exhibited a 6% reduction in body weight after 4 days of treatment with CE-178253 at an oral dose of 1.0 mg/kg and DIO-mice exhibited 15% and 21% reductions in body weight after 10 days of treatment with CE-178253 at oral doses of 1.0 mg/kg and 3.0 mg/kg.

**Dose selection** - To assist in selecting an oral dose for evaluating the actions of PF-95453 in obese monkeys, we determined the pharmacokinetic properties of PF-95453. When pharmacokinetic parameters were assessed in obese male cynomolgus monkey after a single 1.0 mg/kg intravenous dose (n = 3) and after a single 3.0 mg/kg oral dose (administered in 0.5% aqueous methylcellulose; n = 3), PF-95453 exhibited an oral bioavailability of 49.8%, a Cmax of 990 ng/mL at the Tmax of 3.3 hrs, a half-life of 16.1 hrs, a Clp of 1.1 mL/min/Kg, a Vdiss at steady state of 0.47 mL/kg, and an AUC(0-∞) of 23.9 mg-hr/ml (oral administration).

The principal clearance pathway of PF-95453 in the cynomolgus macaque (and also in the rat and dog) involves a P450-mediated N-dealkylation to the active primary amine, PF-95382: 1-[9-(4-chlorophenyl)-8-(2-chlorophenyl)-9H-purin-6-yl]-4-aminopiperidine-4-carboxylic acid amide hydrochloride. After a single dose oral administration at a dose of 3.0 mg/kg, the concentration of the parent and metabolite were approximately equal 8 hrs after administration. Twenty-four hrs after oral administration, the concentration of the metabolite was approximately 1.5-fold higher than the Cmax of the parent. Although active, the activity of the metabolite as a functional CB1 receptor antagonist was approximately 40-fold lower than that of the parent (0.18 vs. 6.9 nM) and there was no anorectic activity of the metabolite in the overnight fast-induced re-feeding acute food intake studies at doses of up to 10 mg/kg. The metabolite also exhibited a specificity for CB1 receptors in radioligand binding relative to CB2 receptors similar to that of the parent molecule (5.2 nM vs. >10,000 nM).
Based on quantitative pharmacology assessments and the pharmacokinetic properties outlined above, it was determined that once daily oral administration of PF-95453, given at a dose of 0.5 mg/kg in a 0.5% aqueous methylcellulose vehicle, would be sufficient to allow demonstration of efficacy within a 2-week time-frame. The study was therefore conducted as a dose-escalation study with PF-95453 administered for the first three weeks as a once daily oral dose of 0.5 mg/kg and then for five additional weeks as a once daily oral dose of 1.0 mg/kg, both in 0.5% aqueous methylcellulose.

The appropriateness of the dose selection was confirmed by assessing plasma drug levels at various times throughout the study. Plasma drug level determination at various time-points between 0-24 hrs after the initial 0.5 mg/kg dose of the study confirmed the prolonged bioavailability of the compound with essentially equal 2 hr and 24 hr plasma drug concentrations of 52 ng/ml (108 nM) and a 2-fold higher \( C_{\text{max}} \) of 96 ng/ml (199 nM), which occurred at approximately 4 hrs. Based on plasma protein binding of 99.87% for PF-95453, the calculated steady-state free fraction of 0.14 nM is approximately equal to the \( K_i \) for \textit{in vitro} functional CB\textsubscript{1} receptor antagonism. Similarly, the steady-state free fraction of 0.76 nM (based on plasma drug concentrations of 280 ng/ml; 581 nM) obtained between 0-24 hrs after the initial 1.0 mg/kg dose of the study (which occurred immediately after 3 weeks of 0.5 mg/kg/day treatment with PF-95453) was 4.2-fold greater than the \( K_i \) for \textit{in vitro} functional CB\textsubscript{1} receptor antagonism.

**Baseline study parameters** - A comparison of the mean baseline values of the appetite behaviors, body weight and body fat indices, and lipid and glycemic parameters for the three cohorts of obese monkeys, collected -3 and -6 wks prior to commencement of dosing, is shown in Table 1. There were no significant differences among baseline measures, demonstrating an appropriate distribution of the animals among the treated and control cohorts.
Food consumption reductions in the treated and pair-fed groups - As shown in Fig. 2, when obese monkeys were treated once daily with PF-95453, food intake was reduced by 15% after treatment with PF-95453 at a dose of 0.5 mg/kg/day and by 23% after escalation to a treatment dose of 1.0 mg/kg/day (p<0.05). Food consumption in the drug treatment group returned to baseline and was equal to food consumption of the vehicle-treated animals within one week of treatment cessation (Fig. 2). The pair-fed group, had their ration adjusted weekly to reflect the amount of food that had been eaten by the drug treatment group during the prior week, such that there were only intermittent differences between the food consumption in the treated and pair-fed groups, such as that observed at week four (Fig. 2), where three of the ten monkeys in the pair-fed group ate less than 50% of their allotted food during the three-day measurement, resulting in a mean food consumption for the group that was lower than that of the drug-treated group. The slight differences in food consumption between the drug-treated and pair-fed groups after 2 and 6 weeks of treatment, that were less than 4%, were likely a consequence of inherent difficulties in collecting all of the small fragments of uneaten food during the 3-day food consumption assessment.

Body weight and body fat reduction in treated animals were greater than in pair-fed animals - Total body weight was measured at baseline and after 3, 5, and 8 weeks of treatment with PF-95453. There were no significant differences in average body weight between the vehicle treated, drug-treated and pair-fed animals at the start of the study (Table 1). However, treatment with PF-95453 reduced total body weight in a time-dependent and dose-dependent manner with a maximal reduction of 10% (p<0.05) relative to the vehicle-treated control group after 8 weeks of treatment that trended toward a return to baseline within 4 wks after treatment cessation (Fig. 3). The 10% reduction in body weight in the drug-treatment group relative to vehicle-treated control animals was the result of a 39% (p<0.001) reduction in whole
body fat mass, as determined by DEXA analysis (Fig. 4a and b), with no reduction in lean body mass (Fig. 4c).

By contrast, the pair-fed control group showed no reduction in overall body weight relative to vehicle-treated control animals throughout the course of the study (Fig. 3), even though their food consumption had been reduced to the same or slightly greater degree as the drug-treated animals (Fig. 2). There was however a slight 11% reduction in whole body fat mass in the pair-fed group relative to the vehicle-treated control group, as determined by DEXA analysis, that nearly reached significance (p=0.05), with no difference in lean body mass between the pair-fed group and vehicle-treated control group (Fig. 4b and 4c). The magnitude of this difference in percent body fat was presumably too small to be notable as a significant change in body weight.

Taken together, these observations suggest that the superior weight loss and body fat reduction of the drug-treatment group relative to the pair-fed group (p<0.01 and p<0.001, respectively) was primarily the consequence of effects of the compound that are independent of its anorectic activity.

**Improvements in adiponectin and leptin** – Plasma adiponectin, a fat-derived hormone produced in an inverse relationship with the amount of fat stores (Yang et al., 2001), and plasma leptin, a sensitive measure of whole body fat load (Wagner et al., 2010), were measured at baseline, and after 3, 5, and 8 weeks of treatment with PF-95453. Consistent with the magnitude of reductions in whole body fat mass noted above in the drug-treated and pair-fed cohorts relative to vehicle-treated control animals, PF-95453 increased plasma adiponectin levels and reduced plasma leptin concentrations in a time-dependent and dose-dependent manner, with adiponectin concentrations increased by 78% (p<0.001) relative to vehicle-treated control animals after 8 wks of treatment (Fig 5a). Leptin, which was essentially unchanged in vehicle-treated control animals at all time-points evaluated relative to baseline, was reduced by 34% in drug-treated animals relative to baseline and was significant at 5 and 8 weeks post
dosing (Fig 5b, p<0.01). Both the increase in adiponectin and reduction in leptin that resulted after treatment with PF-95453 showed trends toward a return to baseline within 4 wks of treatment cessation (Fig. 5a and Fig. 5b).

Plasma adiponectin was also significantly increased by 14.5% (p<0.01) in the pair-fed group relative to vehicle-treated animals after 8 wks of treatment but the magnitude of the increase was substantially lower than that noted in the drug treated group (p<0.001; Fig. 5a), consistent with the intermediate effect on body fat in the pair-fed group (Fig. 4b). By contrast, there were no significant differences in plasma leptin levels between the pair-fed and vehicle-treated animals at any of the time-points evaluated relative to baseline (Fig. 5b). This significant difference in leptin response to drug-treatment versus pair-feeding (p<0.01) may suggest a threshold fat mass loss required to elicit a leptin reduction response in these animals that was achieved in the drug-treatment group but not in the pair-fed animals.

**Changes in lipid and glycemic measures** - There were no differences in plasma total cholesterol, HDLC, triglyceride, or free fatty acid concentrations between the vehicle-treated, drug-treated or pair-fed groups at any of the time-points evaluated (Table 2). Likewise, there were no sustained differences in the inflammatory mediators, CRP or MCP1, between the vehicle-treated, drug-treated or pair-fed groups at any of the time-points evaluated (Table 3). Although there were no differences in fasting glucose and insulin between the vehicle-treated, drug-treated or pair-fed groups, other than a transient increase in the drug-treated group at week 3 (Table 3) and no differences in the glucose or insulin AUCs obtained from the GTT (Table 4) between the vehicle-treated, drug-treated or pair-fed groups, there was a slight decrease in the rate of glucose disappearance (Kg) between the drug-treated and vehicle-treated groups, which led to a small but significant increase in the glucose AUC (Table 4).
Discussion

The regulation of energy intake and energy metabolism by the endocannabinoid system was initially thought to occur centrally. However, much evidence with herbal cannabinoids, endogenous cannabinoids, cannabinoid receptor knockout animals, and cannabinoid receptor antagonists has demonstrated a complex interplay of mechanisms for controlling eating behaviors and regulating energy metabolism that involve both central and peripheral mechanisms (Di Marzo and Matias, 2005; Pagotto et al., 2006; Kunos et al., 2008; Nogueiras et al. 2008; Osei-Hyiaman et al., 2008; Nogueiras et al. 2009; Cota et al., 2009; Quarta et al. 2010). Indeed, the brain endocannabinoid system controls food intake at two levels; it tonically reinforces the motivation to find and consume foods with a high incentive value by interacting with mesolimbic pathways involved in reward mechanisms and it is activated on demand in the hypothalamus after short-term food deprivation and transiently regulates action of other orexigenic and anorectic mediators to induce appetite (Di Marzo and Matias, 2005; Pagotto et al., 2006; Quarta et al. 2010). Further, endocannabinoids and cannabinoid receptors are not confined to the CNS and are produced by and act as local mediators in many tissues including adipose tissue, liver, pancreas, skeletal muscle, and intestine, where they directly regulate various aspects of carbohydrate, lipid, and energy metabolism (Pagotto et al., 2006; Kunos et al., 2008; Nogueiras et al. 2008; Osei-Hyiaman et al., 2008; Cota et al., 2009).

The role of the endocannabinoid system in eating behaviors and regulation of energy metabolism has suggested a therapeutic role for central as well as peripheral CB1 receptor blockade in the management of obesity, and CB1 receptor antagonists such as rimonabant, taranabant, CP-945598, and CE-178253 have demonstrated marked weight loss in preclinical and clinical settings (Pagotto et al., 2006; Lin et al., 2006; Griffith et al., 2009; Cao et al., 2007). However, the relative importance of the effects of these agents on eating behaviors relative to modulation of energy metabolism has not been adequately evaluated. This has been due, in
part, to differences in the relative contribution of central versus peripheral CB₁ receptor-mediated mechanisms in old versus young rodents, lean versus obese rodents, and rodents with various defects in the mechanisms controlling energy intake and metabolism (Pagotto et al., 2006), suggesting a need to utilize animals in steady state to adequately conduct the comparison. Such evaluations in rodents have been further complicated by the observation that toleration to the anorectic activity of these agents begins to occur after the first few days of treatment, whereas body weight loss is sustained throughout treatment, suggesting a time-dependent variation in the relative contribution of central versus peripheral actions of CB₁ receptor blockade during prolonged treatment in rodents (Pagotto et al., 2006).

While a comparison of the relative contribution of central versus peripheral actions of CB₁ receptor blockade might be more instructive in human beings, the difficulties associated with inter-human variability in the mechanisms controlling eating behaviors together with the inherent difficulties in conducting adequately controlled pair-feeding studies in humans would make interpretation of the results of such studies difficult. We therefore chose to evaluate the relative contribution of anorectic as well as food intake-independent effects of a potent and CB₁ receptor-selective antagonist, CP-95453 in obese cynomolgus monkeys.

In these studies, we demonstrated that eight weeks of treatment with PF-95453 significantly reduced food consumption by 23%, reduced body weight by 10%, and reduced body fat by 39% without altering lean body mass. Further, treatment increased adiponectin concentrations by 78%, and reduced leptin concentrations by 34% relative to vehicle-treated control animals. However, pair-fed animals that consumed the same amount of food as the drug-treated animals did not exhibit any significant reduction in either body weight or leptin but did show a reduction in body fat of 11% (~1/3rd of the effect noted in the drug-treated animals) and an increase in adiponectin of 14.5% (~1/5th of the effect noted in the drug treated animals) relative to vehicle-treated control animals. The greater effects of drug treatment relative to pair-feeding with respect to increased adiponectin and reduced leptin concentrations, together with
the significant differences noted between the drug treated and pair-fed groups with respect to body weight reduction and body fat reduction, suggest that, in monkeys, a substantial portion of the anti-obesity effects of PF-95453 may be independent of its effects on appetite behaviors. This is consistent with observations made in young versus old mice, suggesting a lesser contribution to weight loss of anorectic activity relative to food intake-independent effects of CB₁ receptor blockade in aged animals (Cota et al., 2003). The absence of any body weight reduction in the pair-fed animals is presumably a consequence of compensatory mechanisms, such as decreased metabolic rate, that come into play at times of food restriction to favor maintenance of body weight (Morton et al., 2006).

In addition to their roles in regulating fat metabolism, leptin and adiponectin concentrations are also associated with various metabolic processes that play additional roles in the cardiometabolic risks associated with obesity. For example, adiponectin, which is a fat-derived hormone produced in an inverse relationship with the amount of fat stores (Wagner et al, 2010), is also associated with improvements in inflammation, insulin sensitivity, and vascular reactivity (Fernandez-Real et al., 2004; Tan et al., 2004). Whether the improvements in inflammation, insulin sensitivity, and vascular reactivity that have been demonstrated in experimental animals treated with rimonabant and other CB₁ receptor antagonists (Pagotto et al., 2006; Lin et al., 2006; Griffith et al., 2009; Cao et al., 2007) are in part a consequence of increased adiponectin levels are unknown.

While rimonabant has been reported to induce changes in lipid metabolism and glycemic parameters in experimental animals and in clinic (Pagotto et al., 2006), we observed no changes in either lipids, fasting glucose, or fasting insulin concentrations in either drug-treated or pair-fed monkeys in this study. However, consistent with those data there was a slight reduction of ~25% in triglyceride concentrations with PF-95453, but this was not significant. It is likely that the magnitude of the effect on fat mass in these monkeys was insufficient in this short-period of time to induce notable changes in markers of lipid and insulin metabolism.
Taken together, these observations demonstrate that the effects of chronic CB₁ receptor blockade on energy intake and energy expenditure that result in weight loss in obese monkeys likely involve both food intake-independent effects on energy metabolism and drug-dependent reductions of food intake. In addition, these observations suggest that there may be fundamental differences in the role of the endocannabinoid system on lipid metabolism and insulin sensitivity in the obese monkey relative to the obese rat.

Although it was hoped that CB₁ receptor antagonists might provide effective therapeutic options for the management of obesity and associated metabolic disorders, and that the initial market successes of rimonabant (Acomplia) indicated the potential benefits of this mechanism of action, adverse effects of Acomplia that became apparent after considerable market exposure that were related to anxiety and depressed mood mandated its withdrawal from the anti-obesity market (Butler and Korbonits, 2009; Akbas et al., 2009), with discontinuation of development of additional CB₁ antagonists, such as Merck’s taranabant and Pfizer’s CP-945598 & CE-178253, shortly thereafter.

In this regard, it is generally believed that the THC-mediated euphoria of Cannabis sativa, that results from its centrally-mediated CB₁ receptor agonism, is pharmacologically linked to its centrally-mediated appetite-enhancing actions through effects mediated at the HPA axis (Pagotto et al., 2006), and that the appetite suppression of CB₁ receptor antagonists and the corresponding anxiety and depressed mood are likely inexorably linked. However, evidence from animal studies, including this report, suggest that the beneficial metabolic effects of CB₁ receptor antagonism are mediated in part by CB₁ receptor blockade in peripheral tissues. If a pharmacological agent that retains the peripherally-mediated actions of CB₁ antagonism without concomitant centrally mediated modulation of eating behaviors and mood (for example a peripherally restricted CB₁ receptor antagonist that does not cross the blood-brain barrier, such as those described by LoVerme et al., 2009 and McElroy et al., 2008) can be developed, and
whether such a pharmacological agent can produce the degree of modulation of energy metabolism necessary to be an effective anti-obesity agent remains to be determined.
Acknowledgements

All procedures involving animals adhered to the American Society of Primatologists Principals for the Ethical Treatment of Nonhuman Primates and were conducted in compliance with state and federal laws of the US Department of Health and Human Services and guidelines established by the Wake Forest University Animal Care and Use Committee. We thank David Griffith for preparing the CP-95453 used in these studies, Amit Kalgutkar for assessing the drug concentrations in the plasma samples obtained from the pharmacokinetic studies conducted in the obese monkeys, Margaret Landis for developing the formulation used in these studies, and Joel Collins, Mickey Flynn, Sam Rankin, and Aida Sajuthi for technical assistance with the monkeys.
References


Chlorophenyl)-3-(4-chlorophenyl)-7-(2,2-difluoropropyl)-6,7-dihydro-2H-pyrazolo[3,4-f][1,4]oxazepin-8(5H)-one (PF-514273) a Novel, Bicyclic Lactam-Based Cannabinoid-1 Receptor Antagonist for the Treatment of Obesity. *J Med Chem* **52**:2652-2655.


acetyl-CoA carboxylase inhibitors reduce tissue malonyl-CoA concentrations, inhibit fatty acid synthesis, and increase fatty acid oxidation in cultured cells and in experimental animals. *J Biol Chem* **278**:37099-37111.


Footnotes

This work was supported by a grant from Pfizer, Inc.
Legends for Figures

Figure 1. The structure of PF-95453.

Figure 2. Food consumption differences and pair feeding – Food consumption was measured at baseline, after 2, 4, and 6 weeks of treatment, and 2 and 4 weeks after cessation of treatment (washout) as described in Methods. At baseline, animals consumed approximately 60% of the food that was made available to them. During the treatment phase of the study, the food made available to the pair-fed group was adjusted on the day after the food consumption assessment was completed to reflect the amount of food that had been eaten by the drug treatment group during the prior week. Therefore, chronologically, the pair-fed group trailed the vehicle-treated and drug-treated groups by one week. Shown is the average of the mean daily food consumption measured during the three-day assessment for the ten vehicle-treated animals (open circles) ten drug-treated animals (closed squares), and ten pair-fed animals (open triangles) as a function of duration of administration/washout.

Figure 3. Reduction of body weight by PF-95453 – Total body weight was measured at baseline, after 3, 5, and 8 weeks of treatment, and 2 and 4 weeks after cessation of treatment (washout) as described in Methods. Shown are the mean body weights ± SEM for the ten vehicle-treated animals (open circles), ten drug-treated animals (closed squares), and ten pair-fed animals (open triangles) as a function of duration of administration/washout.

Figure 4. Reduction of body fat by PF-95453 – Whole body fat mass (a), change in percent body fat (b) and lean body mass (c), were measured at baseline and after eight weeks of treatment by DEXA scanning as described in Methods. Shown are the mean ± SEM for the ten
vehicle-treated animals (open bars), ten drug-treated animals (closed bars), and ten pair-fed animals (grey bars) at baseline and after seven weeks of treatment.

Figure 5. Changes in adiponectin and leptin by PF-95453 - Plasma adiponectin (a), a marker of inflammation and insulin sensitivity, and plasma leptin (b), a measure of whole body fat load, were measured at baseline, after 3, 5, and 8 weeks of treatment, and 4 weeks after cessation of treatment (washout) as described in Methods. Shown are percentage of baseline adiponectin (a) and leptin concentrations (b) for the ten vehicle-treated animals (open circles), ten drug-treated animals (closed squares), and ten pair-fed animals (open triangles) as a function of duration of administration/washout. Data are expressed as mean percentage of average baseline values ± SEM.
### Table 1

**Baseline food intake, body weight, and biochemical parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle-treated</th>
<th>Pair-fed</th>
<th>PF-95453-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appetite indices</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>228</td>
<td>200</td>
<td>218</td>
</tr>
<tr>
<td>Food intake (% of available)</td>
<td>61</td>
<td>53</td>
<td>58</td>
</tr>
<tr>
<td><strong>Weight loss indices</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>6.6 ± 0.9</td>
<td>6.8 ± 0.9</td>
<td>6.7 ± 0.9</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>18.3 ± 4.3</td>
<td>23.1 ± 5.1</td>
<td>16.3 ± 3.9</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>17.4 ± 8.1</td>
<td>16.6 ± 6.2</td>
<td>18.9 ± 7.4</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>9.8 ± 4.2</td>
<td>9.3 ± 2.6</td>
<td>5.4 ± 1.9</td>
</tr>
<tr>
<td><strong>Glycemic indices</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>57.6 ± 2.3</td>
<td>55.0 ± 1.8</td>
<td>54.2 ± 2.1</td>
</tr>
<tr>
<td>Insulin (μU/mL)</td>
<td>32.9 ± 7.2</td>
<td>23.1 ± 3.5</td>
<td>22.3 ± 4.0</td>
</tr>
<tr>
<td><strong>Lipid indices</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free fatty acids (mEq/L)</td>
<td>1.10 ± 0.09</td>
<td>0.84 ± 0.11</td>
<td>0.83 ± 0.10</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>79 ± 17</td>
<td>67 ± 9</td>
<td>71 ± 23</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>126 ± 7</td>
<td>131 ± 10</td>
<td>127 ± 8</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>57 ± 4</td>
<td>58 ± 4</td>
<td>61 ± 3</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-Reactive Protein (ng/ml)</td>
<td>361 ± 55</td>
<td>339 ± 54</td>
<td>331 ± 38</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>154 ± 19</td>
<td>152 ± 15</td>
<td>127 ± 14</td>
</tr>
</tbody>
</table>

*Blood samples for baseline blood measures were collected at -3 and -6 weeks prior to commencement of dosing as outlined in Methods. Data are the mean of the average baseline values for each parameter ± SEM (n = 10 animals per group).*
### Table 2

**Treatment effects on lipid parameters**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 wk Tx</th>
<th>5 wk Tx</th>
<th>7/8 wk Tx</th>
<th>4 wk WO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>126 ± 7</td>
<td>123 ± 7</td>
<td>127 ± 6</td>
<td>125 ± 7</td>
<td>128 ± 7</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>131 ± 10</td>
<td>124 ± 9</td>
<td>124 ± 9</td>
<td>124 ± 9</td>
<td>121 ± 9</td>
</tr>
<tr>
<td>PF-95453</td>
<td>127 ± 8</td>
<td>122 ± 16</td>
<td>118 ± 11</td>
<td>124 ± 13</td>
<td>138 ± 13</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>57 ± 4</td>
<td>52 ± 4</td>
<td>61 ± 4</td>
<td>66 ± 5</td>
<td>62 ± 4</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>58 ± 4</td>
<td>52 ± 5</td>
<td>62 ± 5</td>
<td>63 ± 7</td>
<td>61 ± 6</td>
</tr>
<tr>
<td>PF-95453</td>
<td>61 ± 3</td>
<td>47 ± 3</td>
<td>59 ± 2</td>
<td>63 ± 4</td>
<td>68 ± 4</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/ml)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>79 ± 17</td>
<td>76 ± 15</td>
<td>66 ± 14</td>
<td>63 ± 12</td>
<td>84 ± 20</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>67 ± 9</td>
<td>67 ± 14</td>
<td>78 ± 13</td>
<td>87 ± 19</td>
<td>77 ± 14</td>
</tr>
<tr>
<td>PF-95453</td>
<td>71 ± 23</td>
<td>100 ± 55</td>
<td>74 ± 34</td>
<td>56 ± 12</td>
<td>97 ± 60</td>
</tr>
<tr>
<td><strong>Free Fatty Acids (mEq/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.10 ± 0.09</td>
<td>0.98 ± 0.16</td>
<td>1.05 ± 0.11</td>
<td>0.96 ± 0.11</td>
<td>1.16 ± 0.06</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>0.84 ± 0.11</td>
<td>0.85 ± 0.11</td>
<td>0.86 ± 0.10</td>
<td>0.77 ± 0.09</td>
<td>1.08 ± 0.17</td>
</tr>
<tr>
<td>PF-95453</td>
<td>0.83 ± 0.10</td>
<td>0.72 ± 0.13</td>
<td>1.04 ± 0.11</td>
<td>0.75 ± 0.14</td>
<td>1.01 ± 0.16</td>
</tr>
</tbody>
</table>

*a* Fasted plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and free fatty acid concentrations were measured at baseline, after 3, 5, and 7/8 weeks of treatment, and after four weeks of washout, as outlined in Methods. Data are expressed as mean values ± SEM (n = 10 animals per group).
Table 3

Treatment effects on glycemic and inflammatory parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>3 wk Tx</th>
<th>5 wk Tx</th>
<th>7/8 wk Tx</th>
<th>4 wk WO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose (mg/dl)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>57.6 ± 2.3</td>
<td>52.0 ± 1.2</td>
<td>53.7 ± 1.3</td>
<td>56.1 ± 2.0</td>
<td>56.2 ± 1.3</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>55.0 ± 1.8</td>
<td>51.3 ± 1.8</td>
<td>56.7 ± 2.0</td>
<td>55.3 ± 2.7</td>
<td>57.4 ± 2.6</td>
</tr>
<tr>
<td>PF-95453</td>
<td>54.2 ± 2.1</td>
<td>61.4 ± 3.6*</td>
<td>55.7 ± 2.9</td>
<td>56.4 ± 3.3</td>
<td>51.5 ± 2.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Insulin (μU/ml)</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>32.9 ± 7.2</td>
<td>28.6 ± 6.6</td>
<td>27.5 ± 8.2</td>
<td>35.9 ± 9.4</td>
<td>26.9 ± 9.4</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>23.1 ± 3.5</td>
<td>19.8 ± 3.3</td>
<td>27.8 ± 6.1</td>
<td>33.6 ± 7.8</td>
<td>21.3 ± 4.4</td>
</tr>
<tr>
<td>PF-95453</td>
<td>22.3 ± 4.0</td>
<td>55.5 ± 10.3*</td>
<td>24.1 ± 5.5</td>
<td>27.5 ± 7.6</td>
<td>14.8 ± 4.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>CRP (ng/ml)</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>361 ± 55</td>
<td>356 ± 52</td>
<td>296 ± 42</td>
<td>306 ± 50</td>
<td>318 ± 48</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>339 ± 54</td>
<td>367 ± 62</td>
<td>264 ± 38</td>
<td>265 ± 42</td>
<td>286 ± 54</td>
</tr>
<tr>
<td>PF-95453</td>
<td>331 ± 38</td>
<td>230 ± 33*</td>
<td>259 ± 25</td>
<td>249 ± 32</td>
<td>330 ± 44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>MCP-1 (pg/ml)</strong></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>154 ± 19</td>
<td>165 ± 22</td>
<td>168 ± 16</td>
<td>139 ± 18</td>
<td>162 ± 12</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>152 ± 15</td>
<td>167 ± 15</td>
<td>175 ± 21</td>
<td>168 ± 18</td>
<td>182 ± 21</td>
</tr>
<tr>
<td>PF-95453</td>
<td>127 ± 14</td>
<td>136 ± 15</td>
<td>114 ± 10*</td>
<td>121 ± 11</td>
<td>226 ± 18</td>
</tr>
</tbody>
</table>

* Fasted plasma glucose, insulin, C-reactive protein (CRP), and monocyte chemoattractant protein 1 (MCP-1) concentrations were measured at baseline, after 3, 5, and 7/8 weeks of treatment, and after four weeks of washout, as outlined in Methods. Data are expressed as mean values ± SEM (n = 10 animals per group).

* p < 0.05 vs. vehicle-treated and pair-fed groups.
# Table 4

## Glucose Tolerance Test Parameters

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 wk Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose AUC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>9281 ± 726</td>
<td>9272 ± 748</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>10194 ± 909</td>
<td>9368 ± 806</td>
</tr>
<tr>
<td>PF-95453</td>
<td>9143 ± 1588</td>
<td>10676 ± 1558*</td>
</tr>
<tr>
<td><strong>Insulin AUC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>8481 ± 863</td>
<td>8353 ± 872</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>7796 ± 807</td>
<td>9333 ± 788</td>
</tr>
<tr>
<td>PF-95453</td>
<td>6297 ± 686</td>
<td>6007 ± 783</td>
</tr>
<tr>
<td><strong>K&lt;sub&gt;g&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.60 ± 0.28</td>
<td>3.56 ± 0.38</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>3.17 ± 0.49</td>
<td>3.59 ± 0.40</td>
</tr>
<tr>
<td>PF-95453</td>
<td>3.61 ± 0.58</td>
<td>3.08 ± 0.29*</td>
</tr>
</tbody>
</table>

*Glucose tolerance testing at baseline and after five weeks of treatment with PF-95453, were done as described in Methods. Area under the curve (AUC), for glucose and insulin, and slope of the glucose disappearance curve (K<sub>g</sub>) were calculated as outlined in Methods. Data are expressed as mean values ± SEM (n = 10 animals per group).

* p < 0.05 vs. vehicle-treated and pair-fed groups.
Figure 1.
Figure 2.

* * * p < 0.05 Tx and Pair Fed Vs. Vehicle

- Vehicle (n=10)
- Pair-fed CTL (n=10)
- PF-095453 (n=10)

Diet Consumed (g/day)

Weeks

0 2 4 6 8 10 12

Dosing

Washout
Figure 5.

A

Change in Adiponectin (%)

- Vehicle (n=10)
- Pair-fed CTL (n=10)
- PF-095453 (n=10)

* p < 0.001 vs. Vehicle

Weeks

Dosing Washout

B

Plasma Leptin (ng/mL)

- Vehicle (n=10)
- Pair-fed CTL (n=10)
- PF-095453 (n=10)

* p < 0.01 vs. Baseline

Weeks

Dosing Washout