Selective cannabinoid-1 receptor blockade benefits fatty acid and triglyceride metabolism significantly in weight-stable nonhuman primates

Vidyaya Vaidyanathan, Raul A. Bastarrachea, Paul B. Higgins, V. Saroja Voruganti, Subhash Kamath, Nicholas V. DiPatrizio, Daniele Piomelli, Anthony G. Comuzzie, and Elizabeth J. Parks

1Center for Human Nutrition, University of Texas Southwestern Medical Center, Dallas, Texas; 2Department of Genetics, Texas Biomedical Research Institute, San Antonio, Texas; 3Department of Medicine/Division of Diabetes, University of Texas Health Science Center at San Antonio, San Antonio, Texas; 4Department of Pharmacology, University of California-Irvine School of Medicine, Irvine, California; 5Unit of Drug Discovery and Development, Italian Institute of Technology, Genoa, Italy; and 6Center for Laboratory Animal Sciences, Crown Bioscience at the David H. Murdock Research Institute, Kannapolis, North Carolina

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Vaidyanathan V, Bastarrachea RA, Higgins PB, Voruganti VS, Kamath S, DiPatrizio NV, Piomelli D, Comuzzie AG, Parks EJ. Selective cannabinoid-1 receptor blockade benefits fatty acid and triglyceride metabolism significantly in weight-stable nonhuman primates. Am J Physiol Endocrinol Metab 303: E624–E634, 2012. First published July 3, 2012; doi:10.1152/ajpendo.00072.2012.—The goal of this study was to determine whether administration of the CB1 cannabinoid receptor antagonist rimonabant would alter fatty acid flux in nonhuman primates. Five adult baboons (Papio Sp) aged 12.1 ± 4.7 yr (body weight: 31.9 ± 2.1 kg) underwent repeated metabolic tests to determine fatty acid and TG flux before and after 7 wk of treatment with rimonabant (15 mg/day). Animals were fed ad libitum diets, and stable isotopes were administered via diet (d31-tripalmitin) and intravenously (13C3-palmitate, 13C1-acetate). Plasma was collected in the fed and fasted states, and blood lipids were analyzed by GC-MS. DEXA was used to assess body composition and a hyperinsulinemic euglycemic clamp used to assess insulin-mediated glucose disposal. During the study, no changes were observed in food intake, body weight, plasma, and tissue endocannabinoid concentrations or the quantity of liver-G fatty acids originating from de novo lipogenesis (19 ± 6 vs. 16 ± 5%, for pre- and posttreatment, respectively, P = 0.39). However, waist circumference was significantly reduced 4% in the treated animals (P < 0.04), glucose disposal increased 30% (P = 0.03), and FFA turnover increased 37% (P = 0.02). The faster FFA flux was consistent with a 43% reduction in these fatty acids used for TRL-TG synthesis (40 ± 3 vs. 23 ± 4%, P = 0.02) and a twofold increase in TRL-TG turnover (1.5 ± 0.9 vs. 3.1 ± 1.4 μmol·kg⁻¹·h⁻¹, P = 0.03). These data support the potential for a strong effect of CB1 receptor antagonism at the level of adipose tissue, resulting in improvements in fasting turnover of fatty acids at the whole body level, central adipose storage, and significant improvements in glucose homeostasis.

endocannabinoid; adipose; fatty acid flux; stable isotopes; baboon

THE CB1 CANNABINOID RECEPTOR (CB1R) and its endogenous ligands, the endocannabinoids, have emerged as key players in the control of energy balance. Large-scale, randomized clinical trials of endocannabinoid antagonist treatment (e.g., rimonabant) in humans demonstrated significant improvements in cardiovascular risk factors, although psychiatric side effects caused the drug to be removed from the market (15). Despite this setback, and given the recognition that endocannabinoids (EC) interact with other systems (e.g., inflammation, insulin signaling), research in this area has remained extremely active, and peripherally restricted cannabinoid antagonist-based therapy may still have a place in the improvement of insulin sensitivity. A good deal of recent human research has focused on the tissue distribution, concentrations, and peripheral effects of the endocannabinoids 2-arachidonoyl glycerol (2-AG) and anandamide (AEA) and the fatty acid ethanolamides oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). The levels of these molecules in plasma and tissue are related to obesity (5, 20), insulin resistance (11), and diabetes (25, 39). Cannabinoids are lipids that signal through fatty acid means, and a common theme emerging from the basic (25, 39), cellular, and animal research (31, 61) is that their influence on metabolism may be mediated through alterations in fatty acid balance. Early data from rodent studies demonstrated that treatment with an EC agonist elevated liver fatty acid synthesis (41) and that treatment of ob/ob mice with rimonabant reduced hepatic lipogenesis (42). In humans, obesity is associated separately with elevated plasma endocannabinoid concentrations (24) and increased hepatic de novo fatty acids (23, 54), and thus, whether rimonabant treatment would reduce fatty acid synthesis in an animal model with physiology more close to humans is unknown. Because treatment of human subjects with rimonabant produced weight loss, it has been difficult to separate the beneficial effects of EC antagonist treatment from the metabolic improvements occurring after weight reduction. No tests of fatty acid synthesis or flux have been performed in large animals or humans at weight stability, and metabolic studies available in the literature have often been complicated by changes in food intake and body weight. Therefore, the present investigation utilized repeated kinetic analysis to assess the treatment effect of the CB1 antagonist rimonabant on lipid flux in the baboon (Papio) at weight stability. The close evolutionary relationship between humans and nonhuman primates suggests that they share many of the specific genetic mechanisms involved in determining differential susceptibility to disease (18). Not only do nonhuman primates offer a large, long-lived animal for the study of chronic diseases associated with metabolic dysregulation (13, 14, 27, 66), they provide a model that is genetically very similar to humans. The present study was designed to test whether the insulin-sensitizing
effects of rimonabant would result in a significant reduction in de novo lipogenesis in the baboon and whether improvements in glucose metabolism would be coincident with improvements in adipose lipid flux. The dosage of rimonabant (15 mg/day) was set to not cause a change in food intake and body weight or produce characteristics of depression in the baboons. As described below, these findings have implications for the role of EC blockade to mediate improvements in metabolic dysfunction through the adipose and support the potential for modulation of the peripheral EC system in the development of future treatments for metabolic diseases.

METHODS

Animals and study design. The baboons studied herein were selected from a population at the Southwest National Primate Research Center located at the Texas Biomedical Research Institute (San Antonio, TX). The overall study design is shown in Fig. 1. From a larger group of animals (Papio), five male baboons that were 7.5–18.1 yr of age (i.e., fully sexually mature) were chosen. Each animal was housed in an individual cage. Given the labor-intensive nature of these studies, animals were studied one at a time, sequentially, over a 3-yr period. The light cycle in the clinic room was set every day from 0600 to 1800. During an acclimation phase (Fig. 1), each animal was observed for aggressive/submissive behaviors, daily food consumption was monitored, and the animal was accustomed to the tether jacket system (16). After it was determined that the animal would acclimate to these surroundings it underwent ketamine sedation, and baseline assessments were made as follows: body composition by dual-energy X-ray absorptiometry scan (DEXA; Lunar Prodigy whole body scanner; GE Medical Systems, Madison, WI), measurements of body weight, waist circumference, and body surface area, and blood sampling for clinical biochemistries. A hyperinsulinemic euglycemic clamp study was performed the same day using previously published methods (13, 14). Following these assessments, the animal underwent surgical tether implantation, as described in detail previously (16). On the same day, biopsy samples of liver, subcutaneous adipose tissue (SubQ), and omentum, i.e., visceral adipose tissue (VAT), were taken. Tissue was frozen in liquid nitrogen immediately after biopsy.

During a 24-h period after catheter placement, the animal was carefully monitored. After a recuperation period of 7 wk, the animal began treatment phase, metabolic study procedures were repeated. Following these studies the tether and catheters were removed surgically under isoflurane, and the animal was monitored again until it recuperated. All study interventions and animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of the Texas Biomedical Research Institute. All animals received humane care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication no. 86-23, revised 1985).

Dietary intake and isotope-labeling scheme. For all animals, food was made available throughout the study from 0800 to 1600 each day, and animals were acclimatized to this feeding time. This protocol was used to obtain a definitive time when animals would have been actively eating (0800–1300) to investigate postprandial metabolism. Food intake and changes in behavior (e.g., depressive posture, interactions with handlers) were monitored daily by technical staff. As described in detail elsewhere (4), the standard diet (Monkey Diet 15%, Constant Nutrition Purina 5LE0) contained 57.7% carbohydrate (g/100 g weight), 15.3% protein, and 4.7% fat (ether extract). The animals were offered a quantity of food daily that was based on the estimated metabolizable energy requirements for adult captive baboons (39a) and designed to meet an expected energy requirement to sustain constant body weight (40–51 kcal/body wt in kg). This quantity of energy was adjusted based on the weekly measurements of the animal such that food was provided just in excess of that needed to maintain weight. Water was provided to the animals ad libitum, and fresh fruits and vegetables were given for enrichment. Immediately before receiving their food each morning, animals received a single peanut butter sweet treat, which contained the dose of rimonabant. Only on the day of the isotope study was the drug also combined with glyceryl-d31-tripalmitin, which allowed for identification of dietary fatty acids in the blood of the animals. The dietary label and the intravenous (iv) isotope studies were performed by modifying human protocols used previously while taking into account the different metabolic body size of the baboon (4, 33, 62, 63). At 0800 on the day of the isotope infusion study, an iv infusion of [13C1]sodium acetate (5 g dissolved in ½ normal saline, infusion rate of 1.0 ml/min) was begun to achieve labeling of fatty acids made through the de novo lipogenesis pathway. This infusion continued for 23 h. Metabolite and hormone data from the fasting state represent analysis of blood taken before 0800. Blood was drawn at 1100, 1200, and 1300 in the postprandial state, and data presented in Table 1 for the fed state represented the average of these three values. At 1600, the food was removed as per daily protocol. To reduce stress, fasting metabolism in the baboons was assessed at night and under light sedation, as

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Prescreening adult baboons

<table>
<thead>
<tr>
<th>Anthropometrics</th>
<th>Blood chemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>morphometrics</td>
</tr>
<tr>
<td>DEXA clamp</td>
<td>blood chemistry</td>
</tr>
<tr>
<td>Blood chemistry</td>
<td></td>
</tr>
<tr>
<td>Acclimate to</td>
<td>tether system, n=5, maintain on chow</td>
</tr>
<tr>
<td>Rimonabant (15 mg/d in sweet treat (1/day)</td>
<td></td>
</tr>
<tr>
<td>Week 5</td>
<td>begin treatment</td>
</tr>
<tr>
<td>Week 8</td>
<td>blood chemistry</td>
</tr>
<tr>
<td>Morphometrics</td>
<td>DEXA clamp</td>
</tr>
<tr>
<td>Blood chemistry</td>
<td>blood chemistry</td>
</tr>
<tr>
<td>Remove cannula</td>
<td>discharge</td>
</tr>
</tbody>
</table>

Fig. 1. Study timeline. Five male baboons were studied over a 12-wk period to determine the effect of 15 mg·kg⁻¹·day⁻¹ of the cannabinoid (CB₁) antagonist rimonabant. For details of procedures, see METHODS. DEXA, dual-energy X-ray absorptiometry.
Analysis of fatty acid composition and fatty acid and glycerol turnover. The fatty acid compositions of TRL-TG fatty acids and plasma FFA were analyzed by a HP 6890 series gas chromatograph, as described previously (4). To determine isotopic enrichments, the GC-MS method utilized selected ion monitoring for mass-to-charge ratios (m/z) of 270, 271, 272, 274, 300, and 301. Palmitate methyl ester enrichments were calculated using five-point standard curves for M4 and (d30 + d31) analysis. The measurement of newly made fatty acids was performed with m/z 270, 271, and 272 and calculated using the mass isotopomer distribution analysis method (28). Isotopomers of the propionic ester of glycerol (m/z 171, 172, 173, 175, and 176) were assessed in the electron impact mode, as described by Sunehag et al. (60). The fragment assessed for the derivatized d5-glycerol had a m/z of 173, and the 175/176 ions were derived from the internal standard that was added (13C5, d5-glycerol). Comparable ion peak areas between the standard curve and biological samples were achieved by either diluting or concentrating the sample.

Calculations and statistical analysis. The fatty acid infusate compositions and enrichments were analyzed by GC and GC-MS, as described previously (4). In the present analysis, palmitate is used as the fatty acid marker for all fatty acids in TG (i.e., the contribution of plasma palmitate in the PGA pool is assumed to contribute to liver-TG synthesis as efficiently as other fatty acids in the PGA pool). The contribution of dietary fatty acids to the plasma FFA pool was determined as follows: %FFA from dietary spillover:

\[
\text{fraction of d31-palmitate in FFA} = \frac{\text{fraction of d31-palmitate in the sweet treat-TG}}{\text{fraction of d31-palmitate in the sweet treat-TG}}
\]

Fasting lipolysis was assessed by measuring the rate of appearance of plasma FFA (Ra FFA) and of plasma free glycerol (Ra glycerol) during the night. The final Ra FFA and Ra glycerol data for a single baboon were determined from steady-state values collected between 0300 and 0700 (from 4–8 h after the start of the infusion). The measurement of TRL-TG turnover, in units of mmol·kg lipid·h⁻¹, represents the production of lipoprotein-TG from the liver and the clearance of TG from the blood. This turnover was calculated by modeling the rise to plateau of the plasma FFA label (M4-palmitate enrichment) in TG over the time frame 2400 to 0700. To test for changes in TRL-TG assembly in the liver, the variously labeled methyl palmitate isotopomers in TG were analyzed as the proportion of total methyl palmitate found in TG (2). The values used to obtain these fasted sources were derived from the average contributions of sources in TRL-TG from samples collected between 0400 and 0700. Thus, the contributions of various sources (dietary fat, plasma FFA derived from adipose tissue, and fatty acids made via de novo lipogenesis) of TRL-TG fatty acid are presented here as a proportion, which reflects the fluxes of fatty acids into the intrahepatic TG synthesis machinery (VLDL-TG synthetic processes). The proportions of palmitate derived from the sources were then multiplied by the absolute concentration of TRL-TG fatty acids to determine their quantitative contributions of these sources to blood-TG concentrations. It is possible that not all fatty acid sources will be identified using this scheme, as we have demonstrated previously (23, 45). The fatty acids that remain unlabeled at the end of the 23-h study could be derived from visceral stores or from intrahepatic-TG droplets. Calculations were performed using Excel (version 2007; Microsoft, Seattle, WA), and statistical analysis was conducted using Statview for Windows (Version 5.0.1; SAS Institute, Berkeley, CA). Data were tested for skewness and log-transformed when parametric analysis assumptions were not met. For paired t-tests, statistical significance was taken as a P value of ≤0.05. Repeated-measures ANOVA was used to test baseline, 3.5-wk, and 7-wk concentrations of plasma rimonabant concentrations, with a P value of 0.05 to denote significance. The association between outcome variables was assessed with Pearson’s correlation coefficients. Simple linear regression was performed using a layered Bonferroni correction for multiple comparisons.

### Table 1. Animal characteristics

<table>
<thead>
<tr>
<th>Variable (Anthropometrics)</th>
<th>Baseline</th>
<th>7 Wk</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>31.6 ± 1.1</td>
<td>31.2 ± 1.6</td>
<td>0.141</td>
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<tr>
<td>Body fat mass, kg</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.6</td>
<td>0.302</td>
</tr>
<tr>
<td>%Body fat mass</td>
<td>8.7 ± 1.8</td>
<td>7.8 ± 1.6</td>
<td>0.256</td>
</tr>
<tr>
<td>Lean mass, kg</td>
<td>27.7 ± 0.5</td>
<td>27.4 ± 0.5</td>
<td>0.293</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>59.0 ± 1.1</td>
<td>56.5 ± 1.9</td>
<td>0.039</td>
</tr>
<tr>
<td>Trunk fat, kg</td>
<td>1.59 ± 0.56</td>
<td>1.12 ± 0.30</td>
<td>0.257</td>
</tr>
<tr>
<td>TRL-TG, mmol/l</td>
<td>0.11 ± 0.03</td>
<td>0.12 ± 0.06</td>
<td>0.372</td>
</tr>
<tr>
<td>TRL-B48, ng/ml</td>
<td>118 ± 47</td>
<td>114 ± 13</td>
<td>0.472</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>7.9 ± 3.4</td>
<td>6.2 ± 2.3</td>
<td>0.298</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.8 ± 0.2</td>
<td>5.0 ± 0.2</td>
<td>0.195</td>
</tr>
<tr>
<td>FFA, mmol/l</td>
<td>0.45 ± 0.09</td>
<td>0.37 ± 0.07</td>
<td>0.170</td>
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<tr>
<td>Ketone bodies, μmol/l</td>
<td>94 ± 26</td>
<td>89 ± 16</td>
<td>0.312</td>
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</tbody>
</table>

Values are means ± SE. TRL, triacylglycerol-rich lipoproteins; TG, triacylglycerol; B48, apolipoprotein B48; FFA, free fatty acids. *Treatment group; n = 5 male baboons. Paired t-tests were used to test for significant differences.

Plasma TG and TRL-TG values were log transformed before analysis. Values are of fasting metabolites derived from the first blood draw on the morning of the isotope infusion study.

Analysis of metabolites, hormones, endocannabinoids, and rimonabant. Triacylglycerol (TG)-rich lipoproteins (TRL) consisting of chylo- microns and VLDL (d < 1.006 g/l) in the fed and fasted states were isolated from plasma by ultracentrifugation, as described previously (4). For measurements of plasma-TG, TRL-TG, glucose, and free fatty acid (FFA) concentrations, enzymatic kits were used (nos. 461-09092, 461-08929, 439-90091, 999-34691, and 991-34891, respectively; WAKO, Richmond, VA). TRL-B48 concentration was measured using a human apoB-48 ELISA kit (Shibayagi), and insulin was measured by Millipore ELISA kit for human insulin (no. EZH1-14K).

Systemic rimonabant concentrations in plasma were measured by Sanofi researchers. Briefly, samples from baseline, 3.5 wk, and 7 wk were mixed with 50 μl of internal standard solution (25.0 ng/ml of SR141716-D10 in acetonitrile). The tubes were vortexed and centrifuged at 10,000 g for 5 min. An aliquot of the supernatant (50 μl) was transferred into an autosampler vial and mixed with 100 μl of water for LC-MS-MS analysis. The lower limit of quantitation for SR141716 and its primary metabolite SR141715 was 0.25 ng/ml. The endocannabinoids AEA and 2-AG and the anandamide-like fatty acid ethanolamides OEA and PEA were measured in plasma and in SubQ and VAT biopsy samples by HPLC-MS, as described previously (26, 22). Plasma EC concentrations were measured at baseline, 3.5 wk, and 7 wk, whereas tissue concentrations were measured in biopsy samples taken at baseline and 7 wk. Liver biopsy samples were used to measure liver-TG content, as described previously (32).

Plasma fatty acid concentrations were measured in biopsy samples taken at baseline and 7 wk. Liver biopsy samples were used to measure liver-TG content, as described previously (32).
RESULTS

Morphometric and biochemical changes, concentrations of rimonabant, and EC. Food intake was monitored daily for each animal and was unaffected by rimonabant treatment (data not shown), nor was treatment associated with changes in baboon body weight, body fat, or lean mass (Table 1). Waist circumference was reduced 4% starting from 59.0 ± 1.1 cm at baseline, achieving 57.1 ± 0.8 cm at the midpoint of the study (3.5 wk; data not shown), and ending at 56.5 ± 1.9 cm at 7 wk (P = 0.039; Table 1). No changes in fasting concentrations of plasma lipids, FFA, ketone bodies, glucose, or insulin were observed. Figure 2A displays the concentrations of rimonabant (SR141716) and its primary metabolite (SR141715) in plasma, which verifies that the sweet treat containing the compound was a sufficient vehicle for daily drug delivery. Concentrations of both compounds were elevated significantly above the respective baseline levels by 3.5 wk, and the 3.5- and 7-wk concentrations were not different from one another (Fig. 2A). The interanimal variability of concentrations of the metabolite SR141715 was low compared with the variability of plasma rimonabant between animals. Steady-state concentrations were reached for the metabolite, which suggested that the capacity for rimonabant metabolism had been maximized. The apparent continued rise of plasma rimonabant concentrations between 3.5 and 7 wk supported the concept of recycling of the compound out of tissues, which would have reached a maximum at 100 days. Indeed, plasma rimonabant concentration at 3.5 wk was associated significantly with total body weight (r = 0.940, P = 0.05), but no significant relationships were detected between plasma rimonabant concentration and any measurement of body composition at 7 wk.

Concentrations of the endocannabinoids AEA and 2-AG and the anandamide-like non-CB1 receptor-binding fatty acid ethanolamides OEA and PEA were measured in plasma and in SubQ and VAT (omental) biopsy samples (baseline and 7 wk). To our knowledge, in nonhuman primates, plasma and tissue EC concentrations have not been published before. At baseline, fasting plasma AEA concentrations (2.2 ± 1.3 pmol/ml) were similar to those reported previously in humans (10, 24, 65), as were 2-AG concentrations (45.3 ± 34.6 pmol/ml) (65). At baseline, plasma concentrations of OEA were 16.0 ± 8.7 pmol/ml and PEA 3.7 ± 0.8 pmol/ml. Within adipose depot types, OEA concentration was significantly higher in VAT (81.0 ± 62.2 pmol/g) compared with SubQ (38.2 ± 41.8 pmol/g, P = 0.05). At 7 wk, the higher the SubQ concentrations of OEA and PEA, the greater the animal’s body fat.

A

B

C

D

E

Fig. 2. Plasma concentrations of rimonabant and its primary metabolite and the association between subcutaneous oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) and body fat. Values are means ± SE. A: plasma concentrations of rimonabant (SR141716) and its primary metabolite (SR141715) at baseline, 3.5 wk, and 7 wk posttreatment in 4 baboons. For both the drug and its metabolite, concentrations at 3.5 and 7 wk were significantly greater (\(* P < 0.05\) than their respective concentrations at baseline (i.e., before drug treatment). B and C: relationships were explored between 7-wk concentrations of subcutaneous adipose tissue (SubQ) OEA (B) and PEA (C) and %body fat content. D and E: at 7 wk, higher visceral adipose tissue (VAT) OEA (D) and PEA (E) concentrations were associated with greater triacylglycerol (TG)-rich lipoprotein (TRL)-apolipoprotein B48 lower and HDL cholesterol, respectively.
percentage (Fig. 2, B and C) and waist circumference (data not shown), whereas the higher the VAT OEA, the greater the fed TRL-apoB48 concentration (Fig. 2D), and the higher the VAT PEA, the lower the fasting HDL cholesterol concentrations (Fig. 2E).

**Glucose disposal, TRL-TG turnover, and kinetic measures of lipolysis.** Treatment with rimonabant for 7 wk did not change fasting glucose concentrations (Table 1). However, measurement of glucose disposal rates by the hyperinsulinemic euglycemic clamp showed a significant 31% increase posttreatment ($P = 0.033$; Fig. 3A). Furthermore, the turnover of plasma TG carried in the TRLs increased twofold ($P = 0.033$; Fig. 3B). Using continuous infusion of both [d5]glycerol and [13C4]palmitate, fasting adipose lipolysis rates were measured from midnight to 0700. Although no change in plasma glycerol turnover was observed posttreatment ($R_a$ Glycerol; Fig. 3C), the $R_a$ FFA from adipose was 37% higher ($P = 0.024$; Fig. 3D). The higher ratio of the FFA and glycerol fluxes suggests that intradipocyte fatty acid reesterification was suppressed by treatment (34). The concentrations of plasma AEA at baseline were positively associated with fasting insulin concentrations ($r = 0.960$, $P = 0.01$; Fig. 3E), whereas at 7 wk, plasma AEA concentrations were negatively associated with glucose disposal ($r = -0.898$, $P = 0.03$; Fig. 3F).

**Sources of plasma FFA.** Plasma FFA can be derived from adipose tissue release or from TG lipolysis intravascularly. The contributions of these sources to total plasma FFA concentrations over time were identified utilizing the isotopic labeling scheme before and after treatment. At baseline (Fig. 4A, left), total plasma FFA concentrations rose after the onset of eating (Fig. 4A, open circles between 1000 and 1300) as a result of both a small increase in adipose fatty acids (Fig. 4A, open triangles) and a large contribution of dietary fatty acids (Fig. 4A, gray squares). During the night (between 2200 and 0200), total FFA concentrations were surprisingly high and did not return to fasting levels until 0430. Food was removed from the animal’s cage at 1600, yet residual dietary fatty acids were present in the plasma FFA pool throughout the night (Fig. 4A,

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**Figure 3.** Changes in metabolic variables over time. A–D: changes in glucose disposal rates (A), TRL-TG turnover (B), rate of appearance of plasma glycerol ($R_a$ Glycerol; C), and rate of appearance of free fatty acids ($R_a$ FFA; D) ($n = 5$). *Significant differences from the corresponding baseline (BL) time points. Relationships were explored between BL fasting plasma anandamide (AEA) concentrations and fasting plasma insulin (E) and posttreatment AEA and glucose disposal rates ($R_a$) (F).

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**Figure 4.** Sources of plasma FFA. Plasma FFA can be derived from adipose tissue release or from TG lipolysis intravascularly.
gray squares). This could have resulted from either nocturnal release of chylomicrons labeled the previous day (11) or recycling of a previously stored label. After treatment with rimonabant (Fig. 4A, right), total plasma FFA concentrations again rose postprandially between 1000 and 1300 in a pattern similar to that observed at baseline. However, compared with pretreatment, significantly fewer dietary fatty acids were found in FFA between 1000 and 1300 posttreatment. During the night, diet-derived fatty acids were also lower compared with baseline.

**TG concentrations and fatty acid sources used for TG synthesis.** As shown in Fig. 4C, total plasma TG concentrations were unchanged by treatment during the fed state (open vs. closed squares), but during the night, plasma TG concentrations were lower posttreatment compared with baseline. A similar treatment-related reduction for the nighttime TRL-TG concentrations was also observed, but the changes did not reach statistical significance (Fig. 4C, closed triangles). When the change in nighttime concentrations of plasma TG was calculated, those animals with the greatest absolute reductions in TG concentrations had the highest rimonabant concentrations in blood (r = 0.996, P < 0.02). The changes in the nighttime plasma TG concentrations were due to an increased uptake of the lipids since treatment increased TRL-TG turnover rate significantly (Fig. 3B).

The sources of fatty acids used to synthesize TRL-TG, made up primarily of hepatically derived VLDL during the night, were identified using stable isotope labeling of adipose FFA release, fatty acids made through the de novo lipogenesis pathway in the liver, and fatty acids originating from dietary TG. Figure 5A presents the analysis of these sources and demonstrates that, at baseline, of all the fatty acids found in TG, the sources of 75.9 ± 3.3% of them were identified using the labeling scheme. Of this proportion, dietary sources made up the smallest percentage (16.2 ± 4.1%), with slightly more coming from de novo lipogenesis and the majority originating from the plasma FFA pool (40.4 ± 2.5%). Posttreatment, a smaller proportion of TG fatty acids became labeled (49.5 ± 7.1%, P = 0.008 compared with baseline). The relative amounts of dietary and de novo fatty acid represented similar proportions as found at baseline (Fig. 5A). However, the proportion of TG fatty acids derived from the plasma FFA pool fell to 57% of the baseline level (P = 0.009).

Finally, the proportions of TG fatty acids derived from the sources were multiplied by the total quantity of fasting TRL-TG fatty acids in the blood for each animal to obtain the absolute contributions of these sources to blood TG (Fig. 5B). Fasting TRL-TG fatty acid concentrations were not different between baseline and posttreatment (0.90 ± 0.23 and 0.77 ± 0.17 mmol/l, respectively, P = 0.214), but the absolute amounts of fatty acids identified by labeling were 26% lower after treatment (P = 0.01). Of that labeled, the amounts of fatty acids derived from the diet and from de novo lipogenesis were not different after treatment, but the quantity of TRL-TG made from the plasma FFA pool was 46% lower (0.35 ± 0.08 vs. 0.19 ± 0.06 mmol/l for baseline and posttreatment, respectively, P = 0.02). After rimonabant, the sources of more TRL-TG fatty acids remained unidentified at the end of the study (P = 0.01). As described above, these fatty acids could have come to the liver through visceral TG depot release or could have been derived from TG stores within the liver. Indeed, analysis of liver TG contents from biopsy samples...
demonstrated an increase in liver stores after treatment (20 ± 2 vs. 35 ± 8 nmol/mg protein at baseline and posttreatment, respectively, \( P = 0.046 \)). Plasma rimonabant concentrations did not correlate with the presence of de novo lipogenesis fatty acids when tested either as a percentage of total TRL-TG or as the absolute quantity derived from lipogenesis. The reduction in TG derived from plasma FFA was negatively correlated with the plasma rimonabant concentration (\( r = -0.998, P = 0.002 \)); i.e., the higher the plasma rimonabant concentration, the greater the reduction in the use of these plasma FFA for lipoprotein TG synthesis in the liver.

**DISCUSSION**

The present investigation utilized kinetic analysis to assess the treatment effect of the CB1 receptor antagonist rimonabant on lipid flux in weight stable, nonhuman primates. Our hypotheses were that 1) the insulin-sensitizing effects of the drug would reduce lipogenesis in the nonhuman primate *Papio*, as has been demonstrated in mice (19, 41, 42); and 2) improvements in glucose metabolism would be coincident with improvements in adipose lipid flux, as implicated previously in rodent studies (49) and in cell culture (6, 43). After completion of 7 wk of treatment, rimonabant did not reduce hepatic de novo lipogenesis, which was a surprise. Rather, in the absence of weight loss we observed significant improvements in glucose metabolism, greater whole body turnover, disposal of fatty acids and TG, and a significant metabolic impact of the drug evident during the night, which included more efficient disposal of dietary fatty acids consumed the previous day. As described below, these findings have implications for the role of CB1 receptor blockade to mediate improvements in metabolic dysfunction through the adipose and support the potential for modulation of the peripheral EC system in the development of future treatments for metabolic diseases.

In the baboon studies here, although rimonabant treatment did not change EC concentrations in plasma or adipose tissues, treatment uncovered significant relationships between the EC and lipid-related variables, similar to relationships observed previously in humans (21). At baseline, plasma AEA concentrations were elevated in those animals with higher fasting insulin concentrations, whereas after treatment, higher AEA concentrations were associated with lower glucose disposal rates (\( P < 0.05 \)). Furthermore, posttreatment, higher concentrations of SubQ and VAT OEA and PEA were tightly associated (\( r > 0.75 \)) with greater amounts of adipose mass, a finding similar to those of Guardado-Mendoza et al. (27), Horeau et al. (30), and Matias et al. (37), who showed that PEA is the ethanolamide secreted in the highest concentration from human primary adipocytes in culture. In line with the ethanolamides’ role in metabolic dysfunction, baboon VAT OEA and PEA concentrations were associated with higher fed-state concentrations of intestinal lipoproteins in plasma (apoB48 concentrations) and lower HDL cholesterol concentrations (Fig. 2E).

That these associations were present only after 7 wk (and not at baselines) is likely due to the uniformity of the conditions of housing in the clinic (same feeding time for all of the animals, common diet, reduced physical activity, etc.). These observations support a negative influence of the cannabinoid system on lipid metabolism and underscore the similarities of the EC/insulin sensitivity axis between baboons and humans (21).

The first of our hypotheses was that hepatic lipogenesis would be reduced significantly after rimonabant treatment,

![Figure 5](https://example.com/figure5.png)
even at weight stability. This hypothesis was based on much data from rodent studies demonstrating that treatment with an EC agonist elevated liver fatty acid synthesis (41), that the liver CB₁ receptor in mice is required for stimulation of fatty acid synthesis during high-fat feeding, and that treatment of ob/ob mice with rimonabant reduced hepatic lipogenesis (42). In humans, obesity is separately associated with elevated plasma EC concentrations (24) and increased hepatic de novo fatty acids, as evidenced by analysis of liver TG directly (23, 48) and of VLDL-TG, a validated marker of hepatic lipogenesis (23, 54). Analyzing the presence of newly made fatty acids in VLDL-TG before and after treatment, we found no significant differences. Our lack of ability to detect a change in lipogenesis was not due to low basal levels of fatty acid synthesis. Compared with humans in which fasting lipogenesis contributes 0–12% of VLDL-TG palmitate, baboons exhibited higher levels (19%), presumably because of the higher content of carbohydrate in their diet (69% of total dietary energy). The lack of treatment-induced changes in lipogenesis observed here suggests that the role of the EC system to increase lipogenesis in rodents and the beneficial effect of rimonabant to reduce lipogenesis are dependent on alterations in food intake. It is well established that lipogenesis is a process exquisitely sensitive to negative energy balance (28, 55).

The second key finding of the present study was a rimonabant-induced improvement in macronutrient metabolism at multiple physiological levels. Blüher et al. (10) showed that high plasma 2-AG concentrations correlated negatively with glucose disposal rates in humans, as we have found here in baboons (Fig. 3F). Furthermore, the 30% improvement in glucose disposal rates during the clamp echoes significant reductions in 2-h glucose post-oral glucose tolerance test found in humans, although the latter occurred against a backdrop of body weight loss (39b). From a lipid metabolism perspective, a 37% increase in plasma FFA turnover was observed after treatment (Fig. 3D), which is consistent with a growing body of research. Evidence of a role for EC to modulate adipose tissue metabolism includes the discovery of cannabinoid receptors CB₁R and CB₂R in human omental and SubQ adipocytes (51), increased glucose uptake into adipocytes and lipid droplet formation following CB₂R stimulation (36), and abrogation of the latter effect by CB₂R antagonism in primary human adipocytes (43). In the baboons, the combined turnover data from both plasma glycerol and FFA suggested that rimonabant reduced adipose reesterification rates, resulting in greater outflow of FFA from adipose. Furthermore, the baboon’s waist circumferences became significantly reduced with treatment. Redistribution of body fat in the absence of loss of total body weight has been observed before with other insulin sensitizers such as pioglitazone (38, 57). Although rimonabant-induced weight loss in humans has resulted in reductions in waist circumference (15), the observations here were completely unanticipated due to the constancy of the animals’ body weights. On the other hand, in accord with our findings, Richet et al. reported that 16 wk of rimonabant treatment in high-fat fed dogs reduced subcutaneous adipose tissue accumulation by 20% and also prevented a diet-induced expansion of visceral adipose fat (50).

The present kinetic data suggest that the reductions in adipose accumulation in mice and dogs observed previously were due to a stimulation of fatty acid release from adipose as a result of lower reesterification intracellularly. Whether the impact of rimonabant on adipose metabolism is direct in primates and/or humans remains an open question (64). Early data from treated mice suggested that peripheral modulation by CB₂R antagonism was not a major mechanism for anti-obesity effects (46, 47, 59, 56). However, in addition to the research cited above, numerous recent studies from primary cell culture (25, 39) and tissue expression analysis (43, 51) provide evidence that CB₂R antagonists can exert their effects directly through adipocyte signaling (35), as reviewed elsewhere (44). Compared with central administration, intraperitoneal administration of rimonabant in rats led to independent effects of lipid mobilization in white adipose tissue (40), and Osei-Hyiaman et al. (42) have clearly shown that, in mice, hepatic-specific deletion of the CB₂R protects against diet-induced steatosis. Hence, one limitation of the present study is that it cannot be definitively determined whether the beneficial effects of treatment to change adipose metabolism were direct or mediated centrally. A test of the true direct effect of endocannabinoid antagonists on peripheral metabolism in vivo will await the development of compounds that do not cross the blood-brain barrier. Of note, the main limitations of this study are the small sample size that characterizes primate studies, a lack of control group, and the requisite isolation of the animals outside their typical social structure (required for the metabolic studies). These characteristics of the study design raise the issue of whether clinic housing alone would change any of these measures. Observations from animals kept in similar housing conditions suggest some variability in waist circumference over time and that metabolic markers such as insulin and FFA may worsen due to stress (Parks EJ and Bastarrachea RA, unpublished observations); if the latter two observations are reproducible, the present findings would be even more significant. Other study limitations include the measurement of whole body adipose tissue fatty acid release rather than depot-specific fatty acid release in vivo and a lack of direct measurement of visceral TG stores. Nonetheless, given the intensive nature of tracer studies, particularly in primates, the present results contribute important new findings of a dominant influence of the drug on adipose fatty acid and plasma TG turnover.

The third finding of this study was a significant increase in the plasma turnover of TG-rich lipoprotein particles in treated baboons. In humans, high plasma 2-AG concentrations correlated positively with serum TG concentrations (10), and SubQ levels of AEA, OEA, and PEA correlated negatively with serum TG concentrations (10), and SubQ expression of the drug on adipose fatty acid and plasma TG turnover.

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Adipose tissue, an indirect effect orchestrated through neural disposal in the fasted and fed states provides the metabolic regulating lipolysis and that improvement in fatty acid and TG provide direct evidence to support the hypothesis that antago
kinetics, and measurement of plasma and tissue cannabinoids weight-stable animals the dominant effect of improved fatty acids and muscle and heart). The mechanism(s) by which rimonabant could improve fatty acid clearance peripherally is presently

The fourth finding of this study involves the effect of rimonabant to lower the proportion of VLDL-TG fatty acids becoming labeled during the 23 h of stable isotope administration (Fig. 5). We have shown previously that the contribu

Forgotten relationships observed between body fat and SubQ levels of OEA and PEA (Fig. 2, B and C) suggest that the ethanolamides, being fat derived, may provide a global signal of elevations in fat storage. Since increased body fat is associated with enhanced inflammation, the increased levels of the potent anti-inflammatory PEA (30) may be evidence of a counter-regulatory response that is proportional to body fat content. Collectively, these data provide a metabolic connection to systematically link the mounting basic evidence of the control of the EC system on adipocyte (29), skeletal muscle (58), and intestinal biology (22) to impair lipid metabolism. Recent reports have also described the presence of both cannabinoid CB1 and CB2 receptors in rat pancreatic β-cells (7, 9) and in isolated human islets (8), adding the endocrine pancreas as a potential site in the regulation of glucose homeostasis and indicating a functional a role for endogenous endocannabinoid signaling in regulation of endocrine secretion in the pancreas. These data extend the strong congruence of evidence from various animal models supporting a principal role of the EC system to improve insulin sensitivity through mechanisms of lipid flux.

In summary, the present study is the first to demonstrate in vivo in baboons.


circuits, or a combination of both of these mechanisms. However, the improvements in metabolic health in the absence of weight loss suggest that the development of new antagonists that do not cross the blood-brain barrier has potential as a future treatment for insulin resistance and diabetes. Finally, the significant relationships between plasma/adipose tissue concentrations of EC and measurements of body composition and insulin sensitivity observed here were similar to those observed in humans. These results underscore the strength of the non-human primate model in investigating pathways of energy metabolism that have relevance to human disease.

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DISCLOSURES

The authors have no conflicts of interest, financial or otherwise, to declare. Portions of this work were presented at the American Diabetes Association National Meeting in Orlando, FL, 2010.

AUTHOR CONTRIBUTIONS

V.V., R.A.B., P.B.H., V.S.V., A.G.C., and E.J.P. did the conception and design of the research; V.V., R.A.B., P.B.H., V.S.V., S.K., and N.V.D. performed the experiments; V.V., R.A.B., and E.J.P. analyzed the data; V.V. and E.J.P. drafted the manuscript; R.A.B., S.K., N.V.D., D.P., and A.G.C. interpreted the results of the experiments; E.J.P. edited and revised the manuscript; E.J.P. approved the final version of the manuscript.

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