Rimonabant-mediated changes in intestinal lipid metabolism and improved renal vascular dysfunction in the JCR:LA-cp rat model of prediabetic metabolic syndrome


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Russell JC, Kelly SE, Diane A, Wang Y, Mangat R, Novak S, Vine DF, Proctor SD. Rimonabant-mediated changes in intestinal lipid metabolism and improved renal vascular dysfunction in the JCR:LA-cp rat model of prediabetic metabolic syndrome. Am J Physiol Gastrointest Liver Physiol 299: G507–G516, 2010. First published May 27, 2010; doi:10.1152/ajpgi.00173.2010.—Rimonabant (SR141716) is a specific antagonist of the cannabinoid-1 receptor. Activation of the receptor initiates multiple effects on central nervous system function, metabolism, and body weight. The hypothesis that rimonabant has protective effects against vascular disease associated with the metabolic syndrome was tested using JCR:LA-cp rats. JCR:LA-cp rats are obese if they are cp/cp, insulin resistant, and exhibit associated micro- and macrovascular disease with end-stage myocardial and renal disease. Treatment of obese rats with rimonabant (10 mg·kg⁻¹·day⁻¹, 12–24 wk of age) caused transient reduction in food intake for 2 wk, without reduction in body weight. However, by 4 wk, there was a modest, sustained reduction in weight gain. Glycemic control improved marginally compared with controls, but at the expense of increased insulin concentration. In contrast, rimonabant normalized fasting plasma triglyceride and reduced plasma plasminogen activator inhibitor-1 and acute phase protein haptoglobin in cp/cp rats. Furthermore, these changes were accompanied by reduced postprandial intestinal lymphatic secretion of apolipoprotein B48, cholesterol, and haptoglobin. While macrovascular dysfunction and ischemic myocardial lesion frequency were unaffected by rimonabant treatment, both microalbuminuria and glomerular sclerosis were substantially reduced. In summary, rimonabant has protective effects against vascular disease associated with the metabolic syndrome that appears to be independent of hyperinsulinemia or oversecretion associated with an animal model of the metabolic syndrome, prediabetic status, and associated micro- and macrovascular dysfunctions.

EXCESSIVE WEIGHT GAIN, PARTICULARLY in the form of abdominal (visceral) adipose tissue, is a major public health problem in prosperous societies worldwide (16, 65). The resultant abdominal obesity has driven a developing epidemic of prediabetic insulin resistance and cluster of associated metabolic abnormalities that are termed the metabolic syndrome (9, 35). Insulin resistance leads to chronic hyperinsulinemia and has been implicated as a major determinant of early development of macro- and microvascularopathy, including atherosclerosis, ischemic cardiovascular disease (CVD), and renal damage, which are strongly associated with the metabolic syndrome (9, 26). The contribution of the metabolic syndrome to the development of cardiovascular and renal disease makes the reduction of obesity and related insulin resistance a critical target for therapeutic interventions. Clinical approaches, to date, have included changes in diet, food intake, and physical activity, but these have proven relatively ineffective in the human population and may well be confounded by environmental, behavioral, and genetic factors (11, 31, 38, 66). Significant efforts have been made to develop effective pharmaceutical treatments, with mixed results and/or the withdrawal of some of the most effective agents from further development and/or use (4, 6, 53). Basic research has been dependent on the use of animal models that mimic the metabolic and pathophysiological aspects of the metabolic syndrome (35). Desirable effects of putative pharmaceutical agents have been reduction of food intake with associated weight loss, improvement in insulin sensitivity, reduction in plasma lipid concentrations, improved vascular function, and anti-atherosclerotic cardioprotective activity.

Rimonabant (SR141716, Acomplia) is a selective antagonist of the cannabinoid-1 (CB₁) receptor and has been shown to have pleitropic effects on metabolism, obesity, and behavioral endpoints, such as addictions (5, 12, 24, 30, 32, 33). Thus rimonabant (and related compounds) offers a possible approach to prevention or treatment of the metabolic syndrome, prediabetic status, and associated micro- and macrovascular sequelae (3). More recently, the role of the CB₁ receptor in intestinal physiology, intestinal inflammation, and conditions of obesity has become apparent (21, 22). In this study, we have explored this possibility with emphasis on metabolic, lipid, and macro- and microvascular endpoints using an established animal model, the JCR:LA-cp rat.

The JCR:LA-cp rat is a unique strain that has been used extensively in the study of the underlying mechanisms of the cardiovascular and renal disease associated with the metabolic syndrome (26, 27, 35, 37, 40, 43, 51, 60), including advanced intimal (atherosclerotic) lesions, macrovascular dysfunction, myocardial ischemic lesions, and microvascular renal dysfunction (34, 49). The obese, disease-prone phenotype is due to the cp mutation that results in a stop codon in the extracellular domain of the leptin receptor (ObR) (65) and absence of all
isoforms of the ObR. This results in significant hyperphagia, a rapid development of profound insulin resistance between the ages of 4 and 7 wk, and progressive development of a very-low-density lipoprotein (VLDL) hypertriglyceridemia with delayed clearance of postprandial chylomicrons (60, 61). More recently, the JCR:LA-cp rat has been established as a model for the oversecretion of intestinal chylomicrons, providing new avenues to develop lipid lowering strategies for CVD risk (63).

Our hypothesis was that rimonabant treatment may have beneficial effects in the presence of the metabolic syndrome, beyond reduction of food intake, and confer protection against development of end-stage renal disease and CVD (3). The study endpoints were focused on insulin/glucose metabolism, intestinal and plasma lipid metabolism, cytokines and thrombosis, macrovascular function, and assessment of end-stage lesions of the kidney and heart. The results indicate pleiotropic effects of rimonabant, in the insulin-resistant cp/cp rat, with a modest reduction in body weight coupled with reduction of hypertriglyceridemia, postprandial lipemia, and a proinflammatory status, with associated protection from renal damage and dysfunction.

MATERIALS AND METHODS

Animals

Male JCR:LA-cp rats, cp/cp (obese) and +/+ (lean; a 2:1 mix of cp/+ and +/+), were bred and maintained in our established rat colony (40) and housed in an isolated HEPA-filtered caging system (Tecniplast, Buguggiate, Italy). Rats were housed individually at 11 wk of age and placed on a reversed light cycle 1 wk before the start of the experimental protocol, to facilitate metabolic studies during the active (dark) phase of their diurnal cycle. All food was Lab Diet 5001 (PMI Nutrition International, Brentwood, MO). Rats were weighed and food intake determined twice per week throughout the experimental period and placed on the appropriate control or treated food from 12 to 24 wk of age. All care and treatment of the rats was in accordance with the guidelines of the Canadian Council on Animal Care and was subject to prior institutional approval.

Drugs and Chemicals

Rimonabant was provided by Sanofi-Synthelabo Recherche, Ruel Malmaison, France, and incorporated into powdered rat chow at a concentration, based on body weight and food consumption of the rats, to maintain a dose of 10 mg·kg⁻¹·day⁻¹, as recommended by Sanofi-Synthelabo Recherche. The food was moistened, pelleted by extrusion through a die, and air dried as previously described (34, 50). Reagents and chemicals were obtained from Sigma Chemical (Oakville, ON, Canada).

Experimental Procedures

At 24 wk of age, a standardized meal tolerance test, fat challenge test, or postprandial lymph collection was executed. The rats were killed in the fed state, at 25 wk of age under isoflurane anesthesia. Blood was taken from the heart, and plasma separated for the measurement of plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1), leptin, and adiponectin. Urine was also collected from the bladder immediately before death. The right kidney and heart were taken for histology, and the thoracic aorta for assessment of vascular function.

Meal Tolerance Test

The meal tolerance test followed a standardized protocol (43). In brief, rats were deprived of food for 16 h over the light (inactive) period, and the test was conducted in the early part of the dark period. Conscious, unrestrained rats were subjected to three blood samplings during each session. Initially, animals were placed on a heated table to ensure vasodilation of the tail, and 0.5 ml of blood was taken from the tip of the tail (0 min). Rats were then replaced in their cages and given a 5-g pellet of rat chow (the test meal). Timing began when 50% of the test meal had been consumed, and samples of blood were taken at 30 and 60 min for the analysis of glucose and insulin. All rats ate the full test meal within 15 min of presentation.

Postprandial Lipemia

Following a 16-h overnight fast, animals were subjected to an oral fat challenge, using a modification of the meal tolerance test described above. Briefly, a 5.0-g pellet made with 5001 laboratory chow was consumed by rats. The pellet consists of 49% carbohydrate, 24.0% crude protein, 10% moisture, 6.5% minerals, 6.0% fiber, and 4.5% fat, but was further supplemented with 25% wt/wt dairy fat from double cream (raising the total fat content of the 5.0-g meal to ~30% wt/wt).

Blood samples were collected in tubes containing Na₂EDTA from the tail at time t = 0 and 2, 3, 4, and 6 h following consumption of the pellet meal. Plasma and serum were immediately separated from whole blood by centrifugation (3,000 rpm, 4°C, 10 min). Aliquots of plasma were stored at −80°C for biochemical analyses.

Lymph Cannulation and Nascent Chylomicron Isolation

Rats were anesthetized with phenobarbitone (60 mg/kg ip). The mesenteric lymph duct was cannulated, and at the same time a gastric tube was introduced into the upper duodenum, as previously described (59). Following recovery from anesthesia, rats were given a gastric infusion of Intralipid (KabiPharmacia) 2% (vol/vol) in a 4% (wt/vol) glucose solution. Lymph was collected into tubes containing EDTA. Contaminating leukocytes were pelleted by short-speed centrifugation, and chylomicrons were isolated from lymph by density gradient ultracentrifugation (63).

Analytic Methods

Plasma assays. Glucose was determined using a glucose oxidase assay procedure (Diagnostic Chemicals, Charlottetown, PEI, Canada). Insulin was assayed by rat ELISA assay (Merodia AB, Uppsala, Sweden). Plasma triglyceride (TG; L-type TG H), total cholesterol (cholesterol E), and low-density lipoprotein (LDL) cholesterol (L-type LDL-C) assays were obtained from Wako Pure Chemicals USA (Richmond, VA). High-density lipoprotein (HDL) cholesterol was assayed using direct HDL assay (Diagnostic Chemicals). MCP-1 was measured by immunoassay (R&D Systems, Minneapolis MN), and PAI-1 activity by an ELISA procedure (Diapharma Group, West Chester, OH). Leptin (Alpco Diagnostics; catalog no. 22-LEP-E06) and adiponectin (Alpco Diagnostics; catalog no. 44-ADPR-0434) were analyzed with commercially available enzymatic immunoassays for rodents. The acute phase protein of inflammation haptoglobin was measured using a colorimetric immunoassay (Tridelta Development, Wicklow, Ireland; catalog no. TP801). Urine albumin and creatinine measurements were performed on a Beckman Coulter LX20i analyzer using immune-turbidimetric and Jaffé methods, as in previous studies (34, 50).

Apolipoprotein B48 quantification. Apolipoprotein B48 (ApoB48) concentration in plasma and lymph was measured using an adapted immune-Western blot method, as previously described (62). Briefly, total plasma or lymph apolipoproteins were separated by SDS-PAGE on a 3–8% Tris-acetate polyacrylamide gel (Invitrogen, NuPage). The separated proteins were transferred onto a polyvinylidene difluoride membrane (0.45 μm; ImmobilonP, Millipore, MA). Membranes were incubated with a goat polyclonal antibody specific for apoB (Santa Cruz Biotechnology), and a secondary antibody tagged with hydrogen peroxidase (Santa Cruz Biotechnology) was used to visualize apoB by

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was treated with the inhibitor of NO synthase, L-NAME (endothelial NO-releasing agent acetylcholine (ACh). One ring in four curve) was assessed by determining the concentration response to the contraction, as calculated from the initial concentration-response.

Two-dimensional gel electrophoresis and protein sequencing. Qualitative analysis of smaller sized proteins in lymph from fasted and fed rats was determined by two-dimensional-gel electrophoresis, as described in the supplemental data. (Supplemental material for this article is available online at the Journal website.) Briefly, lymph samples were separated electrophoretically by pH and then, in the second dimension, by molecular weight. Proteins were visualized using silver staining and quantified by densitometry. Protein spots of interest were manually excised and subjected to tryptic digestion followed by liquid chromatography and tandem mass spectroscopic characterization and identification.

Vascular Function Studies

The vascular function of aortic rings, with intact endothelium, was assessed using established methods (48). In brief, thoracic aorta was excised, trimmed of adhering fat and connective tissue, and cut into 3-mm-long transverse rings, which were mounted on stainless steel hooks under 1.5-g resting tension in 10-ml organ baths maintained at 37°C. Baths contained Krebs solution (in mmol/l: 116 NaCl, 5.4 KCl, 1.2 CaCl2, 2 MgCl2, 1.2 Na2PO4, 10 glucose, and 19 NaHCO3), gassed with 95% O2-5% CO2. Tension was recorded isometrically with Grass FTO3C transducers (Grass Medical Instruments, Quincy, MA) and displayed on a Digi-Med tissue force analyzer (model 210, Micro-Med, Louisville, KY) linked to an IBM-compatible computer that acquired data digitally using DMSI 210/4 (Micro-Med) software.

The contractile response of endothelium-intact rings of aortas to phenylephrine (PE) was assessed through concentration-response curves (1 mmol/l to 50 μmol/l). The basal nitric oxide (NO)-mediated relaxation of aortic rings (precontracted with PE to 80% of maximal contraction, as calculated from the initial concentration-response curve) was assessed by determining the concentration response to the endothelial NO-releasing agent acetylcholine (ACh). One ring in four was treated with the inhibitor of NO synthase, L-NAME (N^ω-nitro-L-arginine methyl ester, 10 μmol/l) to confirm that the relaxation was NO depedent. The relaxant response of rings to the direct NO donor S-nitroso-N-acetyl-dl-penicillamine was also determined to confirm viability of the vascular smooth muscle.

Histology

Kidneys were cut through the hilum on the long axis, fixed in formalin, and subjected to conventional processing and sectioning, followed by hematoxylin-eosin staining. The extent of glomerular sclerosis was determined using a similar process to that of Schäfer et al. (54) and guided by the interpretation of Ferrario and Rastaldi (14). Four fields of view of the right kidney of each rat were recorded at ×2 magnification on a digital camera system (Nikon E600 with DMX 1200 camera and ACT-1 software, Nikon, Tokyo, Japan). The images were visualized using Photoshop (V7.0, Adobe Systems, San Jose, CA) and examined blind, and all glomeruli in each field (minimum of 40 per kidney) were rated as normal or sclerotic. Results were expressed as the percentage of glomeruli that exhibited sclerosis.

Hearts were cut transversely into four blocks, fixed in formalin, subjected to conventional processing, embedded in a single paraffin block, and sectioned, followed by hematoxylin-eosin staining. The heart sections were examined blind by an experienced observer, and the number of ischemic lesions was identified in each of the sections summed for each heart. Lesions were categorized by four stages, as previously described (40, 45, 51).

Statistical Analysis

Results are expressed as means ± SE, were analyzed using SigmaStat (Jandel Scientific, San Rafael, CA), and were plotted using SigmaPlot (Systat Software, San Jose, CA) and Prism (Graphpad, San Diego, CA). Results were compared using one-way ANOVA, followed by multiple-comparison tests. Body weight data from 16–24 wk of age were analyzed by two-way ANOVA. Concentration-response curves were analyzed using the program ALLFIT (8), which fits the complete data set to the logistic equation and permits independent testing of differences between individual parameters. A value of P < 0.05 was taken as being statistically significant.

RESULTS

Food Intake and Body Weight

Figure 1 shows food intake and body weights of cp/cp rats, control and rimonabant treated, over the period from 12 to 24 wk of age, with data from control +/? rats shown for reference. Rimonabant treatment caused a rapid and highly significant 30% decline in food consumption that, within 2 wk, was no longer evident; food intake of rimonabant-treated rats rebounded to the range of cp/cp control rats for the remainder of the experiment. Body weight gain of the rimonabant-treated rats became significantly lower than that of the cp/cp control animals after 4 wk of treatment, and this persisted until the end of the study (P < 0.0001).

Insulin and Glucose Metabolism

Plasma insulin and glucose concentrations, fasting (0 min) and during the meal tolerance test, are shown in Fig. 2. At the end of the treatment period, rimonabant-treated rats had a modestly lower fasting and postprandial plasma glucose concentration (P < 0.01 and P < 0.05, respectively, vs. untreated rats). However, this was accompanied by increased plasma insulin concentration (P < 0.01, fasting; P < 0.05, 30 and 60 min postprandial).

Plasma Lipids

As previously reported, HDL and total cholesterol concentrations were significantly lower in the +/? control rats compared with cp/cp control rats (Fig. 3). Rimonabant treatment
had no effect on plasma total, LDL, or HDL cholesterol concentrations of cp/cp rats. However, the plasma TG concentration of rimonabant-treated rats was decreased by 70%, approaching, but not equal to, concentrations of +/-? control rats.

The plasma apoB48 response to a fat challenge is shown in Fig. 4. At time 0 (fasting), concentrations of apoB48 in +/-? control rats were lower than those in the cp/cp controls (P < 0.0005). Interestingly, rimonabant treatment resulted in a significant reduction of fasting apoB48 concentration (50% vs. untreated, P < 0.05). The difference in apoB48 concentration persisted throughout the postprandial period, as shown by the area under the curve (Fig. 4, right, P < 0.005), suggesting improvements to intestinal secretion and/or clearance of TG-rich lipoproteins.

Postprandial lymphatic apoB48, cholesterol, and TG concentrations are shown in Fig. 5. Rimonabant treatment resulted in a striking reduction of lymphatic apoB48 secretion (50%, P < 0.01) and lymphatic cholesterol (P < 0.01).

**Markers of Inflammation and Thrombosis**

Fasting plasma concentration of the acute-phase protein haptoglobin was not different by genotype or treatment. However, curiously, treatment with rimonabant reduced the area under the curve response for haptoglobin following a lipid challenge, suggesting a relationship with intestinal lipid secretion (Fig. 6). To verify the relationship of haptoglobin and intestinal derived lipid secretion, we performed two-dimensional electrophoresis and tandem mass spectrometry and confirmed a reduction in the mass of haptoglobin present in lymph (see Supplemental Data). Four spots of interest were identified as haptoglobin -chain (nos. 85a, 146, and 157) and apoE (no. 85b), all of which were reduced by rimonabant treatment.

Rimonabant treatment simultaneously reduced fed state plasma PAI-1 concentrations to that not different from +/-? rats (Fig. 7). Rimonabant treatment did not influence plasma MCP-1, adiponectin, or leptin concentrations (Fig. 7).
Vascular Function

The contractile dose response of aortic rings to the noradrenergic agonist PE, and the relaxant response to ACh, is shown in Fig. 8. Aortae from cp/cp rats showed enhanced PE-mediated contractility compared with those from +/+ controls. Aortae from rimonabant-treated rats showed no change in PE-mediated contractility, either in maximal response or in the EC50 for PE. Furthermore, impaired ACh-mediated relaxation of PE precontracted aortic rings of cp/cp rats was not improved by rimonabant treatment (Fig. 8). There was no significant difference in the maximal relaxant response to sodium nitroprusside (~100%) or the EC50 between any of the groups (data not shown).

Renal Function and Glomerular Sclerosis

Urinary albumin excretion is markedly elevated in the cp/cp rats at 24 wk of age, as shown in Fig. 9, and this is accompanied by increased incidence and severity of glomerular sclerosis. Rimonabant treatment significantly reduced the albumin/
that of controls at the end of the treatment period. The antagonize the CB1 receptor, which is linked to many biochemical weight of rimonabant-treated wk to return to baseline intake. In the present study, body showed a similar pattern of reduced food intake, but required 8

Rimonabant and Food Intake/Body Weight Reduction

Rimonabant is a member of a unique class of agents that antagonize the CB1 receptor, which is linked to many biochemical and physiological pathways, including those involving catecholamine and endogenous opioid synthesis in various organs (3). CBs are recognized to be widely distributed throughout the intestine, with regional variation and organ-specific actions (22). Our results provide a novel perspective on the metabolic and intestinal effects of rimonabant in an animal model that exhibits the metabolic syndrome and associated end-stage complications.

Rimonabant-treated cplcp rats showed an initial reduction in food intake (23, 33, 59), consistent with previous reports in animals and humans. Unlike the case of diet-induced obesity in mice (33), there was no corresponding reduction in body weight in rimonabant-treated cplcp rats during this initial period of treatment. Interestingly, Janiak et al. (23) found that falfa Zucker rats treated with rimonabant at 10 mg·kg⁻¹·day⁻¹ showed a similar pattern of reduced food intake, but required 8 wk to return to baseline intake. In the present study, body weight of rimonabant-treated cplcp rats was 13% lower than that of controls at the end of the treatment period. The cplcp rat is resistant to reduction in body weight in the presence of changed caloric intake due to altered composition of food or food restriction, such that if pair-fed to +/?, control rats show only a 21% lower body weight than freely fed cplcp animals at 36 wk. For comparison, the body weight of matched +/? rats is 44% lower than that of cplcp animals (39). In this context, the reduction in body weight of the rimonabant-treated cplcp rats, in the absence of long-term reduction in food intake, is of real significance. In comparison, 6-mo-old falfa Zucker rats in the Janiak et al. (23) study showed a 7% reduction in body weight when treated with rimonabant at the equivalent dose. The origin of these differences between rat strains is not clear, but may be related to the more severe insulin resistance and hyperinsulinemia of the cplcp rat and polygenic differences between strains carrying the fa and cp mutations (52).

While data on humans are inconsistent, two recently published large clinical trials show a similar lag in reduction of body weight and waist circumference following initiation of rimonabant treatment (30, 32). In these clinical studies, reduction in body weight was not accompanied by long-term reduction in food intake, suggesting a fundamental change in metabolism, unrelated to food intake and possibly reflected in increased oxidative activity. It has been suggested, by Pagotto et al. (30), that CB1 antagonists may have an antiobesity effect through direct action on adipocytes. In support of this concept, rimonabant has been shown to increase oxygen consumption and glucose uptake by murine skeletal muscle (24).

Rimonabant and Glucose/Insulin Metabolism

Treatment of cplcp rats with rimonabant was associated with elevated plasma insulin concentration and normalized glucose levels. These changes may reflect an improvement in glucose control and are consistent with the physiological adaptation of the cplcp rat to maintain euglycemia (37). These results do not indicate an increase in insulin sensitivity per se, as seen in this

![Diagram](http://ajpgi.physiology.org/10.1152/ajpgi.00340.2010)

Fig. 9. Urinary albumin-to-creatinine ratio (Alb/Creat), as an index of renal vascular function (A), and fractional glomerular sclerosis (B) of control and rimonabant-treated rats. Values are means ± SE; n = 10 rats per group. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. cplcp control.
model with other agents, such as S15261 or D-fenfluramine (4, 41, 50). In contrast to our results, it has recently been reported that rimonabant inhibits basal insulin secretion by pancreatic islets of fa/fa Zucker and Zucker diabetic fatty rats (15). This was an in vitro study using islets isolated from 7- to 11-wk-old (juvenile) rats, which, at that age, are not highly insulin resistant and never exhibit the marked metabolic dysfunction seen in the cplcp rat. Our results were obtained in fully adult animals exhibiting developed metabolic syndrome. Given evidence that hyperinsulinemia plays a significant role in vascular disease (1, 26, 27, 37, 49, 50), the increased plasma insulin concentrations in rimonabant-treated cplcp rats must be considered carefully.

Rimonabant and TG Metabolism

Hypertriglyceridemia of the cplcp rat has been shown to be, at least in part, due to oversecretion of hepatic TG-rich VLDL particles (46, 60). Reduction of plasma TG by rimonabant treatment, of over 70%, is striking and greater than that previously reported (23, 33). It may be possible that rimonabant acts on both hepatic (29) and intestinal lipoprotein secretion in the JCR:LA-cp rat. Poirier et al. (33) observed a 15% decrease in plasma TG in high-fat-fed mice treated with rimonabant, while Janiak et al. (23) reported a 50% decrease in plasma TG in treated fa/fa rats (at 9 mo of age). The results suggest that the beneficial metabolic effects of rimonabant may be more pronounced in the presence of severe hyperinsulinemia and/or known additional contributions of lipogenesis from the intestine. Since there was no change in total circulating cholesterol concentration per se in treated cplcp rats, the results suggest a marked reduction in TG associated with VLDL and with TG-rich and/or chylomicron fractions.

Rimonabant and Cholesterol Metabolism

Changes in cholesterol metabolism with rimonabant treatment have been reported in animal models and humans, but these are not entirely consistent (10, 23, 33). In the diet-induced obese mouse, rimonabant caused only modest reductions in TG and total cholesterol, with an increase in HDL-C-to-LDL-C ratio (33). In the fa/fa Zucker rat, there was a slight decrease in total cholesterol and modest decrease in LDL cholesterol (23). Our results show no change in any of the plasma cholesterol fractions. For comparison, a large clinical study has shown no change in total cholesterol and modest to small change in LDL and HDL concentrations, together with a 20% decrease in TGs (10). These changes, overall, resemble those seen in the cplcp rat, which we consider to be a more extreme model for the metabolic syndrome than either the diet-induced obese mouse or the fa/fa rat. Given new insights into the ability of the liver and intestine to cooperatively maintain plasma cholesterol homeostasis, it is possible that, in the JCR:LA-cp rat, rimonabant may act selectively on intestinal cholesterol metabolism per se and not on the liver. From a cardiovascular risk point of view, reduction in plasma TG is a potentially very significant finding, particularly with respect to the role of postprandial lipemia and risk of CVD (36). In addition, recent studies in our laboratory have shown intestinal apoB lipoproteins, chylomicrons, and postprandial lipemia may be major contributors to vascular disease in the cplcp rat (25, 34, 61, 62, 63).

Rimonabant and Intestinal Lipid Metabolism

Rimonabant treatment of cplcp rats significantly reduced the plasma level of apoB48-containing particles in the fasting state (49%), as well as following an oral fat challenge (Fig. 4). As shown in Fig. 5, the elevated postprandial intestinal secretion of both apoB48 and cholesterol, reflecting the contribution to plasma chylomicron concentration by the intestine, was significantly reduced by rimonabant, with no reduction in TG secretion. The marked reduction in postprandial apoB (58%) and cholesterol (45%) content of the lymph of rimonabant-treated rats (Fig. 5) indicate a reduced production of chylomicron particles. However, the lack of change in lymphatic TG content per se indicates production of fewer larger chylomicron particles and is suggestive of increased receptor-mediated clearance of intestinal-derived particles from the plasma compartment. Rimonabant may potentially have an impact on the intestine by inhibiting transport of anandamide (64), a CB1 receptor ligand, in enterocytes. We speculate that this could downregulate the formation of chylomicrons or increase clearance of TG in circulation, or both.

Rimonabant and Proinflammation

Haptoglobin is an acute-phase protein that reflects inflammatory response (7), which is an inherent component of micro- and macrovascular disease in the cplcp rat (52). Our laboratory has previously reported that dietary fatty acid composition alters haptoglobin levels of the cplcp male rat following an oral fat challenge (16). The reduction of postprandial plasma haptoglobin levels observed in rimonabant-treated rats (Fig. 6) is consistent with a reduced inflammatory status and may contribute to the reduced renal microvascular dysfunction (Fig. 9). Notably, we demonstrate that haptoglobin can be derived from mesenteric lymph and is associated with intestinal lipid secretion. Moreover, rimonabant appears to influence not only the number and cholesterol content of intestinal-derived lipoprotein particles, but also the corresponding secretion of haptoglobin into plasma. While other acute-phase proteins, such as serum amyloid A, have been shown to readily associate with lipoprotein fractions, little is known about the lipoprotein binding capacity for haptoglobin. Haptoglobin may be present in mesenteric lymphatics associated with gut-associated lymphoid tissue response, or indeed directly with intestinal enterocytes (58); however, this understanding is not yet clear.

The intestine, in addition to the liver, expresses apoE mRNA, and the secretion is dependent on a number of poorly understood variables. The mechanism of the reduced apoE secretion into the lymph of rimonabant-treated cplcp rats, under conditions of a fat challenge (Fig. 6) is not obvious, but is consistent with the known effects of the endo-CB system in the gut (3, 22).

MCP-1 is a principal chemotactic factor in migration of monocytes/macrophages and mediates chronic inflammation (13). Our data show higher plasma MCP-1 levels in the cplcp rat compared with lean controls, which is consistent with our earlier observations of widespread activation and endothelial adherence of macrophages in atherosclerosis-prone adult cplcp rats (44, 51). Schepers et al. (55) and Takebayashi et al. (57) have shown an association between plasma MCP-1 and urinary albumin excretion in type 2 diabetic patients, consistent with our findings in the cplcp rat. In contrast to Takebayashi et al.
cular dysfunction and damage (45, 49, 52). In contrast, rimonabant, insulin sensitizers, and ethanol reduce hyperinsulinemia. For instance, cp/cp rats have elevated circulating levels of PAI-1 (56), consistent with the finding of both macro- and microarterial thrombi (43, 51). Reduction of PAI-1 levels in rimonabant-treated cp/cp rats may indicate an improvement in the prothrombotic status and is consistent with reduced glomerular sclerosis. We know that glomerular sclerosis is associated with accumulation of extracellular matrix, and this is related to increased concentration of PAI-1 (19, 20). Thus, in the presence of an inflammatory status (such as atherosclerosis), the reduction of PAI-1 activity seen in the rimonabant-treated cp/cp rat represents an important therapeutic marker (20) of reduced renal dysfunction. Consistent with this notion, Di Marzo and Szallasi (12) have also reported that rimonabant can ameliorate the prothrombotic and inflammatory status of the f/a Zucker rat.

Rimonabant and Vascular Damage

Rimonabant reduced the level of albuminuria and incidence of glomerular damage, indications of microvascular protection. These results are consistent with a hypothesis that the renal damage is related to elevated VLDL and/or chylomicron concentrations (17). However, contrary to the effects of rimonabant on microvasculature, we did not observe any concomitant benefit in macrovascular function, either on the hypercontractile response to PE or the impaired endothelium-mediated relaxation (Fig. 8). This is, perhaps, consistent with the increase in hyperinsulinemia, which appears to be a critical mediator of macrovascular dysfunction in this model (27, 28, 40, 47). Moreover, rimonabant treatment did not decrease the myocardial damage (see Supplemental Data), possibly due the relatively young age of the rats in this study compared with those used in earlier studies of myocardial lesions (40, 42, 45). The absence of any reduction in insulin levels may also underlie both the absence of reduction in macrovascular dysfunction or ischemic myocardial lesions.

Conclusions

Unlike other pharmaceutical interventions studied in the cp/cp rat, rimonabant has dimorphic effects that do not equally affect micro- and macrovascular dysfunction. For instance, angiotensin-converting enzyme inhibitors, endopeptidase inhibitors, insulin sensitizers, and ethanol reduce hyperinsulinemia, hypertiglyceridemia, vascular dysfunction, and myocardial lesions, with several simultaneously reducing renal microvascular dysfunction and damage (45, 49, 52). In contrast, rimonabant reduces TG levels and renal vascular disease, but does not reduce insulin levels, macrovascular dysfunction, or the incidence of myocardial lesions, supporting the notion of two complimentary etiological processes: one that involves macrovascular disease and appears to be related to hyperinsulinemia; and a second that involves renal microvascular complications and that is plasma TG, and possibly PAI-1, dependent. Furthermore, we show that rimonabant appears to have critical regulatory effects on the enterocyte and the associated lipid absorption pathways. We speculate that the contribution of the intestine to overproduction of lipids and/or the proinflammatory acute-phase response may prove to be important in the etiology of microvascular complications. Given the major role of renal failure in the complications of type 2 diabetes, further study of the underlying mechanisms is clearly indicated.

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DISCLOSURES

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