

Impaired postprandial apolipoprotein-B48 metabolism in the obese, insulin-resistant JCR:LA-*cp* rat: Increased atherogenicity for the metabolic syndrome

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Abstract

Aim: Postprandial lipaemia is a significant contributor to the development of dyslipidaemia and cardiovascular disease, which has more recently been shown as a potential risk factor for obesity and pre-diabetes. Clinically however, the diagnosis of early insulin-resistance remains confounded due to the fact that aberrations in lipid metabolism are not often readily identified using classic indicators of hypercholesterolemia (i.e. LDL).

Methods: In this study, we assessed the metabolism of apolipoprotein-B48 (apoB48)-containing lipoproteins in an animal model of obesity and insulin-resistance, the JCR:LA-*cp* rat. The contribution of lipoproteins from the intestine was assessed by measuring plasma apoB48 concentration in the postprandial period following an oral fat load. Plasma apoB48 was measured by improved enhanced chemiluminescent detection and other biochemical parameters measured by established analysis.

Results: Fasting concentrations of plasma apoB48, postprandial apoB48 area under the curve (AUC), as well as incremental-AUC (iAUC), were all significantly greater in the obese phenotype compared to lean controls. Fasting apoB48 correlated significantly with apoB48-iAUC, triglyceride (TG)-iAUC and insulin-iAUC. In addition, there was a highly significant association with fasting insulin and the postprandial ratio of TG:apoB48, a relationship not often detected in humans during insulin-resistance.

Conclusions/interpretation: We conclude that the JCR:LA-*cp* rat can be used as a model of postprandial lipemia to explore chylomicron metabolism during the onset and development of insulin-resistance, including the increased cardiovascular complications of the metabolic syndrome.

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1. Introduction

Postprandial lipaemia is a significant contributor to the development of dyslipidaemia, atherosclerosis and cardiovascular disease (CVD) [1–5]. The atherogenicity of impaired postprandial metabolism has been attributed to intestinally derived apolipoprotein-B48 (apoB48)-containing lipoproteins (i.e. chylomicrons), in particular, cholesterol-dense chylomicron-remnants (Cm-r) [1–8]. We have demonstrated that Cm-r's readily permeate the arterial wall and are preferentially retained leading to the focal accumulation of cholesterol, a hallmark of atherogenesis [5–7]. In diseases

Abbreviations: AAC, area above the curve; AUC, area under the curve; iAUC, incremental area under the curve; apoB, apolipoprotein-B; apoB100, apolipoprotein-B100; apoB48, apolipoprotein-B48; CVD, cardiovascular disease; Cm, chylomicrons; Cm-r, chylomicron-remnants; *cp*, corpulent; ECL, enhanced chemiluminescence; TG, triglyceride

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such as hypercholesterolemia and diabetes, individuals are predisposed to an accelerated form of atherosclerosis due to dyslipidemia, hyperinsulinemia and associated vascular perturbations [5,8,9–12]. Moreover, evidence now suggests that postprandial lipaemia (associated with elevated circulating levels of chylomicrons), is a significant risk factor for CVD in individuals with visceral and/or central obesity [11,13–16].

Obesity and insulin-resistance are among a cluster of clinical symptoms that comprise the metabolic syndrome, in which individuals are at increased risk of developing type-2 diabetes and premature atherosclerosis [15–18]. However, the current inability to readily identify the metabolic syndrome early enough to be able to reduce relative risk creates a significant clinical dilemma [19–22]. Diagnosis of the early insulin-resistance state is often confounded due to the characterization of dyslipidemia, in particular the continued emphasis on circulating concentrations of low-density lipoprotein (LDL), which is often normal during early type-2 diabetes [19,20]. Indeed, the International Diabetes Federation (IDF) recently affirmed that the clinical definition of the metabolic syndrome can be independent of LDL cholesterol concentration [23]. Thus, animal models offer a means to further characterize the early stages of insulin-resistance in order to understand the metabolic and postprandial profile of this condition, in the search for greater accuracy in CVD risk assessment [19,20,23].

Animal models of obesity and the metabolic syndrome are characterized by defects in the metabolism of leptin and its receptor and include the *ob/ob* mouse, the *db/db* mouse, the Zucker (*fafa*) rat and the JCR:LA-*cp* rat [24–26]. Of these models, only the JCR:LA-*cp* rat spontaneously develops the array of significant pathological complications such as spontaneous atherosclerosis and myocardial ischemia, consistent with complications found in man [26–28]. The plasma lipid profile of the JCR:LA-*cp* rat has been characterized over recent years and notably, the *cp/cp* phenotype has mildly higher plasma cholesterol levels compared to the lean (+/?) counterparts [24,27,29,30–35]. Consequently, the JCR:LA-*cp* model represents a unique opportunity to study dyslipidemia during insulin-resistance, in particular, to understand the potential influence this phenotype has on postprandial apoB48 metabolism.

The postprandial phase predominantly represents plasma apoB48 derived from the intestine in both humans and rodents [36–41]. Thus the approach given in this study was to assess chylomicron metabolism in the JCR:LA-*cp* rat following, a lipid-rich meal (or oral fat challenge) using a modified ECL (enhanced chemiluminescence) technique [11,36,42,43]. The primary aim of the study was to determine whether the JCR:LA-*cp* model has impaired metabolism of plasma apoB48 particles following a lipid-rich meal and establish it as model for postprandial dyslipidemia in conditions of obesity and pre-diabetes.

2. Methods

2.1. Animal model and diets

Male rats of the JCR:LA-*cp* strain, obese (*cp/cp*) ($n = 15$) and lean [+/?, or a 2:1 mix of rats heterozygous (*cp/+*) and homozygous normal (+/+)] ($n = 8$), were raised in our established breeding colony at the University of Alberta, as described previously [28]. The strain has recently been re-derived and established at Charles River Laboratories Inc. (Wilmington, MA, USA) with the designation CrI:JCR(LA)-*Lepr^{cp}*. Rats were weaned at 3 weeks of age and housed with a 12/12-h reversed light cycle to allow for study and testing during the dark phase of the rats' diurnal cycle. At that time, rats on protocol were transferred from the isolated breeding colony areas to a state-of-the-art fully individually ventilated caging environment (Tecniplast™, Exton, PA, USA). Animals were allowed to age for approximately 10 months, in order for the phenotype to develop established pathology of the metabolic syndrome and for dyslipidemia homeostasis. The animals had access ad libitum to standard laboratory rat chow (Lab diet 5001, PMI Nutrition International, Brentwood, MO, USA) and water. The composition (w/w) of the 5001 diet consists of carbohydrate 49%, crude protein 24.0%, moisture 10%, minerals 6.5%, fibre 6.0% and fat 4.5%. Animal care and experimental protocols were conducted in accordance with the Canadian Council on Animal Care and approved by the University of Alberta Animal Ethics Committee.

2.2. Fat challenge and the postprandial response

All animals were fasted overnight (16 h) and then offered a 5.0 g pellet made with 5001 laboratory chow (as described above) further supplemented with 25% (w/w) of dairy fat from double cream (raising the total fat content of the 5.0 g meal to approximately 30% (w/w)). The metabolism of insulin and glucose in the *cp/cp* rat is abnormally responsive to physical stress or behavioral disturbance. Thus, in order to reduce variability, fat challenge experiments are performed using a standardized, conscious, non-restraint protocol [44]. Blood samples were drawn from the tail at times 0, 2, 4, 5, 6, 8 and 10 h following consumption of the pellet meal. Blood was collected into tubes containing Na₂EDTA and plasma was separated by centrifugation at 3000 rpm at 4 °C for 10 min. Aliquots of plasma were stored at –80 °C for biochemical analyses.

2.3. Quantitation of plasma apolipoprotein-B48

The quantitation of apoB48 from rodent plasma samples was established by adaptation from a previous SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis), Western-blot technique coupled to ECL analysis [42]. ApoB48 protein was identified using a recent commercially available antibody to apoB (Santa Cruz Biotech, CA, Cat#sc-

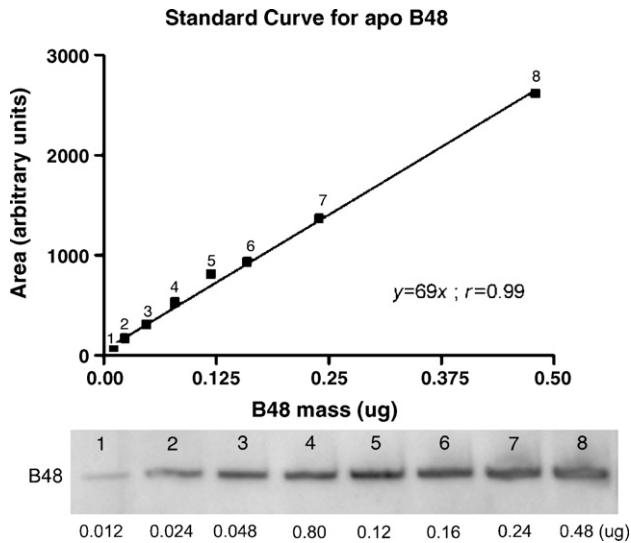


Fig. 1. Increasing concentrations of purified 1.006 g/ml density fraction from JCR:LA-*cp* rat plasma (12 ng/ml–0.48 μ g/ml). Known protein concentrations of rat apoB48 from purified fractions were subjected to SDS-PAGE and Western blot followed by ECL procedure. Intensity of each apoB48 band was measured by densitometric analysis.

11795). The antibody is an affinity-purified goat polyclonal, raised against a peptide that has been mapped near the amino terminus (α -1 region) of human apoB. We have established that the polyclonal antibody effectively detects both rodent and human forms of apoB (Fig. 1). The apoB48 band was visualized using ECL (ECL-Advance, Amersham Biosciences, UK) and the imaging of proteins was conducted using a charge coupled device [CCD]-camera and Fluor-S MultiImager system (Bio-rad Laboratories, CA). The modified ECL procedure has a limit of detection to approximately 10 ng/ml, which is an improvement over previously published ECL methods [39]. The mass of apoB48 from rodent plasma was quantified using linear densitometric comparison with a known mass of the purified rodent apoB48 protein.

2.4. Plasma biochemical profile

The profile of biochemical characteristics in fasting plasma from lean and obese groups from the JCR:LA-*cp* strain were assessed using commercially available homogenous, enzymatic colorimetric direct and indirect assays. Triglyceride (TG) concentrations (WAKO, Chemicals USA Inc., Richman, VA, USA, Cat#998-40391/994-40491), total cholesterol (WAKO, Cat#439-17501), LDL (WAKO Cat#993-00404/999-00504) and HDL-cholesterol (Diagnostic Chemical Ltd., Charlottetown, Prince Edward Island, Cat#258-20) were measured using direct colorimetric chemical enzymatic reactions. Plasma glucose was measured as per the glucose oxidase method (Diagnostic Chemicals Ltd., Cat#220-32). Insulin (LINCO Research, MS, USA, Cat#RI-13K) and leptin (LINCO Research, MS, USA, Cat#RL-83K) were determined using commercially available radioimmunoassays for rodents.

2.5. Analysis of the postprandial response

The postprandial response of plasma apoB48, TG, insulin and leptin from +/? ($n=8$) and *cp/cp* ($n=15$) animals was determined by total area under the curve (AUC) and leptin by total area above the curve (AAC) using Graphpad Prism (CA, USA), which corresponds to the total plasma concentration over the 10-h postprandial period. Fasting concentration of these parameters were further subtracted from the total AUC for the postprandial period to yield the incremental area under the curve (iAUC). Importantly, the iAUC represents the change in the postprandial response (compensating for the initial concentration of each parameter measured during the fasting state), and is expressed as the percent change from baseline. Knowing that the plasma content of both apoB48 and TG during the postprandial phase are primarily due to the synthesis of chylomicrons transporting dietary lipid, changes in iAUC values for apoB48 provide an accurate representation of contributions from the intestine. The TG:apoB48 ratio is an indicator of particle size and was calculated using pair-matched iAUC values, respectively, at each time point following the oral fat load.

2.6. Statistical analysis

All results are expressed as the mean \pm S.E.M. Data were tested for normal distribution and differences between *cp/cp* and +/? groups were analysed using unpaired *t*-test and one-way ANOVA with significance set at $p < 0.05$ (Sigma Stat, Jandel Scientific, San Rafael, CA, USA). Statistical correlation analysis was also performed using pair-matched values of each parameter, from each animal, at each time point for the postprandial curve.

3. Results

3.1. Fasting biochemical profile

Body weight and fasting parameters of both *cp/cp* and age-matched +/? animals are shown in Table 1. At this age, *cp/cp* animals are obese, hypertriglyceridemic, hyperinsulinemic, hyperleptinemic and have progressive development of vascular complications (including atherosclerosis) as previously established [27,44]. Notably, fasting plasma apoB48 concentrations in *cp/cp* animals were more than double that of the +/? controls ($31.1 \pm 4.7 \mu$ g/ml versus $14.0 \pm 1.8 \mu$ g/ml, respectively), indicating that obese animals had a significant accumulation of apoB48-containing particles compared to their lean counterparts (Table 1).

3.2. Postprandial response of apolipoprotein-B48

The postprandial response, as measured by the total AUC and the change in apoB48 (iAUC), showed that *cp/cp* animals had a progressive and significant delay in the clearance

Table 1

Fasting biochemical profile of aged-matched JCR:LA-*cp* obese (*cp/cp*) and lean (+/?) >10 months of age

	Lean (+/?)	Obese (<i>cp/cp</i>)
Body weight (g)	432.75 ± 17.68	970.87 ± 20.14 ^a
ApoB48 (μg/ml)	14.03 ± 1.76	31.15 ± 4.67 ^a
TG (mmol/l)	0.299 ± 0.027	3.782 ± 0.496 ^a
Total cholesterol (mmol/l)	1.506 ± 0.030	2.469 ± 0.193 ^a
LDL cholesterol (mmol/l)	0.559 ± 0.024	1.111 ± 0.015 ^a
HDL cholesterol (mmol/l)	0.906 ± 0.015	1.225 ± 0.007
Glucose (mmol/l)	5.199 ± 0.121	7.029 ± 0.203 ^b
Insulin (pmol)	118.25 ± 20.05	871.63 ± 50.54 ^a
Leptin (ng/ml)	2.97 ± 0.42	62.14 ± 5.07 ^a

Data shown are mean ± S.E.M. for obese (*cp/cp*) (*n* = 15) and lean (+/?) (*n* = 8).

^a *p* < 0.0001 vs. +/? rats.

^b *p* < 0.01 vs. +/? rats.

of apoB48 particles over the 10-h period (Fig. 2). Total AUC indicated that circulating apoB48 mass over the postprandial period was approximately three-fold higher in *cp/cp* rats compared to +/? controls (AUC, 1400 ± 222 and 428 ± 68 area units, respectively, *p* = 0.0114). Corresponding iAUC showed that the change in apoB48 particles (i.e. chylomicrons) during

the postprandial phase was two-fold higher in *cp/cp* animals compared to the +/? controls (iAUC; 1982 ± 117 and 661 ± 39 area units, *p* = 0.0176, see percent change in Fig. 2 inset). At 2 h post the fat challenge, circulating apoB48 concentrations had increased by 100%, progressively increasing to 300% by 10 h, as compared to 20% and 60%, respectively, in the +/? control animals. The slope of the apoB48-iAUC was also calculated between 0 and 5 h to represent the initial phase of the postprandial period. Based on the generation of a first order polynomial equation (including both the intercept and slope), *cp/cp* animals had a significantly greater rate of chylomicron accumulation in plasma compared with +/? animals in the early postprandial period (apoB48-iAUC slope⁰⁻⁵ *cp/cp*: $y = -1.528 + 49.05x$ ($r^2 = 0.98$) versus +/? : $y = -7.837 + 19.39x$ ($r^2 = 0.93$), *p* = 0.0017).

3.3. Postprandial response of triglyceride

In the fasted state, VLDL forms the principle source of TG in the circulation for JCR:LA-*cp* animals [35]. Values observed in this study are consistent with previous reports that *cp/cp* animals have characteristic and severe hypertriglyceridemia compared to the +/? control animals (12-fold difference) as shown in Table 1. Moreover, the response of TG during the postprandial phase highlights the extent of hypertriglyceridemia in *cp/cp* animals (AUC; 4998 ± 469 and 436 ± 41 area units, respectively, *p* < 0.0001) and is shown in Fig. 3. In contrast, it was interesting that the corresponding TG-iAUC over the 10-h period, did not show any statistical difference between *cp/cp* and +/? (Fig. 3, inset). We

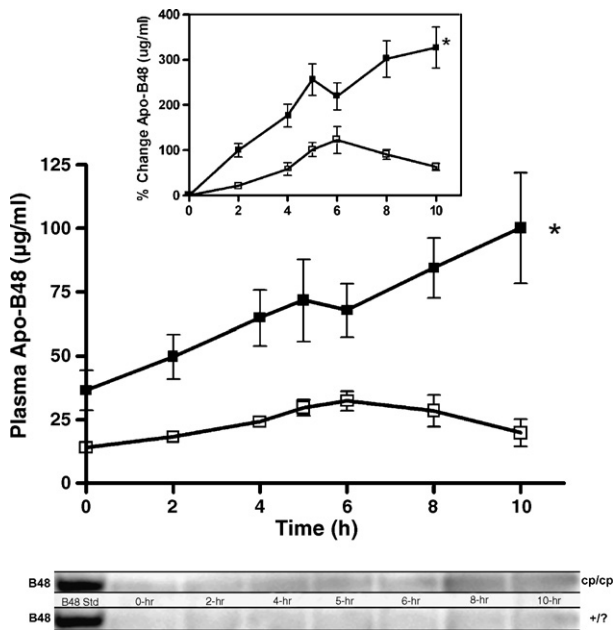


Fig. 2. The postprandial associated plasma apoB48 response (AUC) following an oral fat challenge in JCR:LA-*cp* obese (*cp/cp*) and lean (+/?) controls. Data are shown for *cp/cp* rats (filled squares) (*n* = 15) and +/? controls (open squares) (*n* = 8) as mean ± S.E.M. The percent change in plasma apoB48 from fasting baseline is shown in the inset and represents the corresponding incremental area under the curve (iAUC). Both the postprandial apoB48-AUC and the apoB48-iAUC was significantly greater in the *cp/cp* compared to +/? animals, (*) *p* < 0.05. The lower panel represents a typical western blot detecting plasma apoB48 following an oral fat challenge (0–10 h) in both *cp/cp* and +/? JCR:LA-*cp* rats. The slope of the initial postprandial phase (between 0 and 5 h) for *cp/cp* animals ($y = -1.528 + 49.05x$ [$r^2 = 0.98$]) was significantly greater compared with lean +/? animals ($y = -7.837 + 19.39x$ [$r^2 = 0.93$]); *p* = 0.0017 based on a first order polynomial and both intercept and slope parameters.

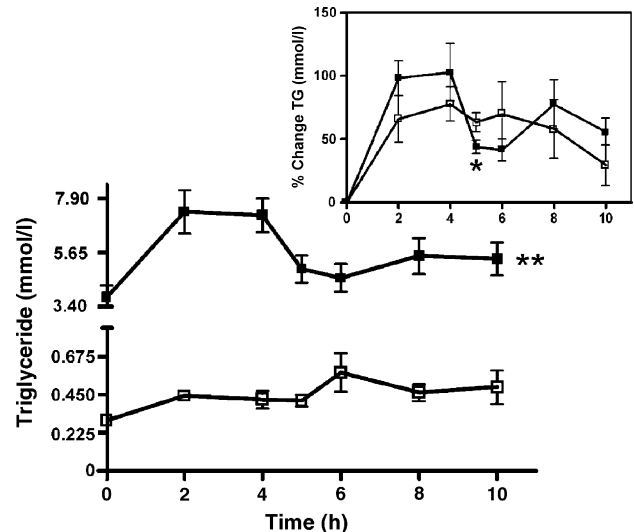


Fig. 3. The postprandial response in plasma TG (AUC) following an oral fat challenge in JCR:LA-*cp* obese (*cp/cp*) and lean (+/?) controls. Data are shown for *cp/cp* rats (filled squares) (*n* = 15) and +/? lean controls (open squares) (*n* = 8) as mean ± S.E.M. The total AUC and the change in TG from fasted concentrations is shown (inset) and represents the incremental area under the curve (iAUC). The AUC for *cp/cp* animals is significantly greater than for +/? animals, (***) *p* < 0.0001. The iAUC for *cp/cp* animals is significantly greater at 4 h compared to 5 h, (*) *p* < 0.05.

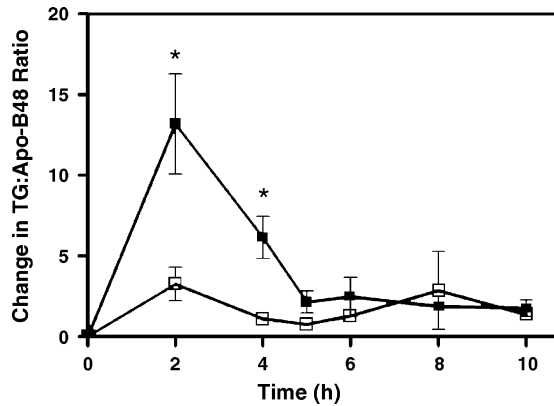


Fig. 4. The change (postprandial) in the corresponding TG:apoB48 ratio (AUC) following an oral fat challenge in obese (*cp/cp*) $n=15$ (filled squares), and lean (*+/?*) $n=8$ (open squares). Data are shown as mean \pm S.E.M. The *cp/cp* animals had a significantly higher TG:apoB48 (AUC) ratio at both 2 and 4 h following the oral fat challenge compared to lean controls, (*) $p<0.05$.

also observed a distinctive biphasic clearance profile of TG from the circulation, which is predominant in the TG-iAUC for *cp/cp* animals. In particular, *cp/cp* animals demonstrated a rapid decline in TG at 4–5 h ($p<0.05$), which appears to rise again at 8 h to 70% above fasting levels, suggesting additional secretion of TG in the hypertriglyceridemic state. This distinction becomes more evident on further analysis of the TG:apoB48 ratio (Fig. 4). The TG:apoB48 ratio reflects the proportion of TG per particle during the postprandial period (for their respective incremental change over time). There was no overall statistical difference in the TG:apoB48 ratio between the *cp/cp* and lean control animals. Obese *cp/cp* animals did have significantly higher TG:apoB48 ratio in the early postprandial phase (i.e. at time points 2 and 4 h following the fat meal), consistent with an initial absorption, appearance and accumulation of larger chylomicron particles (Fig. 4).

3.4. Response of postprandial insulin

Obese *cp/cp* animals are phenotypically hyperinsulinemic and we observed fasting insulin concentrations to be seven-fold higher than those of age-matched *+/?* animals (see Table 1). The total AUC and change in insulin response (iAUC) over the postprandial period for *cp/cp* and *+/?* animals are given in Fig. 5. The total AUC for plasma insulin following the oral fat challenge in *cp/cp* animals was approximately eight-fold greater compared to the lean controls (AUC; 8797 ± 344 and 1145 ± 117 area units, respectively, $p<0.0001$). Despite the hyperinsulinemia in the fasting state, the *cp/cp* phenotype demonstrated an additional and sustained increase in postprandial insulin secretion of approximately 20% above fasting concentrations at 2–5 h, thereafter returning to fasting baseline concentrations. In comparison, *+/?* animals showed a maximal increase in postprandial

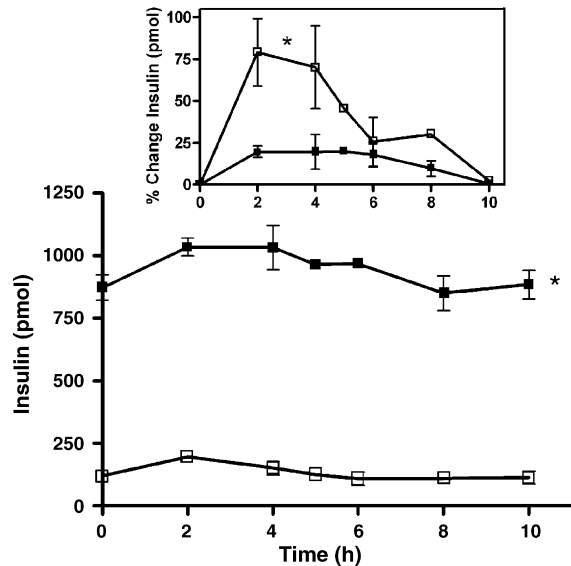


Fig. 5. The postprandial response in plasma insulin following an oral fat challenge in JCR:LA-*cp* obese (*cp/cp*) and lean (*+/?*) animals. Data are shown for *cp/cp* rats (filled squares) and *+/?* lean controls (open squares) as mean \pm S.E.M. The total AUC and the change (iAUC) in insulin from fasted concentrations is shown (inset). The *cp/cp* animals were shown to have a significantly greater total AUC for plasma insulin, (*) ($p<0.0001$) and a markedly lower change (iAUC) in insulin following the oral fat challenge, (*) ($p<0.05$).

insulin of approximately 80% between 2 and 4 h, before declining to fasting levels. Interestingly, the insulin-iAUC showed a transposed relationship (Fig. 5, inset).

3.5. Response of postprandial leptin

Under normal physiological conditions, circulating leptin is thought to functionally switch off both hyperphagia (through the hypothalamic neuropeptide-Y pathway) and sedentary activity through direct intracellular signalling actions of the leptin receptor [45]. However, high fat meals have been shown to suppress circulating leptin concentrations particularly during the first 2–4 h of the normal postprandial phase, which are thought to be independent of intracellular leptin signalling [45,46]. Consequently, it was of interest in this study to determine the potential and corresponding postprandial leptin response following a high fat meal (Fig. 6). The net area above the curve (AAC) analysis demonstrated that there were sustained and overall greater elevation of leptin in *cp/cp* animals (ACC; 535 ± 22.1 area units), compared to *+/?* controls (AAC; 18.4 ± 2.95 area units, $p<0.0001$). However, it was interesting that both the *cp/cp* and *+/?* animals had a net decrease in plasma leptin levels over the 10-h postprandial phase. Further analysis of the iAAC also indicated that *+/?* controls had a consistent, and significantly lower, net suppression in leptin secretion over the postprandial phase (iAAC; -378 ± 59.4 area units, $p<0.05$) compared to *cp/cp* animals (iAAC; -67.23 ± 85.81 area units).

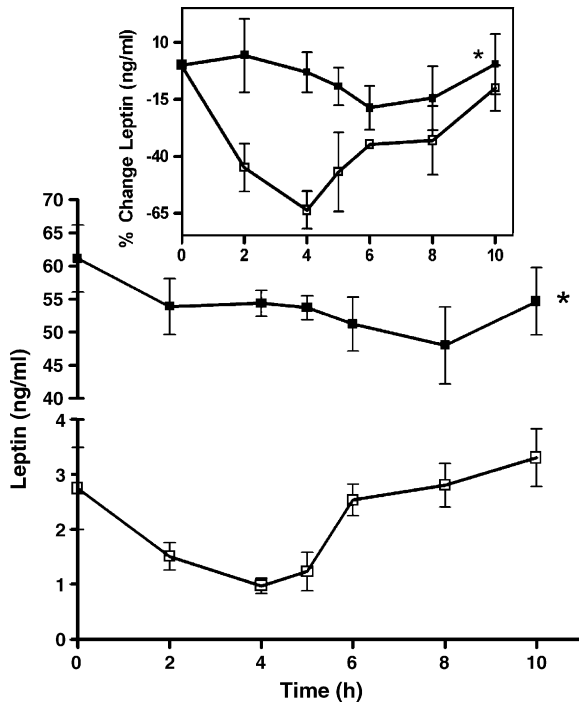


Fig. 6. The postprandial response in plasma leptin following an oral fat challenge in the JCR:LA-*cp* obese (*cp/cp*) and lean (+/?) animals. Data are shown for *cp/cp* rats (filled squares) $n=15$, and +/? controls (open squares) $n=8$ as mean \pm S.E.M. The net area above the curve (AAC) and the corresponding change (iAAC) in leptin from fasted concentrations is shown (inset). The *cp/cp* animals were shown to have a significantly greater total AAC for plasma leptin, (*) ($p<0.0001$) and a markedly lower percentage change (iAAC) in leptin following the oral fat challenge, (*) ($p<0.05$).

3.6. Correlation of postprandial markers and apolipoprotein-B48

Our correlation analysis emphasized aspects of postprandial lipid metabolism that might highlight early markers of risk in the pre-diabetic state. There were moderate to strong associations of fasting apoB48 concentrations with iAUC for

each; apoB48, TG and insulin (Table 2). Although overall TG-iAUC were not different between lean and obese animals, TG-iAUC and fasting apoB48 were shown to have a significant association, which is likely to reflect the obese animals having elevated fasting apoB48 combined with a tendency for increased TG-iAUC. In contrast, apoB48-iAUC (which is more reflective of postprandial chylomicrons), provided a very strong and highly significant association with fasting insulin and the ratio of TG:apoB48, a relationship that is not often detectable in humans. Predictably, fasting concentrations of TG were also strong indicators of almost all the parameters measured, and is likely a reflection of the sustained overproduction of VLDL in the *cp/cp* phenotype. In addition the ratio of fasting TG:apoB48 correlated well with the corresponding AUC of the ratio TG:apoB48 and fasting leptin concentrations. Fasting insulin concentrations provided some of the strongest associations including iAUC for both TG and leptin, consistent with characteristic patterns observed during the development of insulin-resistance.

4. Discussion

The JCR:LA-*cp* rat model has been used to explore the effects of hyperinsulinemia on vascular dysfunction and the development of micro/macro vascular complications in the metabolic syndrome [24]. Despite these studies, there has been no description to date regarding the potential of pre-diabetic rodent models to investigate the role that postprandial lipoproteins may play in accelerating atherosclerotic risk. In addition, clinical studies to date have failed to provide a definitive association between impaired postprandial metabolism (defined in this study as apoB48-iAUC) and the early phases of insulin-resistance and its corresponding risk indices [15,38]. In this study, we demonstrate for the first time, using the unique JCR:LA-*cp* rodent model, that impaired postprandial lipoprotein metabolism is an inherent

Table 2

Pearson correlation coefficients for fasting and change in the postprandial response (iAUC) for JCR:LA-*cp* rodent model following an oral fat challenge

	ApoB48 (hepatic/intestinal)	ApoB48 ^{iAUC} (PP-Cm)	TG (basal-VLDL/Cm)	Ratio TG:apoB48 (VLDL/Cm-size)	Insulin (IR)	Leptin (adipocyte)
ApoB48 ^{iAUC}	0.586 ^a	–	0.310 n.s.	0.148 n.s.	0.874 ^c	0.480 n.s.
TG	0.334 n.s.	0.310 n.s.	–	0.849 ^c	0.765 ^b	0.779 ^c
TG ^{iAUC}	0.575 ^a	0.582 ^a	0.740 ^b	0.520 n.s.	0.821 ^c	0.667 ^c
Ratio (TG:apoB48)	–0.122 n.s.	0.849 ^c	0.849 ^c	–	0.546 n.s.	0.643 ^a
Ratio (TG:apoB48) ^{iAUC}	0.117 n.s.	0.695 n.s.	0.863 ^c	0.838 ^b	0.608 n.s.	0.480 n.s.
Insulin	0.573 n.s.	0.874 ^c	0.765 ^b	0.546 n.s.	–	0.911 ^c
Insulin ^{iAUC}	0.682 ^a	0.287 n.s.	0.746 ^c	0.330 n.s.	0.410 n.s.	0.573 ^a
Leptin	0.430 n.s.	0.480 n.s.	0.779 ^c	0.643 ^a	0.911 ^c	–
Leptin ^{iAUC}	0.275 n.s.	–0.500 n.s.	0.203 n.s.	–0.357 n.s.	–0.486 n.s.	–0.569 ^a

Values are in the fasted state (at baseline 0-h) unless otherwise stated; interpretation of indices in parentheses: PP, postprandial; Cm, chylomicrons; IR, insulin-resistance; iAUC, incremental area under the curve between 0 and 10 h following oral fat challenge; TG, plasma triglyceride; VLDL, very low density lipoprotein; n.s., not statistically significant; (–), not calculated.

^a $p<0.05$.

^b $p<0.01$.

^c $p<0.001$.

component of this condition and is intimately associated with early markers of insulin-resistance.

4.1. Significance of the incremental apoB48 postprandial profile during pre-diabetes

We have revealed significant lipemia of apoB48-containing particles following an oral fat load in the JCR:LA-*cp* animals, particularly in the early phase of the postprandial period. We have also observed that the later phase of the kinetic profile is indicative of small atherogenic remnant-sized particles, which continued to circulate in the plasma compartment throughout the 10-h period. Importantly, we have determined that fasting concentrations of apoB48 in this model (i.e. contributions from both intestinal and hepatic sources), correlate with corresponding changes to apoB48 during the postprandial response (iAUC) (Table 2). We speculate that an oral fat load in these animals would contribute substantially to the atherogenic processes by facilitating saturation of lipolytic pathways, reduce the clearance capacity of cholesterol-rich lipoproteins and exacerbate the permeability/retention of lipid in arterial vessels [15,38]. Furthermore, we show that increases in iAUC for apoB48 correlate significantly with corresponding increases in both iAUC for triglyceride and fasting insulin concentrations. Collectively, these data suggest that impaired postprandial metabolism may be an accurate predictor of the early pre-diabetic phase associated with insulin-resistance, which is an observation that to date has been difficult to detect in humans.

4.2. Oversecretion of VLDL and hypertriglyceridemia in JCR:LA-*cp* animals

Hypertriglyceridemia is an early metabolic abnormality characteristic of the JCR:LA-*cp* rat and appears to develop in response to several lipidogenic factors [27]. Physiologically, it is also important to consider the impact of the hyperphagic behavior in the *cp/cp* phenotype. The obese animals are potentially in a postprandial state continuously for up to 16–20 h per day, which contributes to sustained secretion of TG from the intestine (in the form of chylomicrons). The increase in dietary substrate due to hyperphagia can also facilitate increased carbohydrate derived synthesis of lipid at the site of the liver. Together, these factors are derivatives for significant biochemical modulations including the up-regulation and hypersecretion of VLDL in the hepatocyte [35,47,48].

Closer analysis of the TG:apoB48 ratio (Fig. 4), reveals that at times 2 and 4 h, there is a very clear separation of larger more buoyant particles in the *cp/cp* phenotype (likely to be newly secreted apoB48 chylomicrons). The accumulation of larger particles in the early phase of the postprandial period also infers an initial hydrolytic defect, which is a possible consequence of increased competition for LPL. Previous work in the JCR:LA-*cp* rat has shown that despite the increased production of hepatic VLDL, proportional increases in mRNA for the LPL activator, apoCII have not been found [47].

Intriguingly though, the larger TG-enriched particles did not persist in the circulation of *cp/cp* animals after 5 h of the fat challenge, suggesting that this defect is only affecting the initial phase of the cascade. These later observations are consistent with reports that the activity of endothelial lipases and the cleavage of fatty acids from circulating triglyceride-rich-lipoprotein fractions is not impaired in the JCR:LA-*cp* rat [33].

4.3. Metabolic effects of postprandial insulin

In human obese subjects, studies have reported that a high fat meal does not necessarily stimulate insulin secretion during the postprandial period above that observed during baseline (as assessed by absolute concentrations) [49,50]. Similarly, results in this study demonstrate that a TG-enriched meal does not markedly alter absolute concentrations of plasma insulin during the postprandial phase (Fig. 5). However, when expressed as the percentage change from fasting baseline (iAUC), we reveal that despite the hyperinsulinemia of these animals, plasma insulin iAUC does increase significantly during the postprandial period in response to an oral fat meal (which also contains up to 50% carbohydrate).

4.4. Hyperleptinemia, postprandial metabolism and the JCR:LA-*cp* rat

The JCR:LA-*cp* rat exhibits hyperleptinemia and the circulating values of leptin are similar to that commonly seen in obese humans with leptin resistance [50]. We know that differences in the level of the postprandial leptin response can occur depending on the macro-nutrient composition of the meal [49]. Havel and colleagues have suggested that decreased leptin secretion during the postprandial phase resulting from high dietary fat may facilitate weight gain associated with the regular intake of high fat meals [45]. Moreover, during the insulin-resistant state, as in the case of the JCR:LA-*cp* rat, insulin does not have the capacity to modulate leptin secretion as readily as it does under normal conditions. Therefore, our results suggest that in susceptible individuals, who are both insulin-resistant and hyperleptinemic, the regular consumption of high fat meals may contribute to impaired postprandial metabolism and sustained weight gain.

In conclusion, the results of this study show for the first time that the JCR:LA-*cp* rat has a marked accumulation of postprandial apoB48 particles following an oral fat challenge. In turn, postprandial changes in iAUC for apoB48 appear to correlate significantly with changes in insulin-resistance. The *cp/cp* phenotype is pre-disposed to the development of atherosclerosis and our results support the hypothesis that increased circulating concentrations of apoB48-containing particles can exacerbate the exposure and retention of these cholesterol-rich lipoproteins within the arterial wall. Our findings highlight the potential atherogenic role of postprandial apoB48 Cm-r lipoproteins in the development of

atherosclerosis, particularly during conditions of obesity and early insulin resistance.

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References

- [1] Zilversmit DB. Atherogenic nature of triglycerides, postprandial lipidemia and triglyceride rich remnant lipoproteins. *Clin Chem* 1995;41:153–8.
- [2] Huff MW. Dietary cholesterol, cholesterol absorption, postprandial lipemia and atherosclerosis. *Can J Clin Pharmacol* 2003;10(Suppl A):26A–32A.
- [3] Twickler T, Dallinga-Thie GM, Chapman MJ, Cohn JS. Remnant lipoproteins and atherosclerosis. *Curr Atheroscler Rep* 2005;7(2):140–7.
- [4] Cabezas MC, Erkelens DW. Triglycerides and atherosclerosis: to feast or fast. *Neth J Med* 2000;56(3):110–8.
- [5] Proctor SD, Vine DF, Mamo JCL. Arterial retention of apolipoprotein-B48 and B100-containing lipoproteins in atherogenesis. *Curr Opin Lipidol* 2002;13(5):461–70.
- [6] Proctor SD, Vine DF, Mamo JCL. Arterial permeability and efflux of apolipoprotein B-containing lipoproteins assessed by in situ perfusion and three-dimensional quantitative confocal microscopy. *Arterioscler Thromb Vasc Biol* 2004;24(11):2162–7.
- [7] Proctor SD, Mamo JCL. Intimal retention of cholesterol derived from apolipoprotein B100 and apolipoprotein B48-containing lipoproteins in carotid arteries of Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol* 2003;23(9):1595–600.
- [8] Cabezas MC, de Bruin TW, Westerveld HE, Meijer E, Erkelens DW. Delayed chylomicron remnant clearance in subjects with heterozygous familial hypercholesterolaemia. *J Intern Med* 1998;244(4):299–307.
- [9] Tomkin GH, Owens D. Abnormalities in apo B-containing lipoproteins in diabetes and atherosclerosis. *Diab Metab Res Rev* 2001;17(1):27–43.
- [10] Proctor SD, Pabla CK, Mamo JCL. Arterial intimal retention of pro-atherogenic lipoproteins in insulin deficient rabbits and rats. *Atherosclerosis* 2000;149(2):315–22.
- [11] Taggart C, Gibney J, Owens D, et al. The role of dietary cholesterol in the regulation of postprandial apolipoprotein B48 levels in diabetes. *Diab Med* 1997;14(12):1051–8.
- [12] Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* 1994;106(1):83–97.
- [13] Schaefer EJ, McNamara JR, Shah PK, et al. Elevated remnant-like particle cholesterol and triglyceride levels in diabetic men and women in the Framingham Offspring Study. *Diab Care* 2002;25(6):989–94.
- [14] Chan DC, Watts GF, Barrett PH, et al. Relationships between cholesterol homeostasis and triacylglycerol-rich lipoprotein remnant metabolism in the metabolic syndrome. *Clin Sci (Lond)* 2003;104(4):383–8.
- [15] Mamo JC, Watts GF, Barrett PH, et al. Postprandial dyslipidemia in men with visceral obesity: an effect of reduced LDL receptor expression? *Am J Physiol Endocrinol Metab* 2001;281(3):E626–32.
- [16] Chan DC, Watts GF, Barrett PH, et al. Effect of atorvastatin on chylomicron remnant metabolism in visceral obesity: a study employing a new stable isotope breath test. *J Lipid Res* 2002;43(5):706–12.
- [17] Mekki N, Christofilis MA, Charbonnier M, et al. Influence of obesity and body fat distribution on postprandial lipemia and triglyceride-rich lipoproteins in adult women. *J Clin Endocrinol Metab* 1999;84(1):184–91.
- [18] Despres JP, Marette A. Relation of components of insulin resistance syndrome to coronary disease risk. *Curr Opin Lipidol* 1994;5(4):274–89.
- [19] Moller DE, Kaufman KD. Metabolic syndrome: a clinical and molecular perspective. *Annu Rev Med* 2005;56:45–62.
- [20] Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005;365(9468):1415–28.
- [21] Caballero AE. Metabolic and vascular abnormalities in subjects at risk for type-2 diabetes: the early start of a dangerous situation. *Arch Med Res* 2005;36(3):241–9.
- [22] Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diab Care* 2004;27(5):1047–53.
- [23] The International Diabetes Federation consensus worldwide definition of the metabolic syndrome. Embargo; 2005.
- [24] Brindley DN, Russell JC. Animal models of insulin resistance and cardiovascular disease: some therapeutic approaches using JCR:LA-*cp* rat. *Diab Obes Metab* 2002;4(1):1–10.
- [25] Tofovic SP, Jackson EK. Rat models of the metabolic syndrome. *Meth Mol Med* 2003;86:29–46.
- [26] Pederson RA, Campos RV, Buchan AMJ, et al. Comparison of the enteroinsular axis in two strains of obese rats: the fatty Zucker and JCR:LA-corpulent. *Int J Obes* 1991;15:461–70.
- [27] Russell JC, Amy RM, Michaelis OE, McCune SM, Abraham AA. Myocardial disease in the corpulent strains of rats. In: Shafir E, editor. *Frontiers in diabetes research: lessons from animal diabetes III*. London, UK: Smith-Gordon; 1990. p. 402–7.
- [28] Russell JC, Amy RM. Early atherosclerotic lesions in a susceptible rat model: the LA/N-corpulent rat. *Atherosclerosis* 1986;60:119–29.
- [29] Wu-Peng XS, Chua Jr SC, Okada N, et al. Phenotype of the obese Koletsky (f) rat due to Tyr 763 stop mutation in the extracellular domain of the leptin receptor (Lepr). *Diabetes* 1997;46:513–8.
- [30] Russell JC, Shillabeer G, Bar-Tana J, et al. Development of insulin resistance in the JCR:LA-*cp* rat: role of triacylglycerols and effects of MEDICA 16. *Diabetes* 1998;47(5):770–8.
- [31] Russell JC, Koeslag DG, Amy RM, Dolphin PJ. Independence of myocardial disease in the JCR:LA-corpulent rat on plasma cholesterol concentration. *Clin Invest Med* 1991;14(4):288–95.
- [32] Dolphin PJ, Amy RM, Russell JC. Effect of age on serum lipids and lipoproteins of male and female JCR:LA-corpulent rats. *Biochim Biophys Acta* 1990;1042(1):99–106.
- [33] Russell JC, Koeslag DG, Amy RM, Dolphin PJ. Plasma lipid secretion and clearance in hyperlipidemic JCR:LA-corpulent rats. *Arteriosclerosis* 1989;9(6):869–76.
- [34] Dolphin PJ, Stewart B, Amy RM, Russell JC. Serum lipids and lipoproteins in the atherosclerosis prone LA/N corpulent rat. *Biochim Biophys Acta* 1987;919(2):140–8.
- [35] Vance JE, Russell JC. Hypersecretion of VLDL, but not HDL, by hepatocytes from the JCR:LA-corpulent rat. *J Lipid Res* 1990;31:1491–501.
- [36] Cohn JS, Marcoux C, Davignon J. Detection, quantification, and characterization of potentially atherogenic triglyceride-rich remnant lipoproteins. *Arterioscler Thromb Vasc Biol* 1999;19(10):2474–86.
- [37] Phillips C, Murugasu G, Owens D, et al. Improved metabolic control reduces the number of postprandial apolipoprotein B-48-containing

- particles in type-2 diabetes. *Atherosclerosis* 2000;148(2):283–91.
- [38] Chan DC, Watts GF, Barrett PH, Mamo JC, Redgrave TG. Markers of triglyceride-rich lipoprotein remnant metabolism in visceral obesity. *Clin Chem* 2002;48(2):278–83.
- [39] Smith D, Watts GF, Dane-Stewart C, Mamo JC. Post-prandial chylomicron response may be predicted by a single measurement of plasma apolipoprotein B48 in the fasting state. *Eur J Clin Invest* 1999;29(3):204–9.
- [40] Teng B, Blumenthal S, Forte T, et al. Adenovirus-mediated gene transfer of rat apolipoprotein B mRNA-editing protein in mice virtually eliminates apolipoprotein B-100 and normal low density lipoprotein production. *J Biol Chem* 1994;269(47):29395–404.
- [41] Liu GL, Fan LM, Redinger RN. The association of hepatic apoprotein and lipid metabolism in hamsters and rats. *Comp Biochem Physiol A* 1991;99(1/2):223–8.
- [42] Smith D, Proctor SD, Mamo JC. A highly sensitive assay for quantitation of apolipoprotein B48 using an antibody to human apolipoprotein B and enhanced chemiluminescence. *Ann Clin Biochem* 1997;34(Pt 2):185–9.
- [43] Jackson KG, Williams CM. Apolipoprotein B-48: comparison of fasting concentrations measured in normolipidaemic individuals using SDS-PAGE, immunoblotting and ELISA. *Atherosclerosis* 2004;176(2):207–17.
- [44] Proctor SD, Kelly SE, Russell JC. A novel complex of arginine-silicate improves micro- and macrovascular function and inhibits glomerular sclerosis in insulin-resistant JCR:LA-*cp* rats. *Diabetologia* 2005;48(9):1925–32.
- [45] Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. *Diabetes* 2004;53(Suppl 1):S143–51.
- [46] Teff KL, Elliott SS, Tschop M, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab* 2004;89(6):2963–72.
- [47] Elam MB, Wilcox HG, Cagen LM, et al. Increased hepatic VLDL secretion, lipogenesis, and SREBP-1 expression in the corpulent JCR:LA-*cp* rat. *J Lipid Res* 2001;42(12):2039–48.
- [48] Mantha L, Russell JC, Brindley DN, Deshaies Y. Developmental changes in adipose and muscle lipoprotein lipase activity in the atherosclerosis-prone JCR:LA-corpulent rat. *Int J Obes Relat Metab Disord* 2002;26(3):308–17.
- [49] Havel PJ, Townsend R, Chaump L, Teff K. High-fat meals reduce 24-h circulating leptin concentrations in women. *Diabetes* 1999;48(2):334–41.
- [50] Romon M, Lebel P, Fruchart JC, Dallongeville J. Postprandial leptin response to carbohydrate and fat meals in obese women. *J Am Coll Nutr* 2003;22(3):247–51.