Chylomicron and apoB48 metabolism in the JCR:LA corpulent rat, a model for the metabolic syndrome


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Abstract

Postprandial (PP) lipaemia is a significant contributor to the development of dyslipidaemia and cardiovascular disease (CVD). It is also evident that PP lipaemia is prevalent during conditions of obesity and insulin resistance (IR) and may contribute to increased progression of CVD. Our group has assessed the potential of the obese JCR:LA-cp rat as a model of PP lipaemia in order to explore CM (chylomicron) metabolism during the onset and development of IR in the metabolic syndrome. Studies confirm that both fasting plasma and PP apoB48 (apolipoprotein B48) area under the curve are significantly elevated in the obese JCR:LA-cp phenotype as compared with lean controls. Mechanistic studies have also shown that the concentration of lymphatic CM apoB48 and CM size are significantly increased in this model. Furthermore, PP dyslipidaemia in the obese rat can be improved acutely with supplementation of n–3 polyunsaturated fatty acids. Using a different approach, we have subsequently hypothesized that the vascular remodelling that accompanies IR may explain accelerated entrapment of apoB48-containing particles. Small leucine-rich proteoglycans (including biglycan and decorin) have been observed to co-localize with apoB in human tissue. However, the potential impact of IR on vascular remodelling, particularly in the presence of obesity, remains unclear. Preliminary observations from the JCR:LA-cp model indicate that biglycan protein core content increases with age and is exacerbated by IR, suggestive of pro-atherogenic remodelling. The focus of this review is to contribute to the perspective of PP lipaemia in CVD risk associated with the metabolic syndrome through the use of animal models.

Introduction to the clinical problem

It is evident that PP (postprandial) lipaemia is prevalent during conditions of obesity and IR (insulin resistance) and may contribute to increased progression of CVD (cardiovascular disease). However, a significant clinical dilemma still exists in diagnosing the early phases of the metabolic syndrome (i.e. pre-diabetes) and how this impacts on relative risk of CVD. In part, this has been impaired by the continued emphasis on LDL (low-density lipoprotein), which is often normal during early Type 2 diabetes, leading to undetected yet insidious progression of CVD [1,2]. Indeed it is interesting to note that the recent revision by the IDF (International Diabetes Federation) has defined the metabolic syndrome independent of LDL cholesterol concentration [3].

In the clinic, we continue to recognize the positive effects of LDL-lowering therapy on atherosclerosis and CVD. While these efforts are well documented, increasing evidence supports a causal role between the metabolism of intestinally derived CMRs [CM (chylomicron) remnants] and the development of atherosclerosis [4]. CMs are TAG (triacylglycerol) rich when initially secreted by the enterocyte. Once in circulation, CM particles rapidly undergo hydrolysis to produce cholesterol-dense remnants [5,6]. These TAG-depleted remnants have been shown to be atherogenic as they are able to penetrate arterial tissue and become entrapped within the subendothelial space [7]. It has also been demonstrated that CMRs can induce macrophage lipid loading, which is a hallmark feature of early atherogenesis [8,9]. Moreover, raised fasting concentrations of apo (apolipoprotein) B48, a specific marker for CMs and their remnants [10], have been shown to be elevated in obese, insulin-resistant and Type 2 diabetic subjects [11–14]. However, clinical studies have so far failed to provide a definitive association between impaired PP metabolism and the very early phases of IR and corresponding risk indices [11–14]. Thus animal models offer the potential for further characterization of the metabolic syndrome in order to understand the metabolic and PP profile of this condition. Despite a greater emphasis on the study of CVD risk in the metabolic syndrome, there remains a dearth of well-characterized pre-diabetic models in order to investigate the role of PP lipoprotein metabolism in the development of atherosclerosis.

JCR:LA-cp rat as a model of PP lipaemia

Animal models of obesity and the metabolic syndrome usually have defects in the metabolism of leptin and its receptor. Some of these models include the ob/ob and db/db mice, the Zucker (fa/fa) and the JCR:LA-cp rat [15,16]. Phenotypically, these rodent models develop symptoms...
associated with the metabolic syndrome including obesity, IR, hypertriglyceridaemia and/or hyperleptinaemia [15,16]. Of these animal models, only the JCR:LA-cp rat model has been reported to spontaneously develop pathological complications, such as atherogenesis and myocardial ischaemia that are consistent with CVD complications seen in men [15].

The plasma lipid profile of the JCR:LA-cp (cp/cp) rat has been characterized extensively over recent years [15]. The cp/cp phenotype has mildly higher total plasma cholesterol levels compared with lean (+/−) counterparts [15]. The observed increase in total cholesterol has been associated with the VLDL (very-low-density lipoprotein), and not the LDL fraction, similar to that observed in the pre-diabetic state in humans. As a result, the JCR:LA-cp model provides a unique opportunity to study dyslipidaemia in the pre-diabetic state.

Recently, we assessed the metabolism of PP CMs in the JCR:LA-cp rat. Our approach for these studies has been to develop a novel, oral fat challenge test for our rodents analogous to the existing approach used in clinical studies [17,18]. After an overnight fast (16 h), animals are offered a 5 g food pellet supplemented with dairy fat (approx. 30% carbohydrate, 60% fat, 10% protein w/w). Our methodology uses a well-established standardized conscious, non-restraint protocol [19]. Given that the PP phase (0–4 h) predominantly represents plasma apoB48 derived from the intestine, we utilized this as a guide to assess CM metabolism in the JCR:LA-cp rat [19]. In addition, our group has developed techniques to accurately detect the presence of apoB48 in plasma using a highly sensitive Western blotting/enhanced chemiluminescent procedure [19–21].

Using our approach in the JCR:LA-cp rat, we have observed significant lipaemia associated with apoB48-containing particles, particularly in the early PP phase. Importantly, we have determined that fasting concentrations of apoB48 in this model, despite having contributions from both intestinal and hepatic sources [19,22,23], can predict the corresponding change in PP response, as measured by the incremental area under the curve [19]. We have also observed that the kinetic profile of PP apoB48 demonstrates a significant and exacerbated delay following an oral fat challenge. These results are consistent with reports in human subjects that also show elevated levels of fasting apoB48 in individuals at risk of CVD [11,14]. Thus an oral fat load (and/or challenge) in these animals is likely to contribute to pro-atherogenic processes by either facilitating the saturation of lipolytic pathways, reducing the clearance capacity of cholesterol-rich lipoproteins, or exacerbating the permeability and retention of cholesterol in arterial vessels [11,14]. Consequently, evidence from our studies supports the hypothesis that the JCR:LA-cp model provides a unique means to clarify further the pathological role of PP lipaemia during the pre-diabetic state.

**Hypertriglyceridaemia and VLDL overproduction: a major feature of IR**

An important characteristic of patients with IR is the overproduction of VLDL, with increased secretion of both apoB100 and TAG [24]. Physiologically, it is also essential to consider the PP phase and the often hyperphagia-like behaviour observed during clinical obesity. Humans (and animals) are potentially in a PP state continuously for up to 16–20 h per day. This in turn contributes to a sustained secretion of CM-associated TAG from the intestine, which contributes to circulating TAG levels. Increases in the dietary carbohydrate substrate can also facilitate increased synthesis of lipid at the site of the liver. Thus hyperphagia and IR collectively generate significant biochemical modulations in the liver including the up-regulation and hypersecretion of VLDL [15]. Consistent with observation in humans, the JCR:LA-cp model also presents with classic hypertriglyceridaemia and VLDL oversecretion which appears to develop in response to several lipidogenic factors, including SREBP-1c (sterol regulatory element-binding protein-1c) regulation [15,19].

SREBPs are a family of transcription factors that regulate cholesterologenesis and lipogenesis. *In vivo* studies have shown that the SREBP-1c isoform is primarily regulated by insulin [25]. SREBP-1c levels are increased in the liver of obese, insulin-resistant and hyperinsulinaemic ob/ob mice [26,27], and the oversecretion of VLDL in JCR:LA-cp animals has been attributed to the dysregulation of endogenous fatty acid synthesis via increased expression of SREBP-1c [15]. Further contributions to increased plasma TAG concentrations may be due to down-regulation in muscle LPL (lipoprotein lipase) activity and increased adipose LPL activity observed in the JCR:LA-cp rat [15]. Very recent evidence also implicates the overexpression of PCSK-9 (proprotein convertase subtilisin kexin type 9) in VLDL oversecretion [28]. However, the regulatory aspect of PCSK-9 during IR in influencing LDL (apoB100/E) receptor expression and impact on apoE-containing lipoproteins remains largely unknown.

**The role of insulin in intestinal CM production and metabolism**

CMs are synthesized in the enterocyte and deliver endogenous and dietary lipids to the circulation via the lymphatic system. Insulin has been shown to have an acute inhibitory effect on apoB48 production in enterocytes isolated from chow-fed animals, but this effect is not seen in animals that have hyperinsulinaemia [29,30]. Studies in humans have shown that modest delays in PP apoB48 peak response may be associated with insulin levels in both individuals with fasting hyperinsulinaemia and normal controls [31]. Collectively, it appears that the enterocyte is sensitive to circulating insulin levels, but in conditions of chronic hyperinsulinaemia, the enterocyte may become resistant to these effects [30,31]. However, very little is known regarding the effect of the amount and type of dietary lipid on CM synthesis and clearance in the circulation, particularly in IR. Today, few laboratories have the expertise to undertake *in vivo* lymphatic isolation of CMs in order to measure CM intestinal synthesis and secretion directly. Preliminary data from our laboratory have shown that in the hyperinsulinaemic JCR:LA-cp phenotype, the lymphatic apoB48 concentration...
There is evidence from several studies (using both animal models and humans) that an equivalent fat load (challenge) will result in very divergent response of the intestine when comparing normal and insulin-resistant conditions. The exaggerated secretion of CMs from an ‘insulin-resistant’ intestine is now thought to contribute to the accumulating presence of TAG-rich particles in plasma. Furthermore, delayed clearance of apoE-containing CMR particles via the LDL-R (LDL receptor) and LRP-R (LDL receptor-related protein receptor) pathways ensures a continued exposure of atherogenic particles to the arterial wall. This in turn provides a platform for modified vascular function and exacerbated atherogenesis both during pre-diabetes and the metabolic syndrome.

Effect of $n-3$ PUFA (polyunsaturated fatty acid) supplementation on PP lipaemia in the JCR:LA-cp rat

The literature demonstrates that consumption of $n-3$ PUFAs decreases total plasma TAG and lipogenesis, potentially by inhibiting the activation of SREBP-1c [32]. We have assessed the effect of $n-3$ PUFA supplementation on PP lipid, adipokine and inflammatory markers in the JCR:LA-cp rodent model. We have discovered that relative to obese control animals, treatment of JCR:LA-cp rats with $n-3$ PUFA significantly reduces concentrations of fasting plasma TAG,
total cholesterol, leptin and apoB48. Furthermore, n = 3
PUFAs can significantly improve the PP response (area under
the curve) for both TAG and apoB48 in this animal model.
In this meeting, we report evidence supporting the view that
modest dietary n = 3 PUFA supplementation can potentially
reduce both PP dyslipidaemia and the pro-inflammatory
status associated with IR [33].

**CMRs: increased atherogenicity for the
metabolic syndrome**

Evidence now suggests that remnant lipoproteins (including
intestinal apoB48-containing CMRs) can contribute to atherogenesis
and are a significant risk factor for CVD [4–7,34–36]. We have compared the delivery and efflux of both CMR
lipoproteins and LDL in the vessel wall in order to further
understand the factors that regulate cholesterol accumulation
in early atherogenesis [37]. Our results indicate that, whereas
LDL particles have a higher rate of delivery, they efflux more
readily from arterial tissue compared with the larger
CMRs. Collectively, our findings have highlighted that
lipoproteins permeate through arterial tissue differently, and
the flux of particles may be dependent on the phenotype
and potential interactions with extracellular matrix compo-
ents such as arterial proteoglycans. In disease states, such as
diabetes, the arterial wall demonstrates an exacerbated uptake
and retention of lipoprotein-derived cholesterol, including
apoB48-containing CMR particles [34–36]. Thus we have
hypothesized that the response-to-retention aetiology of ath-
erosclerosis should extend beyond circulating concentrations
of cholesterol to include the rate of lipoprotein particle
accumulation and retention in the sub-endothelial space
within the arterial wall (see the model proposed in Figure 1).

Among the series of pathogenic mechanisms that con-
tribute to atherosclerosis during IR is the remodelling
of extracellular proteoglycans [37,38]. The proliferation
of vascular smooth muscle cells can stimulate the secretion
of arterial proteoglycans, which in turn can increase the
capacity of lipoprotein binding (in vitro) [39,40]. More
specifically, transforming growth factor (TGF) β-1 has been
identified in atherosclerotic vessels and has been shown to
stimulate the synthesis of chondroitin sulfate and dermatan
sulfate-containing proteoglycans by arterial smooth muscle
cells [40]. JCR:LA-cp rats also have increased circulating concentrations of TGFβ-1 [41]. Recent data from our group sug-
gest that relative to lean controls, there is a significant increase
in biglycan protein core content in the obese JCR:LA-cp rats,
which increases with both age and progressive stages of IR
(Figure 2). Moreover, there is also a significant positive corre-
lation between aortic biglycan protein core content and fasting
insulin levels at 6-, 12- and 32-week time points in obese
rats (Figure 2). Chait and colleagues have demonstrated that
TGFβ-1 can increase proteoglycan–lipoprotein binding due
to the increased length of the glycosaminoglycan chain [40].
Furthermore, Scott et al. [42] have shown (using a comparable
cell culture model of fibroblasts) that TGF β-1 can have pro-
found effects on the regulation of both biglycan and decorin.

**Figure 2** | Content of biglycan (protein core) from aorta of lean
and obese (JCR:LA-cp) rats with increasing age

(A) Aorta from at least five animals for both phenotype and each age
6, 12 and >32 weeks (representative of pre-, during and developed IR in
this model) was subjected to proteoglycan extraction and purification,
as well as GAG digestion, separation by SDS/PAGE and Western
blotting and incubation with an antibody cocktail for biglycan. Bars
represent pooled animal samples (n ≥ 5) that were repeatedly blotted
(n = 3) and measured by densitometry (means ± S.E.M.). *P < 0.01
between phenotype at each age; †P < 0.01 for increasing content of
biglycan with increasing age in obese animals. (B) Correlation of aortic
biglycan protein (µg/µl) with increasing fasting plasma insulin
concentrations.

**Conclusion**

More than 25 years after Zilversmit put forth his alternative
hypothesis (reviewed in [34]), endogenous CM cholesterol
metabolism by the arterial wall has been shown to be a
significant contributor to dyslipidaemia and CVD. Evidence
from our studies supports the hypothesis that the JCR:LA-
cp model can provide a unique means to clarify further
the pathological role of PP lipaemia during the onset and
development of IR in the metabolic syndrome. Preliminary
observations from the JCR:LA-cp model indicate that
biglycan protein core content increases with age and is exacer-
bated by IR, supporting the theory that vascular dysfunction
associated with IR can remodel extracellular proteoglycans
(Figure 1). The insights gained from these studies will
help elucidate our understanding of atherosclerosis, obesity
and Type 2 diabetes and may help to identify new forms
of intervention or direct strategies to reduce further the
mortality and morbidity associated with CVD.
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