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Insulin and Testosterone are Associated with Elevated Intestinal Secretion of Lipids and Lipoproteins in a Rodent Model of the Metabolic and Polycystic Ovary Syndrome

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Abstract

The metabolic syndrome is highly associated with the incidence of Polycystic Ovary Syndrome (PCOS). The metabolic aberrations of insulin resistance and hyperandrogenemia have been proposed to contribute to the development of dyslipidemia and increased Cardiovascular Disease (CVD) risk in PCOS. The contribution of non-fasting intestinal lipemia to CVD risk is well established, however little is known regarding the role of androgens in regulating these pathways in conditions of the metabolic syndrome and PCOS. The aim of this study was to determine the contribution of the intestine to lipemia, and the relationship with serum testosterone and insulin in the JCR: LA-cp rodent model of the metabolic syndrome and PCOS. Fasting and non-fasting plasma lipid and intestinal apolipoprotein (apo) B- metabolism were measured in the fasting state, and non-fasting state in response to a high-fat meal. In addition, intestinal mesenteric lymph cannulation was used to determine the intestinal secretion of apoB-lipoproteins and associated lipids. The PCOS-like genotype was observed to have elevated fasting and non-fasting plasma triglyceride, cholesterol and apo-B48 (a marker of intestinal-derived apoB-lipoproteins) compared to control animals. Intestinal lymphatic secretion of apoB48-lipoproteins, triglyceride and cholesterol were elevated in the fasted and non-fasted state. Serum total testosterone and insulin were shown to be positively correlated with fasting and non-fasting plasma triglyceride and apoB48 concentrations. In conclusion, the intestinal oversecretion of apo-B48 lipoproteins and lipids contributes to dyslipidemia in the JCR: LA-cp rodent model of the metabolic syndrome and PCOS. This model may be used to further our understanding of the pathophysiology of dyslipidemia and CVD risk in conditions of hyperandrogenemia and insulin resistance in PCOS.

Keywords: Metabolic syndrome; Prediabetes; Polycystic ovary syndrome; Dyslipidemia; Lipemia; ApoB-lipoproteins; Intestine; Hyperandrogenemia; Testosterone; Insulin; Insulin resistance

Introduction

Prediabetes or the metabolic syndrome is highly associated with the development of polycystic ovary syndrome (PCOS) [1-5]. The clustering of cardiometabolic and endocrine aberrations appears to predispose individuals to the increased risk of Cardiovascular Disease (CVD) and Type 2 Diabetes [5-7]. Dyslipidemia is a predominant cardiometabolic risk factor in PCOS, occurring in 70% of adolescents and adult women with PCOS (**8-11**). The dyslipidemic profile of individuals with PCOS varies but commonly includes elevated fasting plasma concentrations of Triglyceride (TG), LDL-cholesterol, Total Cholesterol (TC), total apolipoprotein-B (apoB), and decreased HDL [8,11-18]. These lipid aberrations contribute to an atherogenic lipid profile, however non-fasting or postprandial lipemia, and elevated plasma apolipoprotein-B48 (apoB48, a marker of intestinal chylomicron lipoproteins) are considered to reflect early and exacerbated risk in the development of CVD [19-22].

Postprandial or non-fasting lipemia represents an overproduction or delayed clearance of apoB-containing triglyceride rich lipoproteins (TRLs) from the circulation after a high-fat meal. Postprandial TRLs predominately include chylomicrons (CM, measured by apoB48), which are secreted from the intestine and transport dietary and endogenous lipids to the circulation, and very low-density lipoprotein (VLDL, measured by apoB100) which are secreted from the liver [23]. Following rapid lipolysis in the circulation, CM and VLDL form smaller cholesterol-dense remnant lipoproteins, CM remnants and LDL, respectively. Evidence to date demonstrates CM remnants permeate and deliver cholesterol to the arterial wall, and initiate macrophage foam cell formation, a hallmark feature of early atherogenesis [24-26]. Furthermore, the arterial retention of cholesterol derived from CM remnants has been observed to be greater compared to LDL (apoB100). In otherwise normolipidemic individuals with Coronary Artery Disease (CAD) or those predisposed to CVD in conditions of diabetes, MetS and obesity, elevated fasting and non-fasting excursions of CM (apoB48) and TG have been reported to contribute to increased risk of CVD and end-stage CAD events [25,27]. Further evidence from the ACCORD study demonstrates elevated non-fasting remnant-cholesterol dense apoB-lipoproteins contribute to an increased incidence of end-stage cardiovascular events [21,28]. Non-fasting or postprandial lipemia has been reported in PCOS women and may contribute to early CVD development [29].

Our current understanding of the pathophysiological mechanisms underpinning the development of dyslipidemia in PCOS remains limited [7,8,30]. Hyperandrogenism and insulin resistance have been postulated to act independently and/or synergistically to alter lipoprotein metabolism in PCOS [30]. However, hyperandrogenemia has been positively and independently correlated with the

development of metabolic risk and dyslipidemia in PCOS [31]. Indeed, women and adolescents with excess androgens appear to have an exacerbated dyslipidemic profile compared to those with normal androgen concentrations [1,32]. Furthermore, plasma free Testosterone (T) concentrations have been shown to be positively associated with fasting plasma triglycerides and total apoB, independent of body mass index and insulin resistance in PCOS patients [33]. Androgens have the capacity to modulate lipidogenesis and lipoprotein metabolism via the Androgen Receptor (AR) and other lipidogenic nuclear receptors [34,35]. However, the mechanisms of androgen-mediated changes in lipid metabolism are complex, particularly in the etiology of dyslipidemia associated with the metabolic milieu in the PCOS [1,7,8,18,36].

At present there are several rodent models of PCOS, including androgen-induced and genetic variants, which partially mimic the metabolic and reproductive aberrations observed in the clinical PCOS condition [37,38]. Animal models offer the opportunity to study the mechanisms involved in the pathogenesis of dyslipidemia in PCOS. We have established the JCR: LA-cp PCOS-like rodent model, a model that has a recessive defect in the Ob-gene, and develops features of the metabolic syndrome and cardiometabolic risk [39]. Therefore, the aim of this study was to assess the fasting and non-fasting lipid and lipoprotein metabolism, intestinal CM secretion, and the relationship of these atherogenic lipid biomarkers to T and insulin in the JCR: LA-cp rodent model of PCOS.

Materials and Methods

Animals and study protocol

Adult (12 weeks) JCR: LA-cp strain, PCOS-like genotype (cp/cp, n=9) and control non-PCOS genotype (+/?, n=9), were raised in our established breeding colony at the University of Alberta, as described previously [40]. The animals had ad libitum access to standard laboratory rat chow (Lab diet 5001, PMI Nutrition International, Brentwood, MO, USA) and water. 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. We have previously established the female JCR: LA-cp rodent model as a PCOS-like model which displays hyperandrogenemia, acyclicity, arrested follicular development and cystic follicles, accompanied by obesity and insulin resistance (IR) [39]. This genotype (cp/cp) spontaneously develops obesity, metabolic syndrome, and PCOS-like features because it has a recessive defect in the stop codon of the Ob-gene. Therefore an obese control genotype that does not develop PCOS-like features and is matched for body weight is not available, but rather a control heterozygous genotype without the metabolic syndrome is available and used in these studies.

Postprandial lipid and lipoprotein response

Animals were fasted overnight (16 h) and then given a 5.0 g pellet containing 30% (w/w) lipid, and blood samples obtained using a standardized tail vein procedure at times 0, 2, 4, 5, 6, 8 and 10 h following consumption of the pellet as described previously in our laboratory [41]. Blood was collected into tubes containing K2EDTA (ethylene diamine tetraacetic acid, BD Franklin Lakes NJ USA, Cat#367835) at each time point, plasma was separated by centrifugation at 3000 rpm at 4˚C for 10 min, and aliquots of plasma were prepared for immediate lipid (triglyceride and cholesterol) analysis or stored at −80˚C for apoB-lipoprotein analyses [41].

Intestinal chylomicron and lipid secretion

The surgical procedure for intestinal mesenteric lymph cannulation and isolation of intestinal CMs is a standardized protocol described previously in our laboratory [42]. In brief, rats were anaesthetized (3% Isoflurane) and a mesenteric lymph duct and gastric cannula were inserted. Rats were given a constant gastric infusion of saline (1.5 mL/hour for 5 hours) and then Intralipid (Kabi Pharmacia, Sweden) (1.5 mL/hour for 5 hours), and lymph was collected into EDTA (4 mM). Lymph was centrifuged at 3000 rpm for 15 min, flushed with N2, and aliquots frozen at -80˚C or prepared for immediate analysis (particle size, apoB48 and lipid analysis).

Plasma and intestinal lymph biochemical assessment

Total serum T concentrations were determined with commercial Enzyme-Linked Immunosorbent Assay (EIA) kits from ALPCO Diagnostics, USA. (T, Cat#11-TESHU-E01). TG (WAKO, Chemicals USA Inc., Richman, VA, USA, Cat#998-40391/994-40491), TC (WAKO, Cat#439-17501), LDL-C (WAKO Cat#993-00404/999-00504) and HDL-C (Diagnostic Chemical Ltd., Charlottetown, Prince Edward Island, Cat#258-20) plasma concentrations were measured using direct colorimetric chemical enzymatic reactions. Plasma glucose was measured using the glucose oxidase method (Diagnostic Chemicals Ltd., Cat#220-32) and insulin was determined using EIA (ALPCO Diagnostics, USA, Cat#80-INSRT-E01). The quantification of apoB48 was performed on both postprandial plasma and mesenteric lymph samples using established SDS-PAGE western-blot techniques ECL analysis in our laboratory as previously described [41].

Analysis of the postprandial lipid and lipoprotein response

The postprandial response of plasma lipids and lipoproteins was determined using Area under the Curve (AUC) analyses (GraphPad Prism, CA, and USA). The AUC corresponds to the total plasma concentration over the 10 h postprandial period. The fasting concentration of each parameter was subtracted from the total AUC in the postprandial period to yield the incremental area under the curve (iAUC), which represents the postprandial response to the high fat lipid meal as described previously [41].

Statistical analysis

All results are expressed as the means ± SEM. Data were tested for normal distribution, and differences between PCOS and control groups were analyzed using unpaired t-test with significance set at $p<0.05$. Pearson correlation analysis was performed using pair-matched values of each parameter from each animal (Sigma Stat, Jandel Scientific, CA, USA).

Results

Fasting biochemical profile

Fasting plasma concentrations of Triglyceride (TG), Total Cholesterol (TC) and apoB48 (>30 fold) were markedly higher in the PCOS (cp/cp) compared to control animals, and there was no difference in HDL-C or LDL-C concentrations between groups (Table 1), consistent with previous observations in this model [39]. The PCOS cp/cp animals had fasting plasma insulin concentrations eight-fold higher than control animals (Table 1), consistent with previous

findings [39]. To determine the relationship between Testosterone (T) and fasting plasma TG, apoB48 and insulin concentrations pearson correlations were performed and showed that these parameters were positively correlated with T concentration (Table 2).

	Control (+/?)	PCOS (cp/cp)
Body Weight (g)	195.53 ± 6.81	372.60 ± 9.35b
T (ng/mL)	0.35 ± 0.02	0.70 ± 0.03b
Leptin (ng/mL)	5.95 ± 1.15	141.70 ± 9.15b
Insulin (mU/L)	8.75 ± 0.98	84.16 ± 7.95b
TG (mg/dL)	20.20 ± 2.50	875.50 ± 89.92b
TC (mg/dL)	85.80 ± 4.25	135.50 ± 12.35b
HDL-C (mg/dL)	36.70 ± 2.01	39.82 ± 5.15
LDL-C (mg/dL)	18.57 ± 2.13	17.10 ± 2.95
ApoB48 (μg/mL)	8.42 ± 1.20	295.45 ± 22.38b

Table 1: Fasting plasma biochemical parameters in the JCR: LA-cp PCOS-like rodent model. Values are expressed as means ± SEM. a $p<0.05$, b $p<0.001$

	T	TG	TGiAUC	ApoB48	ApoB48iAUC
T0.838c 0.676b 0.873c 0.860c		0.838c	0.676b	0.873c	0.860c
Insulin	0.672b	0.723c	0.783c	0.822c	0.884c
InsuliniAUC	0.793c	0.880c	0.710c	0.837c	0.791c

Table 2: The relationship between testosterone, insulin and lipid parameters measured in the fasted and postprandial state in the JCR: LA-cp PCOS-like rodent model. Values are expressed as mean ± SEM. Pearson correlation coefficients (r) were performed between fasting plasma insulin, testosterone, triglyceride, apoB48, and between testosterone the non-fasting or postprandial response in insuliniAUC, TGiAUC, apoB48iAUC following a high fat meal challenge. TG, fasting plasma triglyceride; TGiAUC incremental area under the curve for TG following high fat meal; apoB48, apolipoprotein B48, ApoB48iAUC incremental area under the curve for apoB48 following high fat meal. a $p<0.05$, b $p<0.01$, c $p<0.001$, represent the significance of the pearson correlation coefficient (r) shown.

Postprandial response of TG, apoB48 and insulin following a high-fat meal

The postprandial response of TG and apoB48, as measured by AUC and iAUC, were markedly increased in PCOS cp/cp animals compared to controls (Figures 1 and 2). The TG postprandial response in PCOS cp/cp animals was approximately >100 mg/dL compared to 20 mg/dL in controls, reflecting a fivefold increase in plasma TG concentrations in cp/cp animals. Similarly, apoB48 response was elevated twenty fold, approximately >100 μg/ml compared to 5 μg/ml in control animals (Figures 1 and 2).

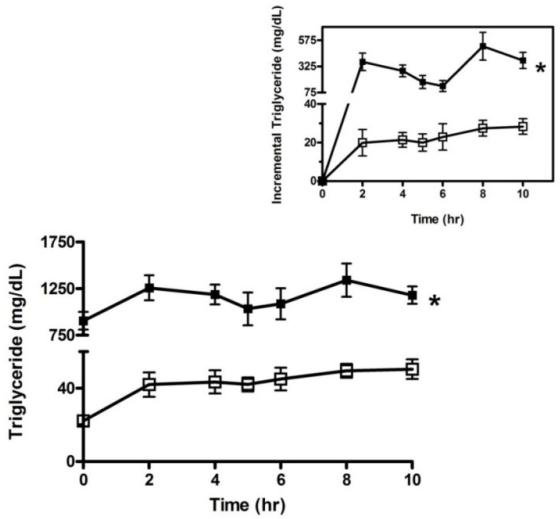

Figure 1: The postprandial response in plasma TG (AUC and iAUC) following a high fat meal in the JCR: LA-cp PCOS-like rodent model. Data are shown for PCOS cp/cp rats (filled squares) and control rats (open squares) as mean ± SEM. The total AUC and the change in TG from fasted concentrations are shown (inset) and represent the incremental area under the curve (iAUC). The AUC for PCOS cp/cp animals is significantly greater than for control animals, (*) $p<0.0001$. The iAUC for PCOS cp/cp animals is significantly greater than for control animals, (*) $p<0.0001$.

Basal fasting TG was positively correlated with total TGAUC ($r=0.976$, $p<0.0001$) and the relative increase in TG response (i.e. TGiAUC) was inversely correlated to fasting TG ($r=0.654$, $p=0.0032$). The postprandial insulin iAUC in PCOS cp/cp animals was significantly increased compared to control animals (Figure 3). Fasting plasma concentrations of T and insulin were positively correlated with the postprandial response of TGiAUC and apoB48iAUC (Table 2). There was a further positive association between the postprandial response of insuliniAUC and both TGiAUC and apoB48iAUC (Table 2), consistent with findings demonstrating insulin's association with lipidogenesis [40].

Intestinal lymph chylomicron and lipid content

The intestinal lymph CM production and content are shown in Table 3. When lymph was collected under conditions of fasting (saline administration), the PCOS cp/cp animals secreted two fold higher the number of CM particles (measured by apoB48, one apoB48 is the core structural component of each lipoprotein particle), compared to controls. The increase in CM particle number was associated with a 50% and 30% increase in TG and cholesterol secretion, respectively. The ratios of total cholesterol (TC)/apoB48 and TG/apoB48 reflect the proportion of TC and TG per CM particle, respectively. Despite the increased particle number in the PCOS cp/cp rats, no difference was observed in the TC/apoB48 or TG/apoB48 ratio between the groups.

As expected, intestinal lymph CM and lipids were increased following intralipid administration, which represents the non-fasting or postprandial state following the high fat meal. Both control and PCOS cp/cp animals had increased secretion of CM lipoprotein particles (apoB48) compared to the fasting state (saline). The PCOS

cp/cp animals secreted >50% more CM particles (apoB48) and TG associated with these particles, compared to controls. In addition, the cholesterol content of the lymph CM was increased 80% in PCOS cp/cp animals compared to their control counterparts. Consistently, the ratio of TC/apoB48 was twofold higher in PCOS cp/cp animals; however there was no difference in TG/apoB48 or particle size between the groups (Table 3).

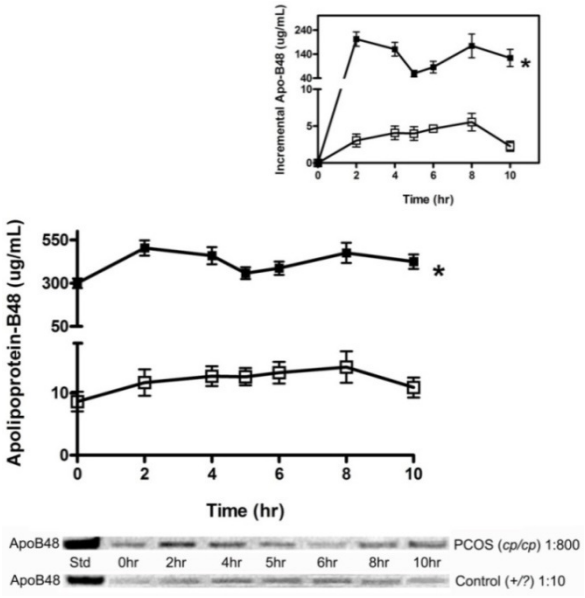

Figure 2: The postprandial plasma apoB48 response (AUC and iAUC) following a high fat meal challenge in the JCR: LA-cp PCOS-like rodent model. Data are shown for PCOS cp/cp rats (filled squares) (n=9) and control rats (open squares) (n=9) as mean ± SEM. The total AUC and the change in apoB48 from fasted concentrations are shown (inset) and represent the incremental area under the curve (iAUC). Both the postprandial apo-B48-AUC and the apoB48-iAUC was significantly greater in the PCOS cp/cp rats compared to control rats, (*) p<0.0001. The lower panel represents a typical western blot detecting plasma apoB48 following the highfat meal (0–10 h).

Discussion

Dyslipidemia is a common metabolic aberration in PCOS and predisposes individuals to increased risk of atheroscerlosis and CVD [7,8,42-44]. Atherogenic lipid profiles in both the fasted and non-fasted state contribute to CVD risk and we are yet to understand fully the pathophysiological mechanisms involved in the development of dyslipidemia in PCOS [7,8,45]. Given the complex metabolic millieu of PCOS, animal models are considered effective tools to study mechanisms associated with these metabolic aberrations in this condition [39,46]. The aim of this study was to evaluate the fasting and non-fasting plasma lipid profile, and associated intestinal CM and lipid secretion in order to facilitate our understanding of postprandial and intestinal lipid metabolism in PCOS. The results of this study demonstrate the JCR: LA-cp/cp PCOS-like rodent has exacerbated postprandial lipemia associated with a marked increase in the secretion of intestinal CM-apoB48, cholesterol and TG, compared to control animals.

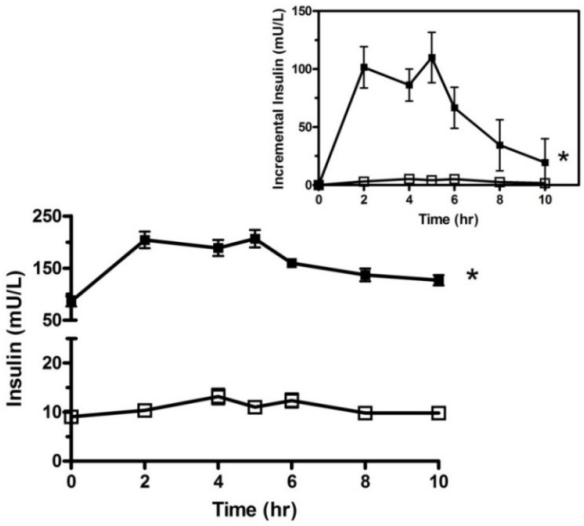

Figure 3: The postprandial response in plasma insulin following a high fat meal in the JCR: LA-cp PCOS-like rodent model. Data are shown for PCOS cp/cp rats (filled squares) and +/? controls (open squares) as mean ± SEM. The total AUC and the change (iAUC) in insulin from fasted concentrations are shown (inset). The PCOS cp/cp animals were shown to have a significantly greater total AUC and iAUC for plasma insulin following the high fat meal challenge, (*) (p<0.0001).

	Saline		2% Intralipid	
	Control (+/?)	PCOS (cp/cp)	Control (+/?)	PCOS (cp/cp)
Triglyceride (mg/hr)	2.14 ± 0.46	5.58 ± 0.76a	13.78 ± 3.58	29.21 ± 2.40b
Total Cholesterol (mg/hr)	0.16 ± 0.02	0.25 ± 0.01a	0.16±0.05	1.03 ± 0.12b
ApoB48 (µg/hr)	232.08 ± 16.21	422.57 ± 44.18a	408.74 ± 40.69	808.40 ± 89.37b
TC/apoB48	0.59 ± 0.08	0.64 ± 0.07	0.47 ± 0.13	1.28 ± 0.20 b
TG/apoB48	8.47 ± 1.53	11.04 ± 1.57	32.72 ± 5.44	36.58 ± 4.40
Particle Size (nm)	130.00 ± 5.47	131.91 ± 4.89	183.50 ± 8.37	197.13 ± 6.84
Data shown are mean ± SEM for control and PCOS cp/cp animals a p<0.01 compared to control rats when given saline (fasted state) b p<0.01 compared to control rats when given 2% intralipid (non-fasted state)				

Table 3: The nascent intestinal lymph chylomicron and lipid content in the JCR: LA-cp PCOS-like rodent model. Values are expressed as means ± SEM.

We have also shown that fasting and postprandial CM-apoB48iAUC and TGiAUC response following a high fat meal are positively correlated with fasting plasma T and insulin concentrations

in this rodent model. Therefore this model may be useful to further examine etiological mechanisms in the development of dyslipidemia associated with hyperandrogenemia and the metabolic syndrome in PCOS. It is well established that adolescents and women with PCOS exhibit a higher incidence of atherogenic lipoproteinemia, usually reported as increased fasting plasma TG, total apoB, LDL-C and low HDL-C [18,31]. However non-fasting or postprandial lipemia, and elevated intestinal-derived CM (CM-apoB48) and their remnants, are now considered critical and independent atherogenic risk factors in in the early development of CVD [19,21,29]. Indeed, increased fasting and non-fasting lipemia associated with elevated intestinal CM-apoB48 may be detected in otherwise normolipidemic individuals who have CAD or those at increased risk for CVD such as obese, diabetic and IR patients [25,28,47,48]. Postprandial lipemia, elevated TG and total apoB, following a high fat meal have been reported in individuals with PCOS, and interestingly this has been observed in both lean and obese women [17]. Plasma total apoB refers to TRL and their remnants, inclusive of both apoB48 (CM/CM-remnants) and apoB100 (VLDL/LDL). However intestinal CM-apoB48 is the predominant apoB protein to change in the non-fasting or postprandial phase following a lipid-rich meal in humans [22]. In this study, we used a standardized high fat meal, comparable to that used in clinical studies, to assess postprandial lipid and lipoprotein metabolism, and we directly measured intestinal secretion of CM-apoB48 and lipids [30,42]. We have demonstrated that PCOS cp/cp rats display non-fasting lipemia, with increased CM-apoB48iAUC and TGiAUC, and this represents over production and impaired clearance of intestinal CM and TG in response to a high fat meal challenge [19,42]. These results further suggest that the non-fasting lipemia and intestinal chylomicronemia contribute to TRL-hypertriglyceridemia in the JCR: LA-cp rodent model [49,50]. In the clinical setting, elevated fasting TG and total apoB, but not elevated apoB48, have been reported in women with PCOS [8,11,13-18]. Overall clinical studies have shown high fasting TG may be predictive of non-fasting or postprandial TG lipemia following a high fat meal [51]. Postprandial lipemia has been reported in women with PCOS in response to a high fat meal and may in part explain the exacerbated CVD risk observed in PCOS [5-7,19-22,30].

Despite the role of hyperandrogenemia in the clinical manifestations of PCOS, little is known about the primary impact of androgens, in particular T, in the development of dyslipidemia in PCOS [7,8,52]. There is clinical correlative evidence suggesting PCOS women and adolescents with excess androgens have exacerbated dyslipidemia compared to those with normal androgen levels [1,53]. In female to male transsexuals, administration of T induces hypertriglyceridemia and androgen-induced animal models of PCOS also present with elevated TG and LDL-cholesterol [54]. A recent study in premenopausal women treated with T demonstrated no effect on hepatic VLDL-TG/apoB100 secretion rates, suggesting T does not regulate hepatic VLDL production [55]. Our results in the JCR: LA-cp rodent model demonstrate non-fasting dyslipidemic markers, intestinal CM-apoB48iAUC and TGiAUC, are positively correlated with plasma T concentrations. The underlying mechanism of hyperandrogenemia driving lipidogenesis has been attributed to T-induction of AR leading to alterations in lipid and lipoprotein metabolism [56,57]. Androgens, non-aromatizable DHT and T, have been shown to induce Peroxisome Proliferator-Activated Receptor (PPAR) and Sterol Response Element Binding Protein (SREBP), to promote lipidogenic pathways in hepatic and prostate cancer cell lines [36,56]. Furthermore, the AR-antagonist flutamide results in significant clinical improvements in atherogenic lipid profile, fasting TG and LDL-cholesterol, in PCOS patients [58,59]. Although not explored in the current study, a flutamide intervention in the JCR: LA-cp rodent model would be useful to elucidate the direct mechanistic role of the AR in mediating increased intestinal lipidogenesis. It is proposed that the intestine is an androgen-responsive organ and intestinal lipidogenesis may be regulated by androgens [56].

In terms of catabolism and clearance pathways, the effects of T on lipolysis and receptor mediated uptake of lipoproteins remain unclear and were not fully explored in this study. Postprandial TG clearance from the plasma is indicative of the extent of lipolysis, which is obligatory for CM-remnant formation and thereafter uptake of remnants by tissues, predominantly the liver. The apolipoprotein compliment of CM modulate lipolysis; apoC-II is a required a co-factor in Lipoprotein Lipase (LPL) activity whereas apoC-I, C-III and apoE inhibit lipolysis [60]. Elevated fasting apoC-III/C-II ratio and apoC-I have been observed in PCOS women, suggesting an inherent impairment in TG hydrolysis [61,62]. Consistently, T administration in female rats following gonadectomy increases total lipoprotein apoC-III which would inhibit TRL lipolysis [63]. T concentrations have been shown to initially increase then decrease following a high fat meal in overweight women with PCOS, inferring an inhibition of lipolysis initially in the non-fasting state [64]. In terms of adipose LPL activity there remains contradictory evidence in the literature. DHT has been shown to induce an increase in LPL protein in isolated adipocytes, whereas elevated T is negatively associated with adipocyte LPL activity in women with PCOS, suggesting a reduction in lipolysis leading to a delayed clearance of TRL's [65,66]. In the JCR: LA-cp rodent model, the male cp/cp genotype has been shown to have enhanced adipose LPL activity favoring uptake of fatty acids and TG storage [67]. Although not measured in this study, an increase in adipose LPL activity is also likely in the female cp/cp genotype but the role of LPL and apoC proteins involved in the impaired clearance of TRL's needs further exploration in the JCR: LA-cp PCOS-like model.

The tissue uptake of TRL-remnants, CM-remnants and LDL, may be altered in PCOS. In-vitro data in hepatocytes has shown androgens reduce the uptake of LDL via a decrease in apoB100/E receptor activity by an androgen-blocking effect on the estrogen receptor [68]. The apoB/E receptor is also the ligand for uptake and clearance of CM-remnants into the liver [60]. Furthermore elevated apoC-I observed in PCOS women may inhibit apoB100/E- or LDL-receptor uptake of apoB-remnant lipoproteins [62]. Collectively, PCOS patients exhibit atherogenic dyslipidemia, which may be associated with impaired lipolysis and receptor mediated uptake, leading to an increased residency time of these cholesterol-dense atherogenic remnant apoB lipoproteins. Increased circulating apoB-lipoprotein remnants have been proposed to exacerbate the atherogenic cascade and CVD risk [69]. The JCR: LA-cp PCOS-like model exhibits increased CM-apoB48 and the ratio of cholesterol per CM particle (TC/apoB48) was twofold higher in PCOS cp/cp rats compared to controls. This suggests these particles may confer exacerbated atherogenicity when delivered and retained by the arterial wall in this PCOS model [70].

The high incidence of IR in PCOS contributes to CVD risk and the link between IR in the development of hepatic and intestinal apoB-lipoproteinemia has been investigated [18,46,71,72]. In this study, we have shown that the postprandial response of plasma TGiAUC and CM-apoBiAUC was positively associated with fasting plasma insulin levels. These results are consistent with findings in lean and obese PCOS patients exhibiting hyperinsulinemia and an exacerbated TG

and total apoB response following a high-fat meal [17]. We have also shown that the postprandial insuliniAUC is associated with TGiAUC and apoB48iAUC, consistent with the physiological insulin-suppressive effect on lipoprotein synthesis [73,74]. It has been proposed that insulin inhibits CM-apoB48 secretion in the postprandial state [75,76]. However, in conditions of hyperinsulinemia and IR, the enterocyte is no longer sensitive to insulin and the secretion of CM-apoB48 is unabated, as observed in our PCOS cp/cp rats [75,77]. It remains unclear if IR and hyperandrogenism have independent effects on lipidogenesis or together exacerbates the dyslipidemia observed in the PCOS cp/cp genotype. T has been proposed to induce AR-mediated IR in PCOS, and T may ligand-activate the AR to down-regulate insulin signaling in peripheral tissues [77]. Further studies using both an insulin-sensitizer, such as metformin, and the AR antagonist flutamide may clarify the independent or synergistic role of T and insulin on lipid metabolism in the JCR: LA-cp PCOS-prone model. Furthermore, the use of non-PCOS or other induced PCOS animal models that do not exhibit MetS and obesity could potentially be used to elucidate the role of androgens and insulin on the pathogenesis of dyslipidemia.

In the clinical setting, a PCOS patient presenting with both IR and elevated fasting apoB48 and non-fasting lipemia in response to a high fat meal may potentially be at increased risk for early development of CVD [8,68,78]. Indeed, the regular consumption of high fat meals may augment postprandial lipemia in susceptible individuals [17,19,51]. Therefore assessing postprandial lipemia and apo-B48 in PCOS may be an important clinical determinant of early CVD risk. Interventions that target both IR and dyslipidemia such as metformin-flutamide combination therapy may be warranted, however it remains unknown whether these drugs modulate postprandial lipemia and chylomicronemia in PCOS [58,79-81]. Certainly evidence from clinical trials suggests statin and ezetimibe therapies may target plasma apoB48-CM and reduce the risk of end stage CVD events [68,78,82]. Alternatively targeting body weight and dietary intake, such as caloric restriction and macronutrient changes, and exercise programs may offer non-pharmaceutical approaches to target non-fasting lipemia and atherogenic lipid profiles in PCOS, and further investigations are warranted in this area [46,83,84].

Conclusions

Dyslipidemia is considered a key metabolic risk factor in the development of CVD risk in women with PCOS. Therefore studies to elucidate the physiological mechanisms associated with androgens and insulin involved in lipidogenesis are important in the development of effective clinical treatments to treat dyslipidemia in PCOS [8,85]. The JCR: LA-cp/cp rat represents a PCOS-like model with non-fasting lipemia associated with intestinal oversecretion of lipids and apoB-lipoproteins and can be used to investigate the etiological mechanisms in the development of dyslipidemia and CVD risk in PCOS.

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