Understanding the Regulation of Endothelial-bound Lipoprotein Lipase Activity in Humans at Risk of Type 2 Diabetes and Cardiovascular Disease

by

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ABSTRACT

Elevated triglycerides (TG) are a hallmark of insulin resistance, which is generally caused by lower lipoprotein lipase (LPL) activity in the vasculature. LPL hydrolyzes TGs into free fatty acids in plasma for use and/or storage in tissues (i.e., adipose tissue, skeletal muscle). Plasma apolipoproteins (Apos) C3 and C2 interact with LPL to modulate its function, and by inhibiting or activating LPL, respectively. Therefore, these proteins play key role in plasma lipid metabolism, but their role in regulating LPL activity in human insulin resistant (IR) (i.e., pre-diabetic) state is not known. Thus, the purpose of this research was to evaluate the concentrations of ApoC3 and ApoC2 in plasma along with the endothelial-bound LPL availability and activity in IR humans and in healthy, insulin sensitive (IS)/control humans. Insulin resistance was evaluated from plasma insulin and glucose responses to an oral glucose tolerance test, and by calculating the Matsuda index. Subjects were placed in the following groups: IR subjects, Matsuda index <4.0 (N=7; 4 males, 3 females); IS, Matsuda index >7.0 (N=11, 9 males, 2 females). IR and IS subjects received an intravenous infusion of insulin (1 mU/kg/min and 0.5 mU/kg/min, respectively) for 30 minutes to stimulate LPL activity. Whole-body endothelial-bound LPL was released from the vasculature by intravenous infusion of heparin. Plasma samples were collected 10 minutes after heparin infusion and analyzed for LPL concentration and activity, and ApoC3 and ApoC2 concentrations. Although plasma LPL concentrations were not different between groups (IR = 457 ± 17 ng/ml, IS = 453 ± 27 ng/ml, $P = 0.02$), plasma LPL activity was higher in the IR subjects (IR = 665 ± 113 nmol/min/ml, IS = 365 ± 59 nmol/min/ml, P = 0.02). IR subjects had higher concentrations of plasma ApoC3 (IR = 3.6 ± 0.5 mg/dl, IS = 2.7 ± 0.2 mg/dl,

P=0.03). However, ApoC2 concentration was not different between groups (IR = 0.15 ± 0.03 mg/dl, IS = 0.11 ± 0.01 mg/dl, $P = 0.11$). These findings suggest that circulating APOC3 and ApoC2 are not key determinants regulating LPL activity during hyperinsulinemia in the vasculature of insulin resistant humans.

DEDICATION

I dedicate this work to my mentor and fellow lab members who have expanded my scientific reasoning skills significantly through the works of this project. I would also like to distinguish my friends and family for exceptional support and motivation throughout my pursuit of this degree. I recognize the School of Life Science for any/all aid and opportunity provided. I would like to especially thank my committee members, Dr. Christos Katsanos, Dr. Elena (Eleanna) De Filippis, and Dr. Richard Herman for their knowledge, dedication, and support.

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CHAPTER 1

INTRODUCTION

The severity of obesity continues to threaten public health with roughly 41.9% of the adult population affected as reported by the Centers for Disease Control in 2021[1]. More specifically, 9.2% of individuals were classified as having severe obesity, and 14.8% had diabetes. Obesity is defined as having a body mass index (BMI) over 30 and entails excessive accumulation of fat which causes risk to an individual's health[2]. The complexity of obesity continues to challenge the scientific community as incident rates continue to increase globally. Metabolic syndrome (MetS) is a cluster of possibly reversible conditions which increase the risk of developing diabetes, stroke, cardiovascular disease, and has been well correlated with obesity[3,4]. It is often diagnosed as having three or more metabolic risk factors (i.e., elevated triglycerides, elevated glucose, reduced HDL cholesterol etc.). Notably, insulin resistance often occurs as a result of obesity-associated MetS. However, the precise pathogenesis remains unknown. Research suggests an oversupply of fatty acids to muscle may contribute to impaired insulin signaling and/or whole-body glucose oxidation[5]. Lipoprotein Lipase (LPL) is critical for lipid metabolism as it breaks down circulating triglycerides and liberating fatty acids for use and/or storage in adipose tissue and skeletal muscle. Interactions between insulin resistance and plasma LPL have been inconsistent and severely understudied in humans.

Mechanisms of Insulin Resistance

In physiological conditions, increased glucose concentration triggers the increase of insulin secretion (and inhibition of hepatic glucose production) to stimulate glucose uptake within tissues. Skeletal muscle is the principal tissue for glucose disposal accounting for roughly 70% of glucose uptake[6]. During a postabsorptive or fasted state, insulin and glucose are low while free fatty acid (FFA) concentrations are higher due to the insufficient suppression of adipocyte lipolysis. After a meal, the elevation of glucose increases insulin secretion from beta cells, subsequently decreasing plasma FFA content (via inhibition of lipolysis)[7]. Concurrently, glucose uptake occurs in the skeletal muscle via the induction of glucose oxidation, as well as storage via glycogen formation. The shift of metabolic muscle energy from oxidation of fat to oxidation of glucose is referred to as metabolic flexibility[8]. In a patho-physiological condition, there is reduced responsiveness to insulin signaling (documented at the levels of insulin receptor substrate, phosphoninositide-3 kinase, protein kinase B, etc.). This defect in insulin-stimulated glucose uptake into muscle is referred to as insulin resistance.

While the exact mechanism(s) of insulin resistance remains unclear, some studies have suggested a role of muscle LPL activity as related to insulin action. Pollare et al. demonstrated an association of LPL activity to glucose infusion rate by comparing muscle LPL activity (downregulated) to adipose LPL activity (unaffected) during a hyperinsulinemic-euglycemic clamp to explain individual variations in fat or lipid tolerance[9]. This finding has been challenged by subsequent research, which found no significant decrease in, at least, total LPL activity[10]. However, the same report noted an inverse correlation between metabolic flexibility and insulin suppression of LPL activity

in postmenopausal women during a hyperinsulinemic-euglycemic clamp. This provides a potential explanation for the reduction in lipid oxidation in insulin resistance, as increased accumulation of fatty acids accompanying increased lipid storage affects insulin signaling via incomplete fatty acid beta-oxidation and decreased glucose metabolism, resulting in decreased responsiveness to insulin suppression of LPL. Understanding the interaction between insulin resistance and LPL could provide valuable insight into metabolic function and contribute to pharmacological intervention for minimizing MetS-associated outcomes, and importantly insulin resistance.

Insulin Resistance and Plasma Lipid Content

The impact of nutrition on insulin resistance is complicated by the physiological process of obesity. It is accepted that increased dietary fat leads to magnified insulin resistance independent of body weight. The elevation of plasma FFAs has been associated with both obesity and insulin resistance. The quality and dose of dietary fat influences insulin sensitivity. For example, decrease in total dietary fat was found to increase insulin sensitivity by 20%[11]. Conversely, total dietary fat content may be less important than quality, as several studies have suggested increased consumption of saturated fat worsens insulin resistance whereas unsaturated fat displays a decreased or 'protective' effect against insulin resistance [12,13,14]. Moreover, dietary fat intake can affect tissue specific lipid content. A study by Boden et al. showed increased FFA content expressed dose like dependence of acute accumulation of intramyocellular lipid (IMCL) content and a subsequent 40% increase in insulin resistance suggesting a close association between FFA content and insulin resistance[15].

Muscle lipotoxicity, or the chronic exposure of muscle tissue to FFA, leads to elevated IMCL concentrations. Krssak et al. used a non-invasive technique to evaluate IMCL content and showed a negative correlation of IMCL and insulin stimulated whole-body glucose update to conclude that skeletal muscle is mainly affected by IMCL rather than plasma non-esterified fatty acids[16]. This study was later supported by additional research which examined the importance of IMCL and insulin resistance including the evaluation of extramyocellular triglyceride content[17]. It was found that whole-body insulin resistance strongly correlated with abnormal muscle triglyceride accumulation. In contrast, Roden et al. speculated the elevation of FFA concentrations increases insulin resistance through the inhibition of glucose transport and reduction of the overall glucose oxidation rate and muscle glycogen synthesis[18]. Regardless the exact mechanisms involved, these findings suggest skeletal muscle insulin resistance is affected by disposal of lipid towards the muscle.

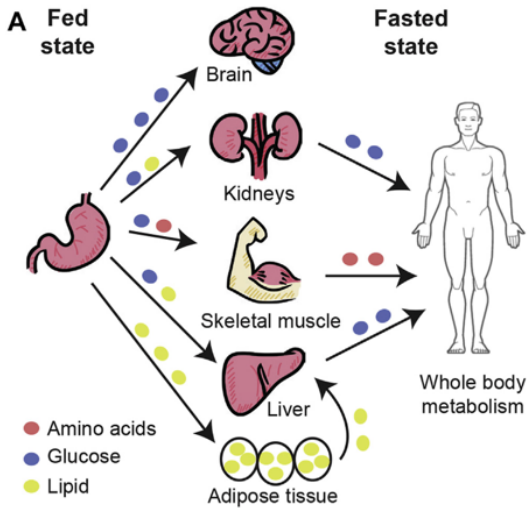


Image 1: Shows glucose metabolism for both fed and fasted states. During the fed state, glucose, lipids, and amino acids circulate for use/storage. During the fasted state, glucose is released from various organs (including gluconeogenesis from substrates originating in adipose tissue and amino acids). Skeletal muscle is the main site for glucose uptake[19] (Image source: Argilés, 2016)[20].

Cellular Regulation of Lipoprotein Lipase

Triglyceride (TG) storage/oxidation is a biological process on which most tissues rely for maintaining energy supply. Plasma TG concentrations increase after ingestion but cannot circulate freely within the blood as a result of their hydrophobic composition. Thus, chylomicrons are formed primarily from digested TG, cholesterol, and apoprotein B48. The chylomicrons are cleared by LPL (also known as "clearing factor" lipase) via catalyzing the breakdown of TG to FFA[21]. Thus, the lipolysis generates FFA to be taken into tissues for oxidation into acetyl CoA, required for initiation of the Krebs cycle, or for storage within these same tissues.

LPL is produced by parenchymal cells of the heart, adipose, and skeletal muscle tissues[22]. While the mechanisms of LPL transport in tissues remains unclear, it is known to bind to the luminal surface of vascular endothelial cells. It is anchored there by heparin sulfate proteoglycans (HSPGs) and transported to that site by glycophosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1)[23,24]. Studies have shown that LPL can be released from endothelial cell binding sites via introduction of heparin[25]. This is thought to be a result of the LPL carboxyl terminal domain, which has binding affinity for heparin and lipoproteins, while the amino terminal domain of LPL is responsible for lipolysis. While LPL can be found as both a monomer and a homodimer, it is generally seen to be active mostly as a homodimer. The regulation of LPL gene

expression in tissues is not yet clear (i.e., transcription, translation etc.). It should be noted, extracellular proteins (i.e., apolipoproteins and angiopoietins) are often evaluated for moderating the function of LPL post-translationally[26].

The human body tightly regulates LPL function during TG influx in plasma (i.e., absorptive state) to meet the nutrient needs of surrounding tissues. In adipose tissue, LPL activity increases after a meal, while LPL activity increases in skeletal muscle in the fasted state[27,28]. This suggests that the regulation of LPL is tissue-specific to adjust lipid distribution across tissues. Wang et al., suggested skeletal muscle LPL deletion not only decreased lipid partitioning in tissues but also affected tissue specific and whole-body insulin resistance in rodents[29]. Other studies have supported this finding by showing that overexpression of LPL in skeletal muscle of rodents leads to increased lipid storage and peripheral insulin resistance[30]. In humans, the mechanisms that affect LPL activity within the vasculature are not clear. The regulation of intravascular LPL in humans is of both physiological and clinical importance because muscle LPL activity may have implications for insulin sensitivity[31]. Also, impaired regulation of intravascular LPL can have metabolic consequences for overall plasma TG concentrations, and that increases the risk for heart disease[32]. Thus, understanding the regulation of LPL activity in the endothelial cells of the vasculature may aid in managing the clinical consequences of obesity.

As mentioned above, the production of LPL is limited to specific tissues (i.e., cardiac, kidney, adipose, and skeletal muscle tissues). The precise physiology of LPL transport to the vasculature remains unclear. Previously it was thought the positively charged heparin domain of LPL (i.e., c-terminal) is bound to negatively charged

HSPGs[33], as heparin injections were observed to release LPL. However, more recent research shows GPIHBP1 is responsible for the transport of LPL into capillaries[34,35]. Once produced by parenchymal cells, LPL remains in the subendothelial space until it is associated with GPIHBP1 to be transported through the endothelium to the luminal side of the capillary.

Rodent studies as early as 2007 have shown GPIHBP1 deficient cell cultures express significantly higher concentrations of plasma TGs and lower LPL function[36]. Interestingly, it was also noted that GPIHBP1 knockout mice express minor amounts of heparin releasable LPL[36]. Speculation regarding a strong heparin binding domain of Apolipoproteins to GPIHBP1 in place of LPL was refuted when Gin et al. found GPIHBP1 bound almost exclusively to LPL[37]. Further, the latter study showed GPIHBP1 does not bind with other members of the lipase family (i.e., hepatic lipase, pancreatic etc.). Subsequent research evaluated GPIHBP1 effects on LPL activity, but established no direct effects, but soluble GPIHBP1 may indirectly affect catalytic activity of LPL via extracellular proteins, such as angiopoietins[38].

In other tissues, GHIPB1 is not seen to interact with LPL. For example, hepatic LPL is thought to access plasma lipoproteins directly via fenestrated capillaries to enhance the liver's primary role of VLDL and ketone production (to spare glucose)[39,40]. The literature has not yet evaluated fed/fasting states of GPIHBP1 and LPL activity. It is thought that higher levels of GPIHBP1 lead to higher levels of LPL in the vasculature. However, variable stability of GPIHBP1 does not significantly affect plasma TG levels dramatically, suggesting LPL modulation is not dependent on GPIHBP1, and because half-normal amounts of GPIHBP1 are sufficient for lipolysis[41]. In humans with

chylomicronemia, Wang et al. revealed severe hypertriglyceridemia was not coupled with GPIHBP1 mutations[42]. It was, however, noted in that study that a rare GPIHBP1 mutation (G56R), was associated with increased levels of plasma TGs and despite normal postheparin-releasable plasma LPL levels.

Endothelial LPL is considered active LPL, as it has the ability to hydrolyze circulating plasma TGs. Dysfunctional LPL results in decreased TG breakdown and thereby elevates plasma TG concentrations. Without an ability to transport to the capillary side, LPL remains in the subendothelial space and appears to be irrelevant for TG breakdown. Although GPIHBP1 is vital for the capture and transport of LPL, it does not appear to affect its activity. This highlights the potential of extracellular modulators of LPL activity and subsequent TG clearance. ApoC2 activates capillary endothelial-bound LPL and increases lipolysis[43]. Accordingly, we speculate Apolipoprotein C2 may be of physiological relevance for endothelial LPL activity.

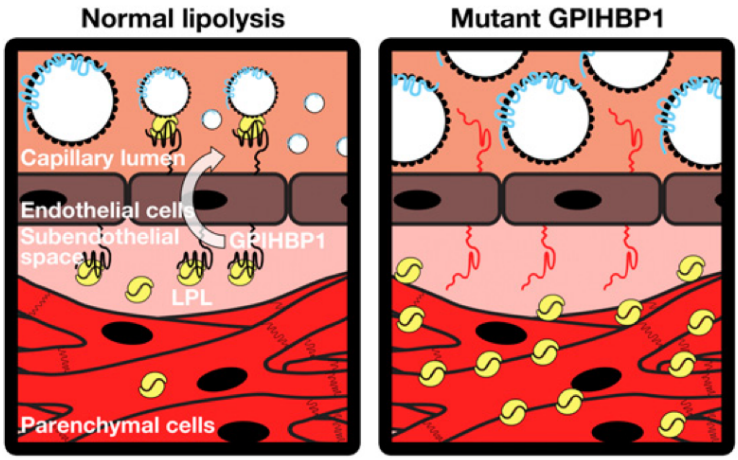


Image 2: Depiction of LPL localization in normal lipolysis and under mutant GPIHBP1 conditions. Failure of LPL to bind with GPIHPB1 results in LPL's inability to localize to the luminal side of the endothelial cells and impaired lipid metabolism. (Image source: Young, 2011)[44].

Apolipoprotein C

Apolipoproteins (Apo), in humans have been implicated in lipid metabolism and insulin resistance. The ApoC family consists of 3 closely related proteins, which are primarily produced by the liver (with measurable amounts from the intestine). ApoC1, ApoC2 and ApoC3 interact with lipoproteins and effect their ability to bind with LPL, and thereby affect TG clearance. While ApoC1 and ApoC3 have been shown to inhibit LPL by preventing binding, ApoC2 has been accepted as an essential activator of LPL[45,46]. Since ApoC3 is often evaluated with respect to heart disease (i.e., atherosclerosis), less is known regarding its role in LPL-mediated lipid metabolism and insulin resistance. Recent studies have suggested potential pathophysiological responses, such as inflammation, may lead to the upregulation of ApoC3 and subsequent exacerbation of insulin resistance[47,48].

In addition to effecting LPL both directly and indirectly, ApoC3 also effects hepatic uptake of TG-rich lipoproteins. In rodents and humans, increased ApoC3 has been associated with increased circulating TG content[49,50] Specifically, research shows ApoC3 loss of function mutations result in lower TG content whereas increased expression of ApoC3 results in hypertriglyceridemia [49,51]. A study by Caron et al. (2011) evaluated subjects with altered glucose metabolism and found a positive correlation between fasting glucose and plasma ApoC3 levels, but found no correlation with fasting plasma insulin[52]. In contrast, decreases in ApoC3 and plasma TGs were also found to

reduce the risk of metabolic abnormalities, including insulin resistance[53]. Thus, it is possible ApoC3 is upregulated in populations with insulin resistance resulting in lower LPL activity and subsequent increased plasma TG content.

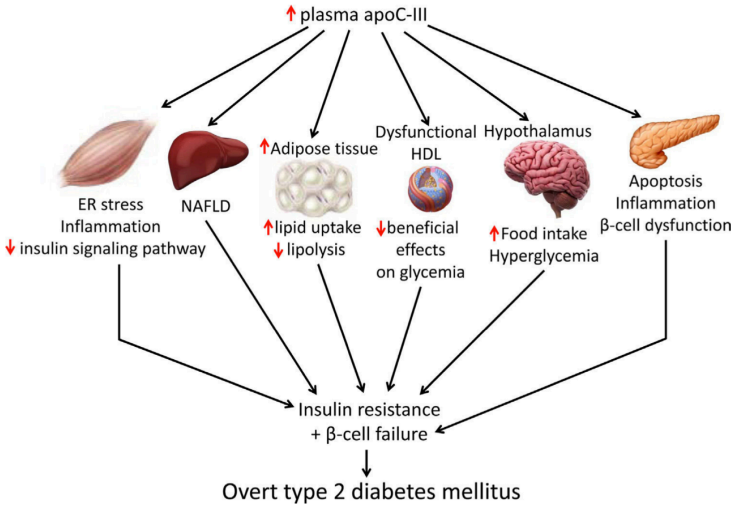


Image 3: The effects of increased ApoC3 for various tissues leading to insulin resistance. (Image source: Aguilar-Recarte, 2021)[54].

While their biological regulation remains unknown, there is currently more evidence available about the role of ApoC2 compared to that of ApoC3 in health and disease. The n-terminal of ApoC2 binds with lipids whereas the c-terminal is primarily associated with LPL activation. It has been shown that there is surface pressure dependent regulation of LPL by ApoC2 when measuring pressure changes at multiple lipid/water interfaces[55]. This suggests LPL may function without the ApoC2, however, critical surface pressures (commonly observed in the human body) require ApoC2 for desorption (movement up the solvent) and to trigger release for LPL activity. Clinical evidence of ApoC2 dysfunction is often associated with hypertriglyceridemia despite

normal LPL concentrations [56]. Various factors have been observed to alter ApoC2 concentration including glucose metabolism and obesity. Subjects with decreased glucose tolerance and type 2 diabetes have higher fasting plasma ApoC2 concentrations [57,58]. Given recent advances in ApoC2 regulation and its clinical relation to glucose metabolism, further studying of ApoC2 is necessary to clarify its potential role as a key regulator of LPL activity.

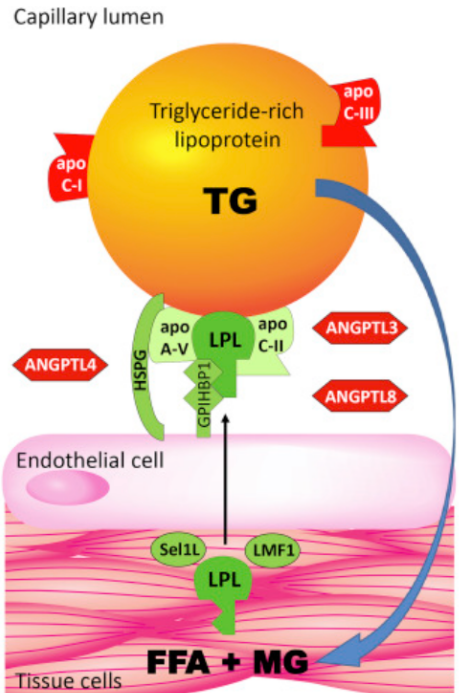
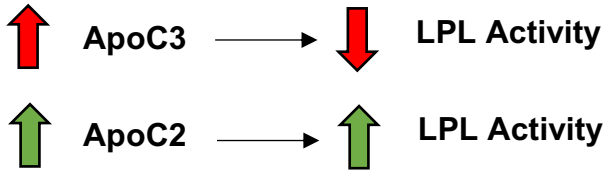


Image 4: Lipolytic complex displaying LPL action dependence on positive (green) and negative (red) regulators of LPL. Note, ApoC2 and ApoC3 are both listed as essential co-factors for this complex for activation and inhibition of LPL activity, respectively. (Image source: Wolska, 2017)[46]



In summary, there is clear need to enhance our understanding of factors that regulate endothelium-bound LPL activity in the metabolic environment of insulin resistance for subsequent lifestyle and/or pharmacological interventions. The impact of obesity and insulin resistance on lipid metabolism is rather complex. Tissue lipid requirements and FFA uptake varies between tissues and nutritional states (i.e., fed/fasted) and requires efficient LPL transport to the capillary endothelium to hydrolyze circulating plasma TGs. Extracellular modulators, such as ApoC3 and Apoc2, which serve to downregulate and upregulate LPL activity, respectively, may serve as critical co-factors to regulate metabolism of circulating TGs and uptake of their fatty acids into tissues. Thus, the purpose of this study was to investigate the concentrations of ApoC3 and ApoC2 in plasma in parallel with the activity of endothelial-bound LPL in insulin resistant (IR) and healthy/non-IR individuals to better understand the regulation of lipid homeostasis in health and disease.

CHAPTER 2

MATERIALS AND METHODS

Subjects

Subjects (mean ± SD; Age 27 ± 8; n = 18) were evaluated for insulin sensitivity using an oral glucose tolerance test (OGTT). Plasma glucose and insulin responses during the OGTT were used to calculate the Matsuda Insulin Sensitivity Index[59]. Subjects were placed in one of two groups: 1) IR or 2) insulin sensitive (IS). For the IR group we targeted individuals with BMI > 30 kg/m^2 and insulin sensitivity index (ISI) ≤ 5. For IS subjects we targeted individuals with BMI of < 25 kg/m^2 and ISI ≥ 8. Subjects were apparently healthy with no history of hypertension, hyperlipidemia, or diabetes. Additionally, subjects were not actively participating in weight loss regimens. Informed consent was obtained prior to the study and ethical approval was granted by the Institutional Review Board at Mayo Clinic.

Procedures

Subjects received a preliminary phone screening to ensure initial criteria was met before scheduling a complete screening visit at the Clinical Studies Infusion Unit in Mayo Clinic Arizona. Complete screening included a physical exam, medical history, urinalysis, electrocardiogram (ECG), pregnancy test (women), the OGTT, and blood testing evaluating cell count, liver function, blood glucose, triglycerides (TGs), cholesterol, and electrolytes. These blood samples were analyzed at the Mayo Clinic Laboratories, at Mayo Clinic Arizona.

Participants maintained their typical diet, avoided alcohol, and did not exercise (apart from daily physical activity) 3 days prior to the infusion study. Additionally, subjects were fasted overnight prior to the infusion study. Venous blood samples were collected from an antecubital vein catheter placed in the arm. Infusion of insulin occurred via an additional IV line in the opposite arm.

Insulin Infusion

Insulin was infused at a rate of 1 mU/kg/min for IS subjects and 0.5 mU/kg/min for IR subjects for 40 minutes. This was done to ensure that subjects have comparable increases in plasma insulin concentrations during the insulin infusion. Plasma insulin concentrations (uIU/ml) changed (i.e., delta change) at a magnitude observed during the postprandial state (IR = 58.1 $\pm$ 26.8 and IS = 48.9 $\pm$ 9.3). During the insulin infusion, blood glucose was maintained at basal concentrations via variable rate of infusion of 20% dextrose. During the last 10 minutes, and in order to release endothelial-bound LPL, subjects received an injection of intravenous sodium heparin (75 UI/kg) infused over a 1-minute period. Plasma samples were collected 10 minutes after the heparin infusion, and they were used for analyses. Samples were stored in a -80^{o}C freezer, until analysis.

Plasma Sample Analyses

Plasma samples were collected in heparin-containing tubes, and the isolated plasma was used to measure LPL concentration and activity, and concentrations of ApoC2 and ApoC3. Post-heparin plasma LPL concentration and activity were evaluated using commercially available ELISA assays kits (ab204721 [LPL concentration]; Abcam

Cambridge MA; Cayman No. 700640 [LPL activity]; Cayman Chemicals Ann Arbor, MI). Plasma apoC2 and apoC3 concentrations were measured using also commercially available ELISA kits (ab168549 [apoC2]; ab154131 [apoC3]; Abcam, Cambridge, MA). Measurements were performed in duplicate for any given sample.

Statistics

Differences in the measured parameters between IS and IR subjects were determined using independent t-tests. The alpha level was set at $p < 0.05$ and data are presented as means ± SD, unless stated otherwise. A Pearson correlation coefficient (r) was used to examine relationships between variables of interest. The obtained R^2 value was used to assess the extent to which one variable explained the other variable.

CHAPTER 3

RESULTS

Our goal was to evaluate ApoC3 and ApoC2 as potentially critical modulators of LPL activity. Key subject characteristics are displayed on Table 1. A dual-energy x-ray absorptiometry (DEXA) scan was used to analyze subject body composistion (i.e., fat tissue, bone density, and lean mass). Human subjects were classified as IR or IS using the Matsuda index calculation[59].

To study differences in plasma LPL, we first evaluated post-heparin plasma LPL concentration (Table 2). Post-heparin LPL concentration was not significantly ($P = 0.46$) different between IS (453 $\pm$ 27 ng/ml) and IR (457 $\pm$ 17 ng/ml) groups. However, plasma LPL activity was significantly ($P = 0.02$) higher in IR subjects (IR = 665 $\pm$ 113 nmol/min/ml) compared to IS subjects (IS = 365 $\pm$ 59 nmol/min/ml).

ApoC3 and ApoC2 were then evaluated as potential modulators of LPL activity within the circulation, and in an effort to explain differences in LPL activity between groups (Table 2). IR subjects displayed higher concentrations of ApoC3 compared to their counterparts (IR = 3.6 $\pm$ 0.5 mg/dl, IS = 2.7 $\pm$ 0.2 mg/dl, P=0.03). However, there was no significant difference between groups in measured ApoC2 concentrations (IR = 0.15 $\pm$ 0.03 mg/dl, IS = 0.11 $\pm$ 0.01 mg/dl, $P = 0.11$). Table 3 shows the concentration of heparin-releasable LPL relative to the concentration of plasma ApoC3 (i.e., LPL activity/ApoC3). The calculated parameter was not significantly different between groups (IR = 145 $\pm$ 22, IS = 176 $\pm$ 15, $P = 0.13$). Similarly, we compared the concentrations of heparin-releasable LPL relative to plasma ApoC2 concentrations (i.e., LPL

activity/ApoC2). This response was not different between groups (IR = 4104 ±844, IS = 4391 ±483, P = 0.38; Table 4).

Pearson correlation coefficient showed a significant positive correlation between TG concentrations and both ApoC2 and ApoC3 (Figure 1) across all study subjects (n=18). ApoC2 alone explained 58% of the variability in TG content ($R^2 = 0.58$). Only 23% of the variability in TG levels was explained by the plasma ApoC3 concentrations alone. Furthermore, Figures 2 and 3 illustrate significantly negative correlations within IS subjects between ApoC2 and both total body fat and gynoid fat. However same correlations were not observed within the IR subjects. Additionally, neither IR or IS groups displayed significant correlations with android or visceral fat. Interestingly, it was shown that LPL activity correlated significantly with total body fat (Figure 6). However, as seen in Figure 5 there were no significant correlations for within either IR or IS groups between ApoC2, ApoC3 and LPL activity. Furthermore, statistical analysis revealed no significant correlations between LPL concentrations and either ApoC2 or ApoC3 concentrations and for within either IR or IS subjects (Figure 4).

Table 1: Subject Characteristics:

	Insulin Resistant	Insulin Sensitive
Total	7	11
Male/Female	4/3	9/2
Age (years)	28 ± 9	27 ± 7
Body mass index (kg/m^2)	34.9 ± 6.8*	22.5 ± 2.1
Total triglycerides (mg/dl)	132 ± 46*	66 ± 33
Insulin (μIU/mL)	12.1 ± 4.0*	2.8 ± 0.9
Glucose (mg/dl)	89.7 ± 10.6	83.5 ± 5.7
HOMA-IR	2.7 ± 0.8*	0.6 ± 0.2
Matsuda ISI	2.9 ± 0.8*	13.9 ± 6.0
Total Body Fat (kg)	40.5± 15.1*	12.6± 4.5
Gynoid Fat (kg)	6.6 ± 2.0*	2.6 ± 0.9
Android Fat (kg)	412.2 ± 67.8*	77.4 ± 11.2
Visceral Fat (g)	755.7 ± 149.5*	286.9 ± 55.6

*Asterisks indicate significant difference between insulin resistant and insulin sensitive subjects at $P < 0.05$

Table 2: Plasma concentrations of ApoC3, ApoC2, and LPL, as well as post-heparin plasma LPL activity differences in the Insulin Sensitive and Insulin Resistant groups.

	ApoC3	ApoC2	LPL	LPL Activity
Insulin Sensitive	2.7± 0.2 mg/dl	0.11 ± 0.01 mg/dl	453 ± 27 ng/ml	365 ± 59 nmol/ml/min
Insulin Resistant	3.6 ± 0.5 mg/dl	0.15 ± 0.03 mg/dl	457 ± 17 ng/ml	665 ± 113 nmol/ml/min
P-value	0.03	0.11	0.46	0.02

Table 3: Ratio of LPL concentration to ApoC3 concentration (LPL/ApoC3) in the Insulin Sensitive and Insulin Resistant groups.

	LPL per ApoC3
Insulin Sensitive	176 ± 15
Insulin Resistant	145 ± 22
P-value	0.13

Table 4: Ratio of LPL concentration to ApoC2 concentration (LPL/ApoC2) in the Insulin Sensitive and Insulin Resistant groups.

	LPL per ApoC2
Insulin Sensitive	4391 ± 483
Insulin Resistant	4104 ± 844
P-value	0.38

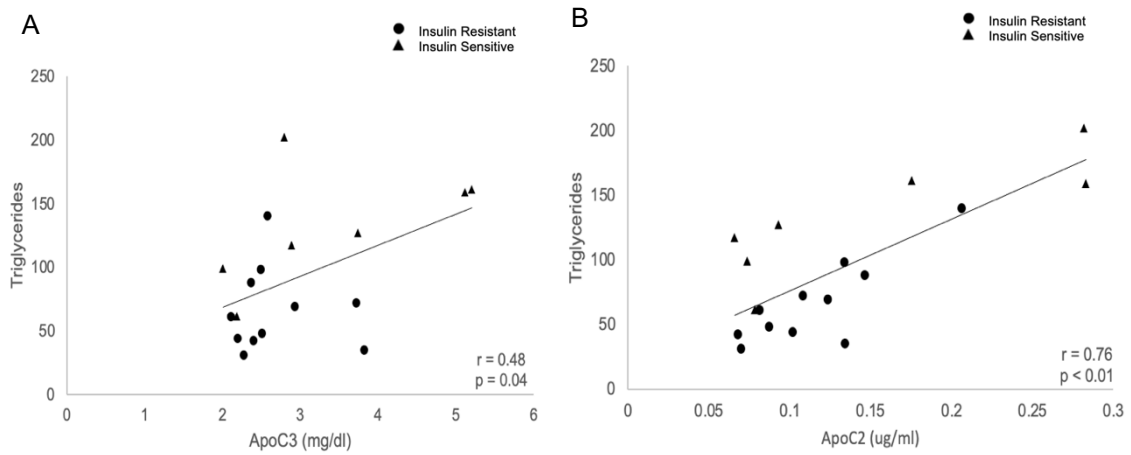


Figure 1: Pearson (r) correlation coefficient between variables of interest and across the entire subject population (n=18). IR subjects are denoted by triangles and IS subjects by circles.

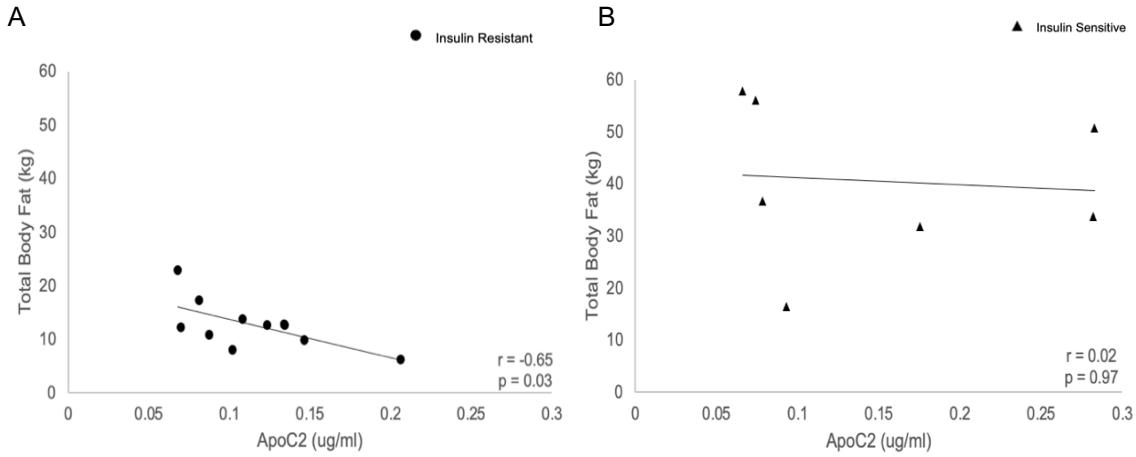


Figure 2: Pearson (r) correlation coefficient between plasma ApoC2 and total body fat (kg) for 2A: IS subjects (n = 11) and 2B: IR subjects (n = 7).

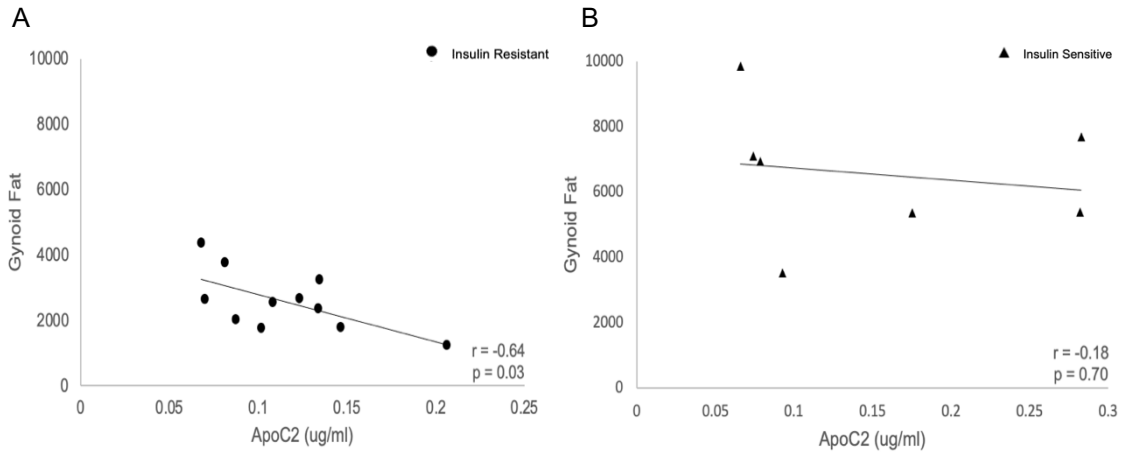


Figure 3: Pearson (r) correlation coefficient between plasma ApoC2 and gynoid fat for 3A: IS subjects (n = 11) and 3B: IR subjects (n = 7).

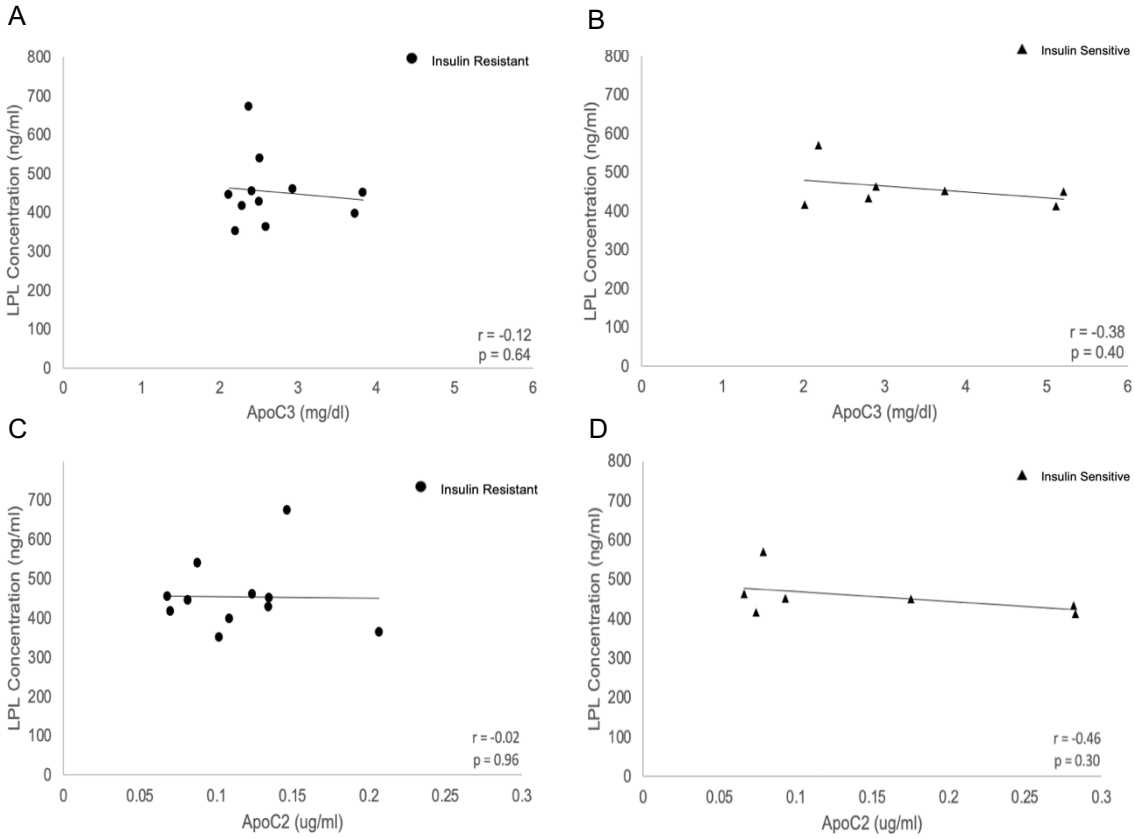


Figure 4: Pearson (r) correlation coefficient 4A and B: between ApoC3 and plasma LPL concentration for IS and IR subjects, respectively and 4C and D: between plasma ApoC2 and plasma LPL concentrations. IR subjects are denoted by triangles and IS subjects by circles.

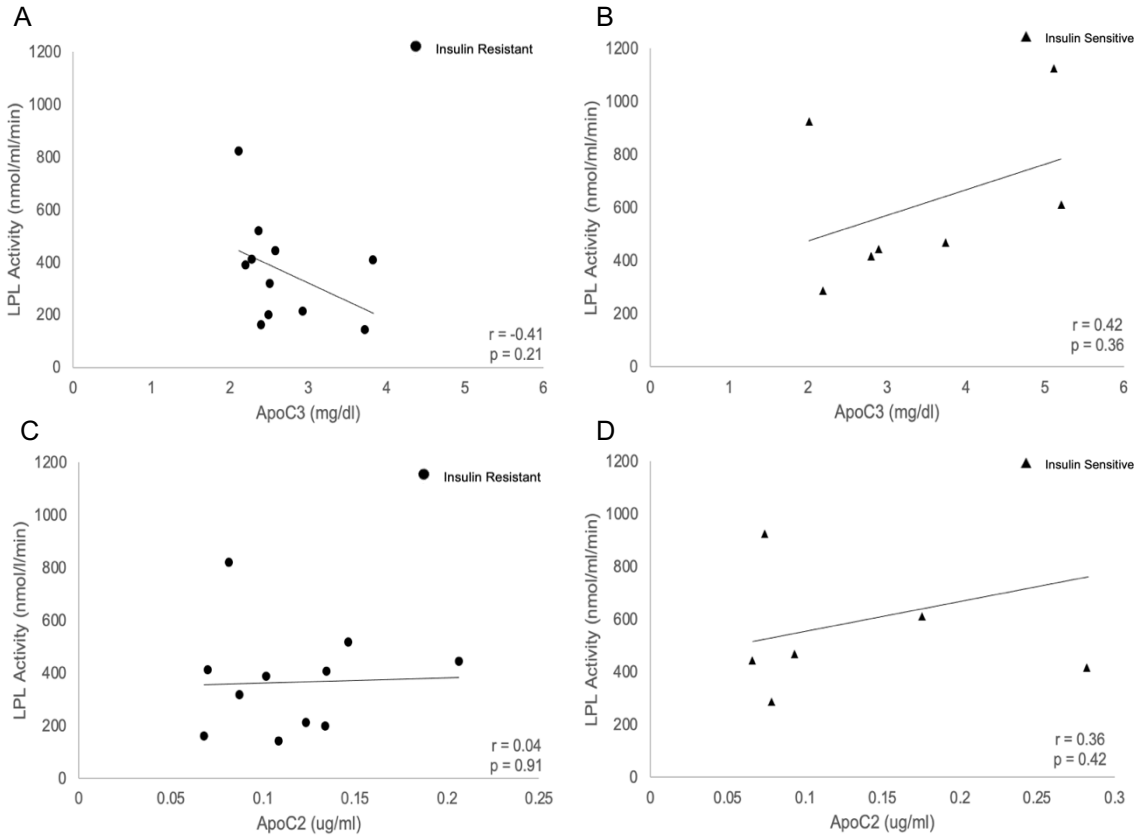


Figure 5A and B: Pearson (r) correlation coefficient between ApoC3 and plasma LPL activity for IS and IR subjects, respectively, and 4C and D: between plasma ApoC2 and plasma LPL activity. IR subjects are denoted by triangles and IS subjects by circles.

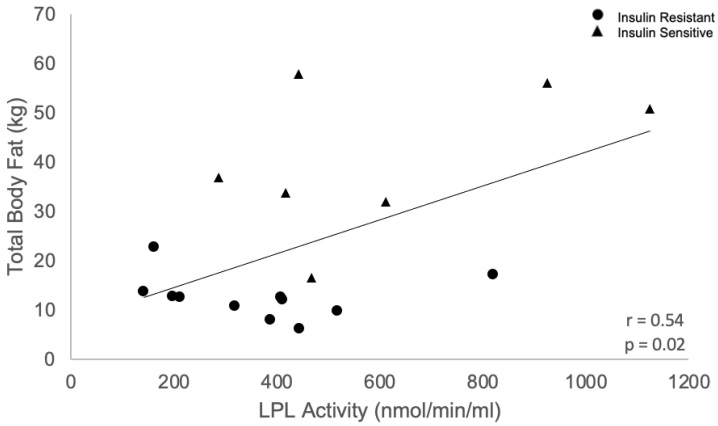


Figure 6: Pearson (r) correlation coefficient across the entire subject population (n=18) between LPL activity (nmol/ml/min) and total body fat (kg). IR subjects are denoted by triangles and IS subjects by circles.

CHAPTER 4

DISCUSSION

Apolipoprotein C2 and C3 are considered key regulators of LPL[60,61]. This is due to observed increases in plasma ApoC3 concentrations occurring concurrently with increases in plasma TG concentrations[62]. Further, decreased plasma ApoC2 concentrations result in a rapid increase of circulating TG and this response displays no phenotypical difference when compared to LPL deficiency[63]. Due to the complexity of factors regulating LPL (i.e., interacting proteins, extracellular availability, anchorage/binding) the role of specific apolipoproteins on LPL activity are not yet clear. Thus, we hypothesized that an increase in post-heparin LPL activity is observed concurrently with distinct apolipoprotein concentrations in IS versus IR subjects. Increased ApoC2 concentrations was hypothesized to explain higher activity of LPL in IR subjects. Conversely, we hypothesized decreased ApoC3 concentrations in IR subjects and because of its ability to decrease LPL activity. However, our findings do not support our hypotheses. While there was significantly more ApoC3 in IR subjects, plasma concentrations of ApoC3 and ApoC2 relative to LPL concentrations were not statistically significant. Additionally, correlations for between both ApoC3 and ApoC2 and LPL activity were not statistically significant. These findings indicate that plasma concentrations of ApoC3 and ApoC2 may not have major biological role in regulating LPL activity in IR subjects.

Previous research has suggested ApoC3 may be more relevant with respect to LPL-independent TG metabolism (i.e., hepatic lipase activity)[64]. In patients with chylomicronemia syndrome, ApoC3 reduction resulted in significantly lower plasma TG

levels despite having no LPL-dependent pathway for TG removal[65]. In the latter study, it was suggested that ApoC3 may play more important role with respect to impairments in hepatic triglyceride rich lipoprotein clearance.

Recent studies have indicated ApoC2 regulates LPL in a pressure-dependent model, suggesting that cellular pressures might be important for relative LPL concentrations and subsequent activity[55]. The pressure dependent model states that ApoC2 is not required for the binding of LPL to lipids but is required for LPL activity on the lipid surface. When LPL binds with plasma TGs it increases local surface pressures and allows ApoC2 to modulate its function. When evaluated at a triacylglycerol/water interface, it has been seen that ApoC2 expresses high affinity for lipid binding and retention pressure compared to other apolipoproteins (including ApoC3). This implies that changes in surface pressure may have primary role in determining direct effects of ApoC2 on LPL activity. Accordingly, lipoprotein size has been reported to affect LPL activity more than ApoC2 content[66]. One could speculate, larger lipoprotein particles change the critical surface for ApoC2, and thus make ApoC2 more relevant for regulating LPL activity. Potential differences in interfacial pressures (i.e., VLDL at >25 mM/m and HDL at < 20 mM/m comparatively) may contribute to the lack of detectable effects of ApoC2 on LPL activity [67]. Regardless, decreased ApoC2 concentrations appear to be (indirectly) associated with the severity of IR, via decreased regulation of LPL[68]. Further evaluation of the role of ApoC2 in plasma and how it might be regulating LPL activity in the context of the surface pressure is critically important.

Interestingly, only IS subjects displayed negative correlations between ApoC2 and both total body fat and gynoid fat. Across studies, increased ApoC2 has been

positively correlated with obesity and TG content[10,69,70]. Also, normolipidemic individuals display ApoC2 distributed to HDL (rather than VLDL and LDL as in hypertriglyceridemia)[71]. Our findings in metabolically healthy individuals indicate that ApoC2 is biologically relevant with respect to body weight/fat accumulation. Moreover, both ApoC3 and ApoC2 were positively correlated with plasma TG concentrations across subjects. Given ApoC3's ability to inhibit LPL activity, this positive correlation between ApoC3 concentrations and plasma TG concentrations is consistent with the literature[72,73,74]. Conversely, studies have observed increased plasma TG concentrations in the presence of ApoC2 deficiency[75,76]. However, obesity is also generally associated with elevated ApoC2 values, most likely as a result of increased ApoC2 production with increased dietary TG consumption[46,77].

Our study is limited by the sample size in each group. Larger study cohorts can provide better reflection of population responses, and a separate study can confirm these findings. Because of lack of previous research on LPL with respect to ApoC3 and ApoC2 in IR humans, this research has generated new investigational avenues. Despite limitations, subjects were well characterized metabolically and anthropometrically. Additionally, subjects displayed considerably different degrees of insulin sensitivity (i.e., IR vesus IS), and the samples analyzed were obtained under rather physiological insulin response conditions. Thus, this study offers valuable insight of possible mechanisms that might explain differential regulation of LPL function in the insulin resistant state.

In summary, IR subjects display higher levels of intravascular LPL activity compared to IS subjects, despite having comparable plasma LPL concentrations. Additionally, we show no differences in plasma ApoC2 concentrations between IR and

IS groups. However, IR subjects displayed higher plasma concentrations of ApoC3. Although higher intravascular LPL activity is observed in IR subjects, our findings show that circulating ApoC2 and ApoC3 concentrations are not key modulators of LPL activity in these same subjects.

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