

Metabolic Health with Obesity: A Novel Role for Cholesteryl Ester Transfer Protein

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Abstract

Obesity is an increasingly prevalent condition that increases risk factors for type-2 diabetes and heart disease. Weight loss reverses the complications of obesity. Long-term maintenance of weight loss, however, is difficult. Mechanisms that improve metabolic health in obese people are therefore attractive targets for study. In my dissertation work, I have identified a novel role for Cholesteryl Ester Transfer Protein (CETP) to protect female mice against insulin resistance and exercise intolerance caused by obesity. CETP is a lipid transfer protein that shuttles lipids between lipoproteins, culminating in delivery of cholesterol esters to the liver for secretion as bile. Bile acids are known to have insulin-sensitizing effects. Mice naturally lack CETP expression. I discovered that female mice transgenic for CETP were protected from high fat diet-induced insulin resistance. This effect was modest in males. In female mice I found activation of bile acid signaling pathways in liver and muscle as well as increased glucose rate of disappearance and increased muscle glycolysis. These results suggest that CETP can ameliorate insulin resistance associated with obesity in female mice by promoting muscle glucose utilization.

Based on the observations of improved muscle function in the CETP mice, I hypothesized that CETP could improve exercise capacity by increasing muscle oxidative metabolism. While there is no difference in exercise capacity between lean, chow fed CETP-expressing mice and their non-transgenic littermates, CETP-expressing female mice are protected against the decline in exercise capacity caused by obesity.

This improvement in exercise capacity corresponded with increased mitochondrial oxidative capacity.

My dissertation work has demonstrated a novel role for CETP to promote metabolic health in obese animals potentially through its effect on bile acid signaling to muscle. I propose that targeting bile acid signaling pathways could promote metabolic health in obese people. The sexual dimorphism observed adds to the growing body of evidence that CETP likely has a positive impact on metabolism in females. Further understanding the role of CETP and bile acid signaling will help to provide new strategies for promoting metabolic health in obese people.

To Mom and Dad

You have always emphasized the value of education.
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List of Abbreviations

CETP	Cholesteryl Ester Transfer Protein
ERK1/2	Extracellular related kinase
Fxr	Farnesoid X Receptor
Shp	Small Heterodimer Partner
Akt	Protein Kinase B
Dio2	Type 2 Thyroid Deiodinase
CE	Cholesterol Ester
TG	Triglyceride
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
VLDL	Very Low Density Lipoprotein
ApoA1	Apolipoprotein A1
SR-B1	Scavenger Receptor B1
Fgf15	Fibroblast Growth Factor 15
Gpbar1	G-Protein Coupled Bile Acid Receptor
BMI	Body Mass Index
CHD	Coronary Heart Disease
MI	Myocardial Infarction
PGC1alpha	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
FGFR4	Fibroblast growth factor receptor 4
G6pase	Glucose 6 phosphatase
Pck1	Phosphoenolpyruvate carboxykinase
Glp1	Glucagon like peptide 1
TUDCA	Taurosoodeoxycholic Acid
TCA Cycle	Tricarboxylic acid cycle
ATP	Adenosine Triphosphate
HFD	High Fat Diet
EndoRa	Endogenous Rate of Glucose Appearance
Rd	Rate of Glucose Disappearance
DPM	Disintegrations per minute
CSE	Chemical Standard Evaporated
SA	Specific Activity
CRS	Chemical Recovery Standard
BCA	Bicinchoninic acid
IL-6	Interleukin 6
RT-PCR	Real Time Polymerase Chain Reaction
LC/MS	Liquid Chromatography/Mass Spectrometry
GC/MS	Gas Chromatography/Mass Spectrometry
MMPC	Mouse Metabolic Phenotyping Center
VO ₂	Volume of Oxygen Consumption
VCO ₂	Volume of Carbon Dioxide Production
SEM	Standard Error of Measurement
ANOVA	Analysis of Variance
LDLR	Low Density Lipoprotein Receptor

VLDLR	Very Low Density Lipoprotein Receptor
WT	Wild Type
SI	Insulin Sensitivity Index
Hnf4alpha	Hepatic nuclear receptor 4 alpha
Lrh-1	Liver receptor homolog 1
GIR	Glucose Infusion Rate
G6P	Glucose 6 Phosphate
F6P	Fructose 6 Phosphate
F1,6BP	Fructose 1,6 Bisphosphate
G1P	Glucose 1 Phosphate
Cpt1b	Carnitine Palmitoyltransferase 1b
Hk2	Hexokinase 2
AUC	Area Under the Curve
ERalpha	Estrogen Receptor Alpha
RCT	Reverse Cholesterol Transport
LCAT	Lecithin—cholesterol acyltransferase
Cyp7a1	Cytochrome P450 7 subfamily A, polypeptide 1

CHAPTER I

BACKGROUND AND SIGNIFICANCE

Overview

Obesity leads to insulin resistance, diabetes, and heart disease. Weight loss resolves the morbidities of obesity. Most people who lose weight, however, have great difficulty maintaining their level of weight loss [1, 2]. Additionally, obesity causes a reduction in exercise capacity, making weight loss through exercise more difficult and creating a vicious cycle of increasing weight and insulin resistance [3, 4]. Understanding factors that improve metabolic health in obese individuals could lead to improved treatment of obesity. Additionally, mechanisms that improve exercise capacity with obesity could help obese people to lose weight through exercise. Overall, my dissertation work has demonstrated that Cholesteryl Ester Transfer Protein (CETP) protects against the negative effects of obesity on insulin sensitivity and exercise capacity.

CETP is a lipid transfer protein that facilitates the transfer of lipids between lipoproteins. Specifically, it allows for the exchange of Cholesteryl Esters (CE) and triglycerides (TG) between serum High Density Lipoprotein (HDL) and either Low Density Lipoprotein (LDL) or Very Low Density Lipoprotein (VLDL) [5]. CETP-mediated flux of TG to HDL destabilizes the scaffold of the HDL Apolipoprotein A1 (ApoA1) particle, resulting in decreased HDL cholesterol [6]. Animals that naturally lack CETP, such as mice, carry a much higher percentage of their total cholesterol in HDL. Since

CETP activity leads to reduced HDL, current models of HDL function suggest that CETP would increase risk of cardiovascular disease. Therefore, CETP inhibitor drugs were developed to increase HDL cholesterol and thereby protect against heart disease [7]. While CETP inhibitors potently increase HDL in humans, none to date has demonstrated clinical efficacy in reducing the incidence of heart attack [8, 9]. This lack of clinical efficacy despite robust increases in HDL suggests that CETP does not have a simple direct relationship with risk of cardiovascular disease and invites further study to better understand the specific role of CETP in metabolism.

In contrast to its perceived negative effect on cardiovascular risk, CETP has a beneficial role in promoting reverse cholesterol transport, the removal of cholesterol from peripheral tissues and its delivery to the liver for secretion as bile. CETP expression improves hepatic uptake of cholesterol in a mouse model that is otherwise unable to clear HDL due to deficiency of Scavenger Receptor B1 (SR-B1) [10, 11]. In women, higher serum CETP activity correlated with increased radiolabeled cholesterol uptake from macrophages to serum in an *ex vivo* study. This effect was not observed in men [12]. Additionally, clinical studies have suggested that CETP has a beneficial effect on glucose homeostasis, especially in females. For example, a study of obese women who had undergone gastric bypass surgery showed that higher CETP mass correlated with improved glycemia 12 months post-op, an effect not observed in males [13]. Overall, studies in both mice and humans have shown that CETP can promote reverse cholesterol transport and potentially improve insulin sensitivity. These studies also suggest an important sexual dimorphism in the metabolic effects of CETP.

The increase in reverse cholesterol transport mediated by CETP likely leads to increased gut bile acids [11]. In the liver, CE are converted into bile acids and secreted into the gut. Beyond their role in digestion, bile acids activate three major signaling pathways that improve glucose metabolism: 1) Fibroblast Growth Factor 15 (Fgf15) signaling to the liver, 2) Farnesoid X Receptor (Fxr) signaling in the liver and gut, and 3) g-protein coupled bile acid receptor (Gpbar1 also known as Tgr5) signaling in muscle [14, 15]. Gpbar1 upregulates type-2 thyroid deiodinase (*Dio2*), causing increased energy expenditure and glucose oxidation by mitochondria [16-18]. Since Gpbar1 is associated with increased muscle glucose disposal, I hypothesize that bile acid signaling induced by CETP activity leads to improved insulin sensitivity

I propose that CETP also has a role in increasing exercise capacity, which is known to decline with obesity [4]. Mechanisms that help improve exercise capacity could help to improve metabolic health and quality of life for obese people. Exercise capacity relies heavily on glucose oxidative capacity as a fuel source for muscle, especially at higher levels of exercise intensity [19]. Since I discovered that CETP expression alters pathways that lead to improved glucose oxidative capacity, I hypothesized that CETP expression might increase exercise capacity by allowing for increased muscle glucose oxidation.

Overall, recent studies of CETP suggest that it may have a beneficial role in metabolic health. My dissertation project demonstrates that CETP expression improves both insulin sensitivity and exercise capacity in obese female mice. Thus, CETP expression improves major complications of obesity such as insulin resistance and impaired exercise capacity.

Obesity significantly increases risk of chronic metabolic disease

Obesity, defined as the accumulation of excess body fat, is the greatest public health challenge of the 21st century. It is linked to the metabolic syndrome, a condition characterized by increased fasting blood glucose, reduced HDL cholesterol, increased serum triglycerides, and hypertension. These factors combine to create an increased risk of cardiovascular disease [20]. Body mass index (BMI), defined as an individual's weight in kilograms divided by the square of their height in meters, provides a metric definition of obesity. An individual is classified as obese with a BMI of over 30 and overweight with a BMI of 25-30. Increasing BMI correlates with increased risk of developing diabetes and cardiovascular disease. Compared to people with a BMI of 25 and adjusted for age, a man with a BMI of over 35 has a 42-fold greater relative risk of diabetes [21], and a woman with a BMI of over 35 has a staggering 93-fold greater relative risk of diabetes [22]. This large increase in relative risk of diabetes reflects not only the low prevalence of diabetes in lean individuals but also illustrates that obesity dramatically increases the risk of diabetes. Similarly, obesity increases the risk of coronary heart disease (CHD) by 2.0-fold in men and 2.4-fold in women [23]. Understanding risk factors that contribute to the development of metabolic disease with obesity will improve outcomes for patients who are affected by the increasingly prevalent condition of obesity.

Obesity is a quickly increasing problem throughout the world. Once thought to be a problem limited to the developed world, obesity has increased worldwide to the point that it was declared a global epidemic in 1997 [24]. In 2011, the World Health Organization estimated that approximately 500 million people worldwide, or nearly 10%

of the world population, were obese [25]. As of 2012, 35% of the population of the United States, over 100 million people, was reported as obese [26]. Since obese people make up a large portion of the patient population, the costs of treating the metabolic complications of obesity have been estimated to be 20.6% of the total healthcare expenditure in the United States, representing a significant financial burden [27]. Yearly spending on health care for obesity-related complications in the United States is estimated to increase by as much as 66 billion dollars by 2030 [28]. Preventing the metabolic complications of obesity could therefore help to alleviate the great financial burden that obesity presents to governments and health care systems as well as improve quality of life for people with obesity.

Weight loss reverses the deleterious effects of obesity. For example, a study in obese patients who reduced caloric intake and increased physical activity showed that for every kg of weight lost the risk of diabetes dropped by 16% [28]. Weight loss of between 5% and 10% of body weight correlates with improvements in glycemia, serum HDL and triglyceride levels, and blood pressure [29]. Maintaining weight loss over time, however, is difficult; people often regain weight and reach their former body weight. A 2005 study showed that only 20% of people who lost more than 10% of their body weight maintained this level of weight loss for at least one year [2]. Individuals who successfully maintained weight loss were more likely to adhere to diet and exercise plans and were less likely to suffer from depression than those who regained weight. Since the rate of successful maintenance of weight loss is so low, factors that improve metabolic health in the context of obesity are attractive targets for study.

A known factor that improves metabolic health in the context of obesity is female sex. Estrogen, a major female sex hormone, has been proposed to promote insulin sensitivity. Premenopausal women, who have high levels of estrogen compared to postmenopausal women or men, show improved glucose tolerance, reduced risk of developing insulin resistance, and higher levels of Glut4 in adipose tissue [30, 31]. Estrogen signaling is important to insulin sensitivity even in men, as men with aromatase deficiency that completely lack estrogen are insulin resistant [32]. After menopause, when estrogen levels drop, women are prone to develop increased body weight, hyperinsulinemia, and insulin resistance [33, 34]. Estrogen signals through two major receptors, Estrogen receptor alpha (ER α) and Estrogen receptor beta (ER β). The major form of estrogen receptor in the liver is ER α [35]. Estrogen receptors are nuclear receptors that dimerize in response to ligand binding. Once activated, they bind to estrogen response elements (ERE) in the promoter of target genes to activate transcription. Knockout of estrogen receptor alpha in mice results in impaired glucose tolerance and insulin resistance [36]. Overall, female sex and specifically estrogen signaling are factors that promote healthy glucose metabolism.

Additionally, women are protected against the effects of obesity on increased risk of CHD, a condition caused by blockages of blood vessels that supply blood to the heart that can manifest as an acute event known as a myocardial infarction (MI), also known as a heart attack. One of the major complications of obesity is increased risk of CHD [22, 23, 37]. CHD is a subset of cardiovascular disease, which includes a number of different disorders of the heart and blood vessels including coronary heart disease, cerebrovascular disease, peripheral artery disease, congenital heart disease, deep vein

thrombosis, and pulmonary embolism. Women have a lower risk of heart attack compared to men of a similar age, with risk of death from MI in women being that of a man ten years younger [38]. Women are also relatively protected against the increase in CHD risk with weight gain, as increasing body weight correlates with a much larger increase in CHD incidence in men compared to women [23]. CHD, however, remains a major cause of death in women, and incidence of CHD increases sharply in postmenopausal women [39]. Since CHD is a major cause of death, medical science has focused on understanding and treating this disease

Cardiovascular risk factors were defined in a seminal research study performed between the 1940s and 1960s called the Framingham Heart Study. The study followed a population of 5400 individuals from the town of Framingham, Massachusetts and identified risk factors for the development of cardiovascular disease. Over the course of the Framingham Heart study, the following classical risk factors for cardiovascular disease were identified: 1) hypertension [40], 2) increased LDL cholesterol [41], 3) decreased HDL cholesterol [41], 4) male sex [42], and 5) smoking [40].

Based on these findings, clinical guidelines were developed that have helped to improve the treatment and prevention of cardiovascular disease. These improvements in treatment have been largely effective, and the rates of cardiovascular disease within the United States have declined sharply since the 1970s. In the 1970s nearly 1,000,000 people died each year from cardiovascular disease. By 2007 the number of deaths from cardiovascular disease had been reduced by 20% to 800,000 [43]. Our understanding of cardiovascular risk factors, however, is still largely based on the Framingham study, which was performed in a patient population that was mainly lean. By contrast, in the

2010s, the general population is approximately 30% obese and nearly 50% overweight. Obesity presents a challenge to the treatment of CHD, as it causes insulin resistance and diabetes, which are linked to increased risk of cardiovascular disease [22, 23, 37].

Insulin signaling

Insulin signaling is crucial to regulation of metabolic processes in tissues throughout the body. Insulin is a 51-amino acid (5.8 kd) peptide hormone that is produced in pancreatic beta cells and secreted into the serum in response to elevated blood glucose. Insulin signals to the liver to reduce glucose production, promote storage of nutrients as glycogen and fatty acid, and inhibit the release of triglyceride from the liver in the form of VLDL [44-49]. Insulin also promotes the uptake of glucose by muscle and adipose tissue [50, 51]. Insulin achieves these effects by activating a complex signaling network that has three major nodes: the insulin receptor, phosphoinositide 3-kinase (PI3K), and protein kinase b (AKT). The insulin signal is initiated when insulin binds to the insulin receptor, which is a transmembrane protein with tyrosine kinase activity. Activation of the insulin receptor causes tyrosine autophosphorylation of the insulin receptor [52]. Phosphorylation of the insulin receptor leads to tyrosine phosphorylation of insulin receptor substrates (IRS) by the insulin receptor [53]. Phosphorylated IRS, in turn, phosphorylates PI3K, which catalyzes the production of the lipid second messenger phosphatidylinositol trisphosphate (PIP3) from phosphatidylinositol bisphosphate (PIP2). PIP3 binds AKT to the cellular membrane and allows for AKT phosphorylation at threonine 308 by Phosphoinositide-dependent kinase-1 and at serine 473 by mTORc2 [54-58].

AKT serves as a signaling nexus that is a master regulator of metabolic processes. AKT downregulates gluconeogenesis by deactivating the transcription factor Forkhead Box Protein O1 (FoxO1), which transcriptionally upregulates genes whose products control gluconeogenesis, including phosphoenolpyruvate carboxykinase (*Pck*), and glucose-6 phosphatase (*G6pc*) [59]. FoxO1 also transcriptionally upregulates microsomal triglyceride transfer protein (*Mttp*), which functions to promote the lipidation of VLDL and has the net effect of increasing TG release from the liver [60]. Therefore, insulin signaling to AKT suppresses release of TG from the liver in the form of VLDL. In addition to its regulation of FoxO1, AKT activates glycogen synthesis by phosphorylating glycogen synthase kinase 3 (Gsk3), leading to activation of glycogen synthase [61]. In addition, AKT can activate mTOR to promote protein synthesis and muscle hypertrophy[62]. Both AKT and IRS signaling can activate the growth factor signal ERK1/2, AKT through activation of Raf and IRS through activation of Ras [63-65].

Insulin signaling also activates *de-novo* lipogenesis in the liver through activation of the transcription factor sterol regulatory element binding partner 1c (SREBP-1c), which transcriptionally upregulates genes whose products control fatty acid synthesis such as fatty acid synthase (*Fas*) and acetyl coA carboxylase (*Acc*). Insulin signaling both increases the transcription of SREBP-1c and promotes its cleavage to its mature, transcriptionally active form [66, 67].

Insulin signaling has an important role in muscle and adipose tissue to promote glucose uptake by these tissues. Insulin treatment of these tissues results in increased glucose uptake through translocation of glucose transporter 4 (Glut4) from intracellular vesicles to the plasma membrane [68, 69]. Insulin signaling to AKT is crucial to the

membrane translocation of Glut4, as constitutively active AKT causes increased Glut4 translocation and glucose uptake [70], and dominant negative AKT impairs the ability of insulin to promote Glut4 translocation [71, 72]. AKT phosphorylates AS160 at multiple sites, and mutation of these phosphorylation sites blunts the ability of insulin signaling to promote Glut4 translocation [73]. AS160 has rabGTPase activity that suppresses Rab proteins that are required for translocation of Glut4 [73]. AKT signaling inhibits the GTPase activity allowing for activation of Glut4 translocation [74]. Uptake of glucose by muscle and adipose tissue is crucial for storage of energy as glycogen in muscle and fatty acid in adipose tissue.

Insulin resistance

Unfortunately, cells and tissues can fail to properly respond to an insulin signal, a condition known as insulin resistance. Insulin resistance is marked by the failure of tissues such as liver and muscle to properly respond to an insulin signal, resulting in impaired glucose tolerance, increased blood glucose, and increased serum triglycerides [75]. The progression from insulin resistance to type-2 diabetes occurs as the pancreas secretes increasing levels of insulin in an attempt to compensate for the insulin resistance [76, 77]. Eventually the pancreas cannot produce enough insulin to keep pace with the level of insulin resistance, and the pancreatic beta cells can fail to continue producing sufficient insulin, resulting in type-2 diabetes [78]. Insulin resistance is associated with obesity and physical inactivity [79, 80].

At the molecular level, insulin resistance is associated with accumulation of excess lipid in liver and muscle, particularly diacylglycerols and fatty acyl coAs. In

muscle, diacylglycerols activate PKC β , which causes serine phosphorylation of IRS1, interfering with normal insulin signaling to promote glucose uptake and glycogen storage [81, 82]. In the liver, a similar process occurs. Diacylglycerols activate PKC ϵ , which causes serine phosphorylation of the insulin receptor, disrupting its ability to phosphorylate IRS, leading to reduced glycogen synthesis and increased gluconeogenesis, as the downstream signals to AKT and GSK3 are disrupted [83]. These changes in signaling have the net effect of altering whole-body metabolism.

At the systemic level, insulin resistance results in reduced muscle glucose uptake, failure to suppress hepatic glucose production, and increased plasma free fatty acids (FFA) [75, 84, 85]. Moderate levels of insulin resistance cause a compensatory increase in insulin secretion from the pancreas, and an individual with modest insulin resistance may have normal blood glucose but chronically elevated insulin and expansion of beta cells in the pancreas [86]. Over time, increasing levels of plasma glucose interfere with the normal process of glucose stimulated insulin resistance, as it is hypothesized that beta cells are attuned to specific levels of glucose above which they do not function properly [86]. Further progression of insulin resistance causes decompensation, the process by which beta cells fail to keep pace with the insulin demand produced by the levels of circulating glucose. This decompensation is thought to be caused by glucotoxicity interfering with the ability of beta cells to produce normal levels of insulin [87, 88]. The progression to insulin-dependent diabetes is caused by deficiency of beta cells. The reduction in beta cells is caused either by increased beta cell apoptosis or reduced beta cell proliferation; however, studies in humans have suggested that increased apoptosis is a greater factor than impaired proliferation [89, 90].

A proposed mechanism that could promote insulin secretion even in the context of type-2 diabetes is glucagon-like peptide 1 (Glp1). Glp1 is an incretin hormone released from the gut in response to a mixed meal [91]. Glp1 promotes glucose-stimulated insulin secretion, and its effects on insulin secretion are seen even in individuals with type-2 diabetes [92, 93]. Additionally, Glp1 has been implicated in beta cell proliferation, as it has been shown to increase beta cell proliferation in mice and upregulate the transcription factor pancreatic duodenal homeobox 1 (Pdx1), which signals to promote beta cell survival and maturation [94, 95]. In a rodent model of diabetes, Glp1 administration resulted in increased insulin secretion, decreased glycemia, and increased beta cell mass [96]. Since Glp1 is secreted from the gut, it suggests an interplay between gut signaling and insulin secretion and sensitivity.

Patients with diabetes have an increased risk of cardiovascular disease. While the treatment of cardiovascular disease has improved, diabetes still correlates with a two to three fold increased risk of death from cardiovascular disease. In a study of the Framingham and Framingham Offspring cohorts, rate of death from cardiovascular disease decreased from 12.8 to 7.7 deaths per 1000 person-years between 1975 and 2001 in people without diabetes. In patients with diabetes, the rate of death dropped from 33.1 to 15.8. While this reflects a significant reduction in both groups, having diabetes still correlates with the same two to three fold higher risk of death from cardiovascular disease in 2001 as it did in 1975 [97]. The fact that diabetes still increases risk of CHD despite improved treatment suggests that additional risk factors in people with diabetes have not been targeted. Understanding these risk factors will help to decrease incidence of CHD in people with diabetes.

Lipoprotein biochemistry and function

Obesity and insulin resistance contribute to dysregulation and dysfunction of lipoproteins, which contributes to increased risk of CHD. Lipoproteins are critical to transport of lipids throughout the circulation, as lipids are not soluble in the aqueous blood. Three major classes of lipoproteins, VLDL, LDL, and HDL, contribute to the circulation of lipids, primarily cholesterol and triglycerides, between cells and tissues.

Both Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) are associated with the apolipoprotein ApoB100. Each VLDL or LDL particle includes one ApoB100 molecule [98]. ApoB100 is a very large protein containing 4536 amino acids (550 kd). ApoB100 is produced in the liver and begins to be lipidated in the rough ER while still being translated. If not lipidated, ApoB100 does not fold properly and is broken down by proteasomal degradation. Lipidation of ApoB100 occurs through the action of microsomal triglyceride transfer protein (MTP) to form a VLDL precursor [99, 100]. Additional lipidation of the VLDL precursor results in the formation of a lipid-poor mature VLDL particle known as VLDL₂ [101]. Further lipidation creates a triglyceride-rich VLDL particle known as VLDL₁ [101]. In the serum, VLDL can be de-lipidated through the action of lipoprotein lipase (LPL), resulting in a smaller and less lipid-rich intermediate density lipoprotein (IDL) [102]. IDL can then be further de-lipidated through the action of hepatic lipase to form LDL, which contains mainly cholesterol [103].

Insulin regulates the production of VLDL, and insulin resistance significantly increases the levels of circulating TG contained in VLDL. Assembly of VLDL is regulated by MTP, which is transcriptionally upregulated by FoxO1 [60]. Since insulin signals to inactivate FoxO1, VLDL assembly is downregulated in response to an insulin

signal. Insulin resistance therefore causes increased activation of FoxO1, increased levels of MTP, and increased VLDL-TG in plasma [104]. Additionally, insulin resistance appears to be “pathway selective,” as in the insulin-resistant state insulin fails to suppress hepatic gluconeogenesis, but insulin still promotes *de novo* lipogenesis through SREBP-1c activation [105]. Therefore, in the insulin-resistant state there is both a higher level of lipid to load onto nascent VLDL and increased levels of MTP, which performs the lipidation of nascent VLDL. These factors combine to increase circulating VLDL triglyceride in insulin resistance.

LDL is also an apoB100 containing lipoprotein and has just one molecule of ApoB100 per LDL particle. LDL particles are about half the size of the larger VLDL particles, with the average circulating human LDL particle being about 250 angstroms in diameter [106]. LDL is primarily a carrier of cholesterol, with over 60% of the LDL particle consisting of CE or FC [107]. The primary function of LDL is to provide cholesterol to cells and tissues, which is critical for normal membrane function. LDL is cleared from the circulation by the activity of LDL receptor (LDLR). LDLR binds to LDL and internalizes it through receptor-mediated endocytosis [108]. Once internalized, the protein structure of LDL is broken down to amino acids, and the cholesterol ester cargo is hydrolyzed to free cholesterol [109, 110]. Through this mechanism, LDL and its cargo are completely removed from the circulation. Normal function of LDL receptor is critical to clearance of LDL from the circulation, as both LDL receptor knockout mice and people with dysfunctional LDL receptor exhibit drastically increased levels of circulating LDL cholesterol [111, 112].

In population studies, LDL cholesterol has been associated with risk of CHD [41]. The modification of LDL contributes to its pro-atherogenic reputation. Triglyceride enrichment of LDL, which is caused by the hypertriglyceridemia associated with obesity and insulin resistance, contributes to formation of small, dense LDL particles, which are associated with increased risk of CHD [113]. Small dense LDL has reduced affinity for the LDL receptor and increased interaction with artery wall proteoglycans. This combination of factors increases the risk of LDL contributing to formation of atherosclerotic lesions that contribute to CHD.

In contrast to LDL and VLDL, Apolipoprotein A-1 (ApoA-1) is central to the structure of HDL. ApoA-1 is a 243 amino acid protein (28 kd) that has 10 alpha helix domains [114, 115]. The c-terminal region of human apoA-1 is highly hydrophobic and is critical for lipid binding [114, 116]. Apo-A1 is produced in the liver and secreted into the circulation. As Apo-a1 binds lipids, a conformational shift occurs that allows for additional lipid binding [117]. As Apo-A1 collects lipids, a nascent HDL particle forms that has a discoidal shape. Two molecules of Apo-A1 wrap around the discoidal HDL particle in a “double-belt” conformation [118]. Mature HDL particles consist of a neutral lipid core mainly composed of CE and TG enveloped by a phospholipid monolayer. Spherical HDL is bounded by three ApoA1 particles that are arranged in a trefoil conformation [119]. The activity of lecithin—cholesterol acyltransferase (LCAT) is crucial to the formation of spherical HDL, as it converts free cholesterol (FC) to CE, which is poorly soluble in the phospholipid bilayer [120]. Mature HDL is cleared of cholesterol in the liver through the activity of SR-B1, which is crucial to HDL function.

HDL's major function is to reverse cholesterol transport (RCT), the movement of cholesterol from peripheral cells to the liver for excretion from the body. The first step in RCT is the uptake of cholesterol from macrophages to nascent HDL involving the loading of FC onto lipid-free/poor apoA1 [121]. ATP binding cassette A1 (ABCA1) plays an important role in this process by actively transporting lipids from macrophages to nascent HDL. Deficiency of ABCA1 is associated with Tangier disease, a condition marked by extremely low levels of HDL and accumulation of cholesterol in peripheral tissues [122]. FC is esterified by LCAT in HDL, and the CEs are then transported to the liver. In the liver, HDL docks to SR-B1, which facilitates the flux of CE into the liver [123]. SR-B1 facilitates movement of CE along its concentration gradient into liver cells; therefore, more CE rich HDL will have a higher rate of CE transfer to the liver [124]. Additionally, CE can be transferred from HDL to LDL, which can then be cleared by LDLR in the liver, contributing to the transport of cholesterol to the liver. Overall, the process of reverse cholesterol transport is important to health, as excess cholesterol in macrophages contributes to atherosclerosis, a significant risk factor for CHD [125].

Obesity and insulin resistance contribute to dysfunction of HDL. Particularly, the hypertriglyceridemia associated with obesity and insulin resistance correlates with decreased levels of HDL [126]. The cause of this relationship is likely TG enrichment of HDL in the context of hypertriglyceridemia. Elevated levels of TG in VLDL result in increased transfer of TG to HDL through the activity of CETP [127]. TG enrichment of HDL makes it a better substrate for modification by hepatic lipase [128]. This modification results in increased uptake of Apo-A1 by the kidney, resulting in decreased HDL levels [129]. TG enrichment of HDL has also been associated with increased

clearance of HDL in humans [130]. Since population studies have shown that reduced levels of HDL correlate with increased risk of CHD, it would suggest that CETP activity contributes to CHD risk.

Cholesteryl ester transfer protein

CETP is involved in both lipoprotein biology and whole-body glucose metabolism and has been studied as a factor that might alter the risk of CHD. CETP is a 78kd glycoprotein that is produced mainly in the liver and adipose tissue and secreted into the serum. In the serum, CETP has lipid transfer activity to shuttle TG and CE between HDL and LDL/VLDL, resulting in TG enrichment of HDL (Figure 1.1) [5]. In the context of obesity and elevated serum TG, CETP activity has the net effect of reducing HDL by transferring TG to HDL, destabilizing the ApoA1 scaffold of HDL and increasing its clearance [6]. Since reduced HDL is an established risk factor for CHD, inhibition of CETP became a drug target with the goal of increasing HDL and reducing CHD. Studies of genetic variants that correlate with varying degrees of CETP activity have shown conflicting results on the relationship between CETP activity and human health. Additionally, CETP inhibitor drugs have not proven effective at reducing the risk of CHD. Recent research has shown that CETP has the ability to promote reverse cholesterol transport and increase bile acid secretion, which may contribute to improved glucose metabolism [10, 11]. Clinical studies of CETP have suggested a sexually dimorphic role for CETP, with CETP having a positive effect on reverse cholesterol transport and glucose metabolism in women [12, 13, 131]. Overall, I propose that CETP has a role to improve reverse cholesterol transport and glucose metabolism in women.

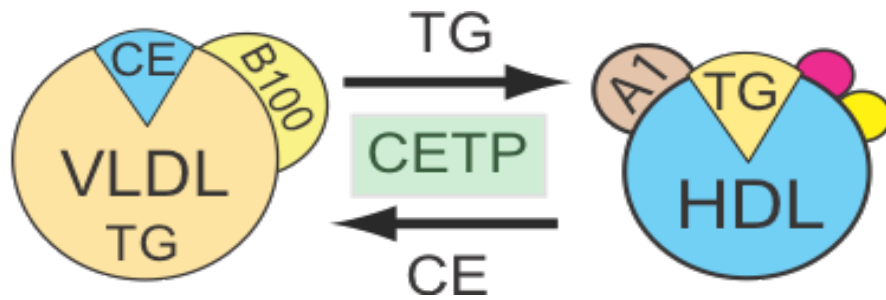


Figure 1.1: Schematic of CETP function. CETP facilitates the movement of lipids between lipoproteins. Specifically, CETP promotes the flux of CE from HDL to ApoB containing lipoproteins LDL/VLDL and TG from LDL/VLDL to HDL.

The structure of CETP defines its function

The function of CETP is tightly linked to its protein structure. CETP facilitates the exchange of lipids between lipoproteins by forming a connection between two lipoproteins, typically HDL and either LDL or VLDL. Structurally, CETP consists of three distinct regions: an N-terminal beta-barrel domain that interacts with HDL, a C-terminal domain that interacts with LDL/VLDL, and an internal structure that creates a hydrophobic pocket (Fig. 1.2) [132, 133]. The N and C terminal ends of CETP attach to lipoproteins and create pores through which lipids can move into the hydrophobic tunnel created by the internal structure of CETP. The terminal ends of CETP serve to specifically recognize HDL and LDL. Both the N and C terminal domains are highly conserved across species, and mutation studies have identified locations in the terminals of CETP that are required for lipoprotein binding activity [134, 135]. Once attached to the lipoprotein, the ends of CETP penetrate the lipoprotein and form a pore through which lipids can move into the central hydrophobic pocket. CETP completely penetrates the phospholipid bilayer of HDL, reaching the core of the lipoprotein, while it incompletely penetrates the phospholipid bilayer of LDL [136]. CETP activity usually is a heteroexchange of lipids, resulting in a one-for-one exchange of TG and CE [137]. In what became known as the shuttle hypothesis, CETP was initially thought to function by accepting lipids from one lipoprotein, detaching, and then binding to a second lipoprotein where it released the bound lipids [138]. More recently, however, a growing body of evidence supports the CETP tunnel hypothesis, which proposes that CETP docks two lipoproteins together and allows for movement of lipid through the hydrophobic core [139]. CETP's selectivity for specific lipoproteins and its ability to

penetrate the lipoprotein surface support the tunnel hypothesis. Regardless of which hypothesis reflects the true nature of CETP function, CETP has a physical structure that allows for it to selectively target lipoproteins and promote exchange of lipids between them.

Intracellular role of CETP

In addition to its role in serum, CETP has intracellular roles in the trafficking and storage of lipids, especially in adipocytes. Expression of CETP in cultured cells resulted in an increase in cholesterol uptake that was not affected by the inhibition of extracellular CETP using an anti-CETP antibody, suggesting that intracellular CETP expression is sufficient to alter cholesterol uptake [140]. Knockdown of CETP in adipocytes, which normally express CETP resulted in a 4-fold increase in CE content and a 50% reduction in TG content. The knockdown of CETP appeared to alter intracellular trafficking of CE and TG, with a large decrease in newly synthesized CE and TG in lipid storage droplets and a corresponding increase in these lipids in the endoplasmic reticulum [141]. A separate study in cultured hepatocytes showed that CETP overexpression or inhibition did not have a significant effect on uptake of CE [142]. Overall, these studies suggest that intracellular CETP has a significant effect on intracellular lipid trafficking but a non-significant effect on CE uptake from outside the cell. The intracellular effect of CETP in the liver is not known. I propose that the major effects of CETP on metabolism are related to serum transfer of lipids between HDL and LDL and their delivery to the liver.

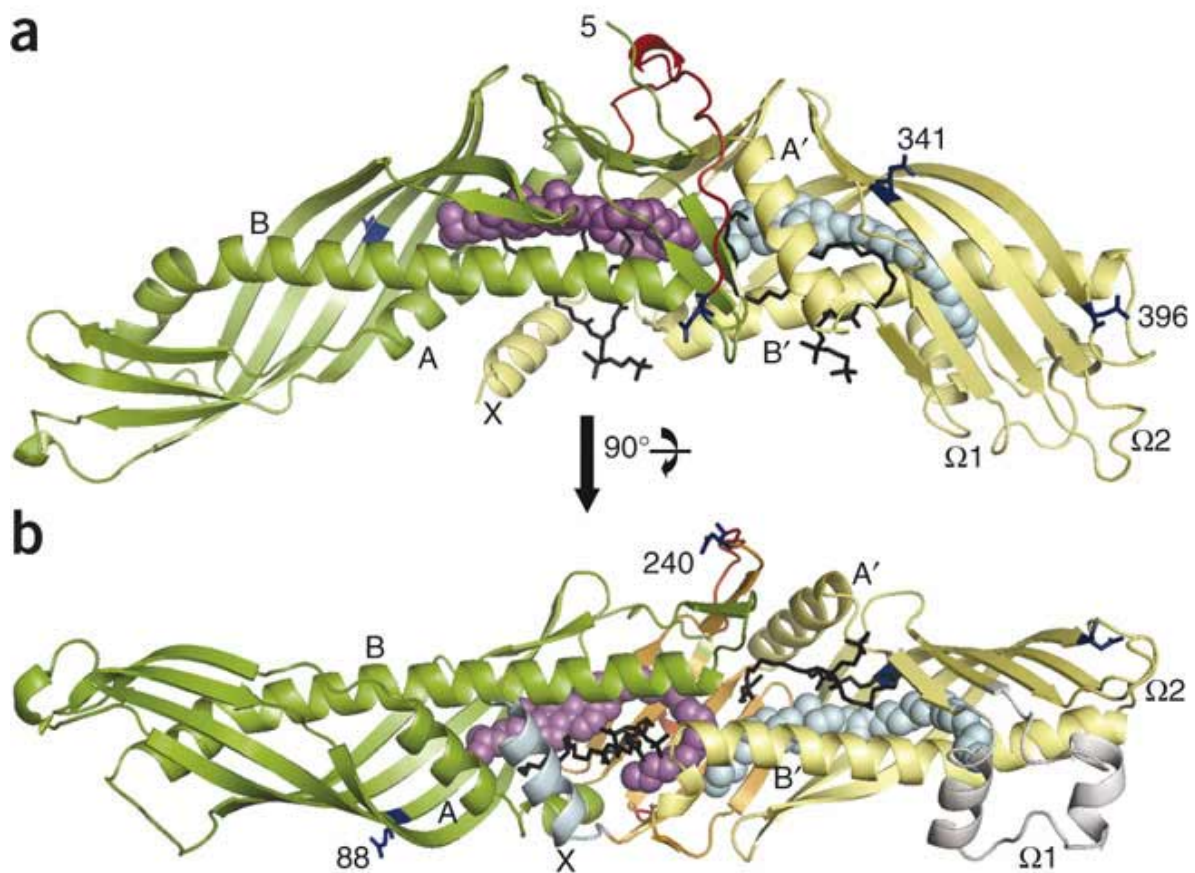


Figure 1.2: Structure of CETP A) Diagram of CETP protein structure highlighting the N-terminal (green), C-terminal (yellow), and linker (red) domains of the protein. Two cholesteryl esters are shown in the interior hydrophobic pocket (magenta and cyan). B) 90 degree rotation of the protein providing an alternate view of the protein. Diagram from [133].

Insights from clinical studies of CETP

Because of its important role in lipoprotein metabolism, CETP has received a significant amount of focus from clinical and basic research. Since CETP reduces HDL and increases LDL cholesterol, it was thought that CETP activity would clearly have deleterious effects on cardiovascular health. Exploration of the effect of CETP on human health, however, has revealed a more complex role for CETP. Human genetic studies have explored the relationships between polymorphisms in the CETP gene and various indicators of human health. These studies, however, appear to have raised more questions than they have answered. Additionally, the failure of CETP inhibitor drugs to reduce risk of CHD has suggested that CETP has additional roles in metabolism beyond affecting HDL level. Finally, studies of CETP in humans have shown a sexual dimorphism in the role of CETP in metabolism and suggest a beneficial effect on reverse cholesterol transport and glucose homeostasis in women. The questions raised about CETP through human studies have provided an important basis for my dissertation work.

Studies of genetic polymorphisms in the CETP gene

Studies of human genetics have identified interactions among genetic variants that affect CETP activity and insulin sensitivity and cardiovascular risk. Unfortunately, these studies have raised more questions than they have answered about the role of CETP in health and disease, as the correlations between CETP polymorphisms and disease have not been consistent across studies in different populations, suggesting a more complex role for CETP than simply negatively impacting health.

The CETP gene has an important single nucleotide polymorphism (SNP) at the Taq1B site in intron 1. A substitution from guanine to adenine at the Taq1b site is known as the B2 variant, whereas the presence of guanine is indicated by the B1 variant. Approximately 65% of humans possess at least one copy of the B2 allele. In a study of Taq1B polymorphisms in diabetic patients, patients homozygous for B1 showed the highest levels of CETP and lowest levels of HDL, whereas those homozygous for the B2 polymorphism showed reduced CETP mass and higher levels of HDL. Overall, the B2 polymorphism is associated with lower levels of CETP and higher levels of HDL in serum [143]. The Taq1b B1 and B2 polymorphisms of CETP, therefore, have been of interest to human genetics researchers as a way to study the effects of variable CETP levels on human health. Based on these studies, it appears that the sex and ethnicity of the study population have a significant effect on whether CETP activity has a beneficial, deleterious, or insignificant effect on human health.

Several genetic studies have identified a correlation between the CETP Taq1b B1 polymorphism and increased risk of disease. Across multiple genetic studies, the B1 polymorphism was consistently associated with lower levels of circulating HDL. A study from the Framingham Offspring population showed that the B1 polymorphism was correlated with reduced HDL [144]. Additionally, studies performed in people from Chinese, Iranian, and Tunisian populations have shown a correlation between the B1 genotype and reduced HDL as well as increased risk of CHD [145-147]. A study in an Iranian population also identified a correlation between the B1 allele and increased risk of CHD that was independent of plasma HDL level [148]. Interestingly, a study of an American population with pre-existing coronary artery disease showed that while the

presence of the B1 allele correlated with reduced serum HDL, it did not have a significant correlation with heart attack events. The study showed, however, that people with the B2 allele benefited more from statin therapy, with a larger reduction in heart attacks seen in the B2 group than the B1 group when they were both put on statin therapy [149]. Finally, a study in a French population showed that the B1 allele was correlated with higher incidence of CHD. Additionally, the study showed that people homozygous for the B1 allele had an elevated risk of sudden death from cardiovascular causes not related to fatal stroke or myocardial infarction (MI) [150]. Overall, a significant body of evidence suggests that the B1 allele correlates with higher CETP activity and reduced serum HDL. These changes in serum HDL do not correlate with increased CHD risk in all studies.

The Taq1b B1 polymorphism does not always correlate with increased risk of disease compared to the B2 polymorphism, and the correlation of the B1 polymorphism with increased risk does not appear to be uniform across study populations of differing ethnicities, particularly in Asian populations. A study in a Singaporean population consisting of people from Chinese, Malaysian, and Indian ethnic backgrounds showed that the Taq1b B2 had a protective effect against CHD in the Chinese population [151]. The same study, however, did not observe an effect of the B2 polymorphism on risk of CHD in the people of Malaysian and Indian ethnicity [151]. A different study in an Indian population, however, showed a correlation between increased risk of CHD and the B1 polymorphism [152]. Overall, genetic studies of CETP have shown that CETP has a significant effect on serum lipoproteins, with increased CETP activity correlating with reduced HDL. While reduced HDL is a classic risk factor for increased risk of CHD,

genetic variation in CETP that is correlated with reduced HDL does not always correlate with increased risk of CHD. Further study of CETP is therefore required to determine its role in whole-body metabolism.

CETP inhibitor drugs

Since CETP correlates with decreased HDL, CETP inhibition became a popular target of drug development efforts that aimed to increase HDL levels and reduce risk of CHD. The studies performed on the development and testing of these drugs have provided further insight into the role of CETP in CHD but have also raised additional questions about the linkage between HDL levels and risk of CHD. Although CETP inhibitors have proven effective at inhibiting CETP activity and elevating serum HDL level, none of the inhibitor drugs tested to date, specifically Torcetrapib and Dalcetrapib, have demonstrated clinically meaningful efficacy in reducing CHD risk. Two other CETP inhibitors, Anacetrapib and Evacetrapib, are currently in phase III clinical trials. The lack of demonstrated clinical efficacy by this class of drugs suggests that CETP may have underappreciated effects on cardiovascular health that are independent of its effect on HDL levels.

Torcetrapib is the most famous CETP inhibitor and the first to enter phase III clinical trials. The drug was considered a failure after the phase III trial was halted due to an increase in myocardial infarction events in the treatment group compared to placebo [153]. Torcetrapib binds CETP in a 1:1 ratio and increases the affinity of CETP for HDL, forming a nonproductive complex that inhibits all of the lipid transfer functions of CETP [154]. While treatment with Torcetrapib reduced LDL cholesterol by 25% and

raised HDL cholesterol by 70%, the hazard ratio for cardiovascular events was 1.25 in the Torcetrapib treated patients compared to the placebo control group. This lack of efficacy suggests that there is a role for CETP that may be independent of its effects on HDL amount. The failure of Torcetrapib was attributed to off-target effects of the drug to increase blood pressure that was associated with increases in aldosterone and corticosterone [155]. The effects of Torcetrapib on CETP activity, however, have not been ruled out as a potential deleterious effect on cardiovascular health. Since CETP inhibition significantly increased HDL, it was expected that it would correlate with reduced risk of CHD. Surprisingly, inhibition of CETP increased risk of death from CHD.

Other drugs that do not have effects on the aldosterone system, such as Dalcetrapib, have also been investigated in clinical trials. Dalcetrapib was the first small molecule inhibitor of CETP to be developed, although it entered phase III clinical trials several years after Torcetrapib. Dalcetrapib is a less potent inhibitor of CETP that covalently binds CETP. Both Torcetrapib and Dalcetrapib increase affinity of CETP for HDL, and they compete with each other for binding CETP, suggesting that they target similar domains of CETP [156]. Dalcetrapib treatment of hamsters resulted in a net increase in reverse cholesterol transport (RCT) as indicated by tracer efflux from radiolabeled cholesterol-laden macrophages to feces in the form of both neutral sterols and bile acids [157]. In contrast, clinical studies of Torcetrapib did not show an increase in fecal sterol secretion in a study of human subjects [158]. When bound to CETP, Dalcetrapib induces a conformational change in the CETP protein that interferes with its ability to bind LDL/VLDL but maintains its ability to bind HDL [157]. In this model, Dalcetrapib allows for CETP-mediated exchange of lipids between HDL particles and

promotes the lipidation of nascent HDL, increasing HDL levels and promoting reverse cholesterol transport[157].

In contrast to Torcetrapib, Dalcetrapib does not alter the aldosterone system and has no effect on blood pressure [159]. Similar to Torcetrapib, Dalcetrapib treatment significantly increases HDL levels by about 30%. Clinical trials of Dalcetrapib, however, did not show a clinically meaningful reduction in CHD risk [9]. Since Dalcetrapib does not alter blood pressure, its failure to protect against CHD risk suggests that the CETP inhibitors as a class of drugs may not be effective clinically despite their ability to increase HDL cholesterol levels. The failure of CETP inhibitors to protect against CHD despite increasing HDL concentration illustrates a need to better understand the role of CETP in metabolism. CETP has effects on metabolism beyond its role in HDL concentration that have recently been identified in human and animal studies.

Sex-specific role for CETP activity in humans

Studies of CETP in humans suggest that CETP has differential effects in men and women, specifically with regards to glucose metabolism. In men, studies suggest a neutral or harmful effect of CETP on glucose homeostasis. An association study in men showed a correlation between high levels of CETP activity and markers of insulin resistance [160]. Additionally, an insulin clamp study performed in men showed no correlation between CETP activity and insulin sensitivity [161]. A *post-hoc* analysis of the ILLUMINATE CETP inhibitor trial showed that diabetic patients treated with the Torcetrapib had a modest reduction in hemoglobin A1c [161]. This study involved 70% men and only examined individuals with diabetes and CHD.

Reciprocally, studies in females suggest beneficial effects of CETP on cholesterol efflux and glucose metabolism. Higher CETP activity is associated with increased transfer of CE from macrophages to HDL in women but not men [12, 131]. This improved RCT capacity with increased CETP was independent of HDL cholesterol [12]. In women, CETP polymorphisms that increase CETP activity correlated with reduced risk of ischemic heart disease [162]. Higher CETP activity is associated with improved glucose metabolism after roux-en-Y gastric bypass in women only [13, 163].

Overall, studies of CETP in humans have revealed a much more complex role for CETP with regards to CHD than a simple effect on circulating HDL levels. The effect of CETP on risk of CHD appears to be influenced by ethnicity based on polymorphism studies. Additionally, the failure of CETP inhibition to reduce heart attack despite increasing HDL suggests that CETP's lipid transfer activity may not directly result in increased CHD risk. Finally, studies in humans have suggested a sex-specific role for CETP, with CETP activity actually correlating with improved health outcomes in women. CETP requires further study to fully understand its effects on human health.

Regulation of CETP in humans

CETP levels are highly variable in humans [164]. Serum CETP levels are known to be increased by obesity [165] and estrogen [166], and CETP expression is acutely suppressed by insulin [167]. Human CETP is highly regulated at the transcriptional level [168, 169]. The human CETP promoter is regulated by SREBP1a [170], LXR [171], LRH-1 [172], and RXR [173]. Overall, CETP expression appears to be linked to intracellular cholesterol content, which activates SREBP1a and LXR, both of which have

response elements in the CETP promoter [170, 171]. The observation that expression of the CETP gene is induced by dietary cholesterol is consistent with this model [174]. Estrogen has been shown to interact with SREBP1a signaling [175], which could be the mechanism by which estrogen upregulates CETP levels.

Because of the variability associated with the CETP gene under control of the human promoter, I chose to use a transgenic mouse model that expresses CETP under control of a constitutive promoter. Mice do not naturally express CETP, so transgenic expression of CETP is required to study CETP in mice. The particular mouse strain that I used expresses a simian CETP gene under control of the metallothionein promoter known as UCTP-20. This mouse line was generated in the early 1990s with the goal of understanding the contribution of CETP to the primate/human lipoprotein profile [176]. The transgenic mouse model expresses CETP in all tissues except lung and spleen, but the levels of CETP RNA are by far the highest in the liver, followed by the brain and heart [176]. The CETP transgenic mice show a more human-like lipid profile, with increased cholesterol in ApoB containing lipoproteins and decreased cholesterol in ApoA containing lipoproteins compared to WT mice [176]. When fed a high-fat/high-cholesterol diet, the CETP-expressing mice showed an increase in total cholesterol [176]. A second study of the same transgenic mice showed increased atherosclerotic lesions in aortas of the CETP-expressing mice when fed a high-fat/high-cholesterol diet [177]. I assert that these mice are an appropriate model to test the contribution of a constant level of CETP on whole-body metabolism. Since human CETP is regulated by diet, insulin, and estrogen, I wanted to use a model that would have consistent CETP activity during high-fat feeding, hyperinsulinemic clamps, and between sexes.

Beneficial role of CETP on reverse cholesterol transport: Insights from model organisms

Recent work in both model organisms and humans has suggested that CETP has a beneficial role to promote RCT and thereby promote bile acid secretion. Bile acids have recently been shown to have beneficial effects on glucose metabolism; therefore, CETP may be a factor that promotes metabolic health.

The study of CETP in mice has required development of transgenic mice or viral expression of CETP. Expression of CETP in mice has demonstrated that CETP promotes RCT in a manner that is SR-B1 independent but LDLR dependent. Expression of CETP in an SR-B1 knockout mouse model almost completely reversed the hypercholesterolemia associated with SR-B1 deficiency [10], suggesting that CETP is able to promote RCT independently of SR-B1, which is the primary method of HDL uptake by the liver. In another study, viral expression of CETP in mice increased efflux of radiolabeled cholesterol from macrophages to feces in SR-B1 knockout mice but not LDLR knockout mice, indicating that CETP promotes RCT in an LDL receptor-dependent manner [11]. The activity of CETP to promote movement of cholesterol from HDL to LDL likely increases the RCT through hepatic cholesterol uptake from LDL through LDLR. The increase in radiolabeled cholesterol to feces in the CETP-expressing animals suggests that CETP may increase gut bile acid content, as cholesterol that is taken up by the liver is largely excreted in the form of bile acids (Fig. 1.3). Recent studies have suggested that bile acids can be signaling molecules that improve glucose metabolism. Therefore, the ability of CETP to promote RCT and increase bile acid content may improve whole-body glucose homeostasis.

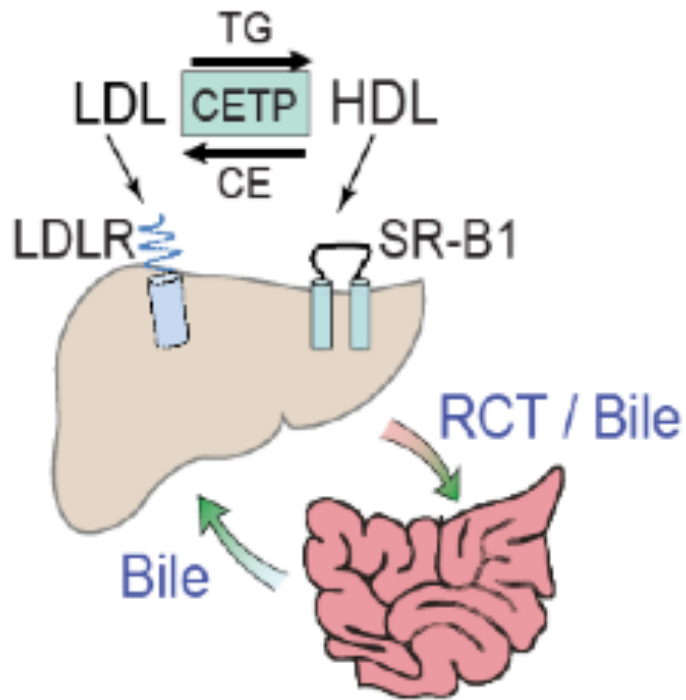


Figure 1.3: CETP promotes reverse cholesterol transport by increasing LDL-mediated delivery of CE to the liver. CETP expression mediates flux of CE from HDL to LDL. CETP promotes reverse cholesterol transport by increasing delivery of CE to the liver through LDL receptor. This process causes increased secretion of bile into the gut, as cholesterol is converted to bile in the liver and secreted into the gut.

Bile acid signaling improves glucose metabolism

In addition to their role in digestion, bile acids are signaling agents that regulate pathways important for glucose metabolism in tissues including liver, adipose tissue, and muscle. Bile acids improve glucose metabolism by inhibiting hepatic gluconeogenesis, promoting hepatic glycogen storage, and promoting energy expenditure in brown adipose tissue and skeletal muscle. Because CETP has been shown to increase RCT and bile acid production, I propose that CETP may potentially have a protective effect against the development of metabolic syndrome with obesity.

Bile acids signal through three major pathways to alter glucose metabolism in liver, adipose tissue, and muscle (Fig. 1.4). 1) Bile acids promote the secretion of the enterokine fibroblast growth factor 15 (Fgf15), which signals to the liver to suppress hepatic glucose production. 2) Bile acids themselves act as ligands of Fxr in the liver. 3) Bile acids activate the g-protein coupled bile acid receptor (GPBAR1 also known as TGR5) in brown adipose tissue and muscle. These pathways combine to promote healthy glucose metabolism by suppressing hepatic gluconeogenesis and promoting muscle glucose uptake.

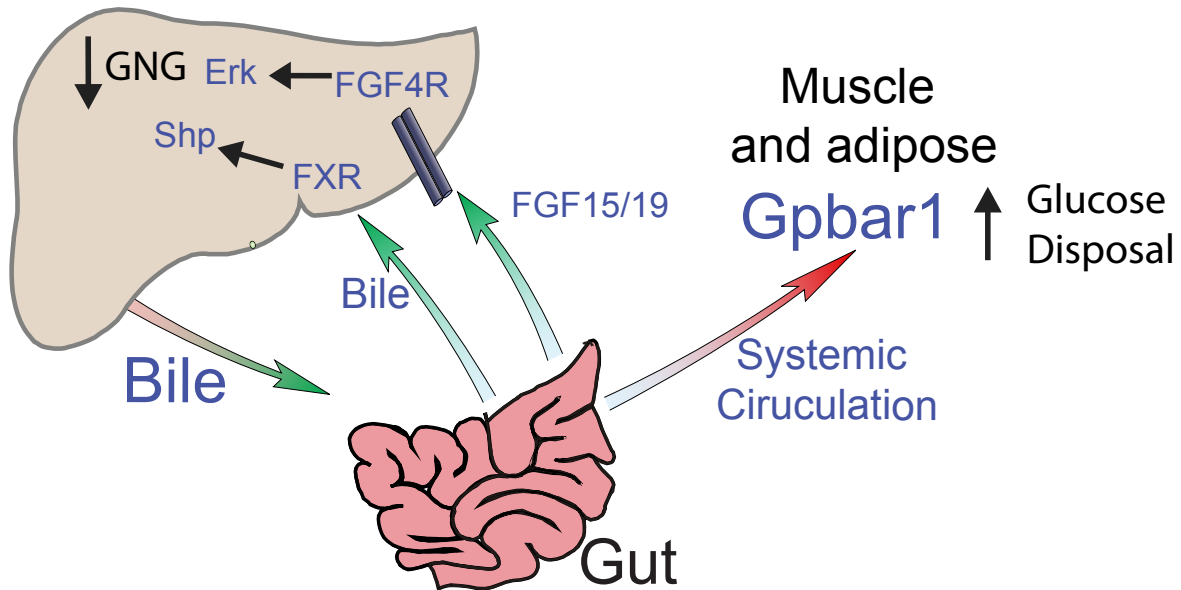


Figure 1.4: Bile acids signal to alter metabolism in liver, adipose tissue, and muscle. Bile acids recirculate from the gut to the liver and serve as a ligand for Fxr, which induces Shp expression, a transcriptional co-repressor that downregulates genes the products of which promote hepatic gluconeogenesis. In the gut, bile acids induce production of FGF15, which signals to the liver through Fgf4r to promote glycogen storage and downregulate genes the products of which promote gluconeogenesis. A small amount of bile acids escape the entero-hepatic circulation into the systemic circulation. Bile acids can signal through Gpbar1 to promote glucose disposal in brown adipose tissue and skeletal muscle.

Bile acids signal through Fgf15 to alter hepatic glycogen storage and gluconeogenesis

The effect of bile acids on hepatic insulin sensitivity is in part mediated by Fgf15. Fgf15 is produced in ileal enterocytes in response to bile acid signaling through farnesoid X receptor (Fxr) [178]. Fgf15 signals to the liver through the fibroblast growth factor 4 receptor (Fgf4r) to activate ERK1/2 (Fig. 1.4) [179]. Fgf15 signaling in the liver has been shown to promote glycogen storage and reduce gluconeogenesis [14, 15]. Fgf15 knockout mice exhibit glucose intolerance and have reduced liver glycogen content compared to control mice [14]. Treatment of mice with Fgf15 resulted in increased hepatic glycogen synthase and increased phosphorylation of glycogen synthase kinase 3 α and β . Administration of Fgf15 to mice with streptozotocin-induced diabetes normalized hepatic glycogen content to levels of control mice [14].

In addition to its ability to promote hepatic glycogen storage, bile acid signaling through Fgf15 suppresses hepatic gluconeogenesis. Treatment of lean mice with Fgf15 results in downregulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α), which is a transcription factor central to activating genes the products of which control gluconeogenesis [15]. Consistent with this result, *G6pc* and *Pck* gene expression was decreased in mice treated with Fgf15. Adenoviral overexpression of Fgf15 similarly reduced gene expression for factors that control gluconeogenesis and also repressed hepatic gluconeogenesis, TCA cycle flux, and fatty acid oxidation [15]. In contrast, Fgf15 knockout mice exhibit hyperglycemia, increased gene expression for *G6pc* and *Pck*, and increased levels of gluconeogenesis during the fed state compared to control mice [15]. Overall, Fgf15 has emerged as an important

new enterokine factor that allows for bile acids to signal to the liver to promote storage of glucose as glycogen and suppress hepatic gluconeogenesis. Fgf15 acts independently of insulin through the ERK signaling pathway and may be a factor that can improve insulin sensitivity in insulin resistant people.

Bile acids signal through hepatic Fxr to suppress gluconeogenesis

Bile acids are ligands for the nuclear transcription factor Fxr, which induces expression of small heterodimer partner (Shp) (Fig. 1.4) [180, 181]. Fxr is required for normal glucose metabolism in the liver, and Fxr knockout mice exhibit a number of metabolic irregularities including fatty liver, elevated serum free fatty acids, elevated serum glucose, and insulin resistance. Feeding mice a 1% cholic acid diet, which is an agonist for Fxr caused a significant decrease in RNA levels for *Pck*, *Ppargc1a*, and *G6pc*, genes the products of which promote hepatic gluconeogenesis. These effects were not observed in Fxr knockout mice, indicating that Fxr is required for the suppressive effects of bile acids on gluconeogenesis [182]. The effect of the cholic acid treatment on metabolism is likely due to a combination of direct Fxr signaling in the liver and indirect signaling through Fgf15, as production of Fgf15 is activated through Fxr signaling in the gut.

Bile acids signal through Gpbar1 to increase energy expenditure and glucose oxidation

Bile acids signal through a third pathway to muscle and adipose tissue through the G-protein coupled bile acid receptor Gpbar1 (Fig 1.4). Addition of the bile acid cholic acid to a high-fat diet resulted in decreased adiposity compared to high fat diet (HFD) without added cholic acid [17]. The authors of this study reported that signaling through Gpbar1 activates type-2 thyroid deiodinase (*Dio2*), which leads to increased energy expenditure in brown adipose tissue [17]. Further study of Gpbar1 has shown it to have a role in regulation of glucose homeostasis. Treatment of diet-induced obese mice with a Gpbar1 agonist resulted in increased energy expenditure, reduced levels of hepatic fat, and decreased adiposity. Additionally, treatment of both diet-induced obese mice and *db/db* mice with a Gpbar1 agonist improved glucose tolerance as measured by oral glucose tolerance tests. Hyperinsulinemic clamps in diet-induced obese mice treated with a Gpbar1 agonist showed that activation of Gpbar1 improves insulin sensitivity. Studies of isolated brown adipocytes treated with the Gpbar1 agonist showed that mitochondrial oxygen consumption was increased by Gpbar1 activation [16]. Additionally, bile acid signaling to Gpbar1 in intestinal L-cells caused increased secretion of Glp1, which is known to promote glucose stimulated insulin secretion [16]. Activation of Gpbar1 by bile acids is protective against HFD-induced glucose intolerance and insulin resistance likely due to its ability to promote glucose disposal. Based on these studies, I propose that factors that increase bile acid production or secretion, such as CETP, might be protective against the effect of obesity on glucose metabolism and insulin sensitivity.

Bile acid treatment improves insulin sensitivity in obese humans

Bile acids also appear to have a significant effect on glucose metabolism in humans. Obese humans treated with a four-week oral course of the bile acid tauroursodeoxycholic acid (TUDCA) showed improved hepatic insulin sensitivity as defined by glucose production during a hyperinsulinemic clamp. Additionally, the TUDCA-treated subjects showed an increase in clamp glucose rate of disappearance, suggesting improved muscle insulin sensitivity [183]. The TUDCA-treated subjects also showed improvement in muscle insulin signaling, with higher levels of insulin receptor substrate tyrosine phosphorylation and increased levels of Akt serine phosphorylation. This study demonstrates that bile acid treatment improves insulin sensitivity in obese humans and that the benefits of bile acid signaling are not restricted to the mouse.

Obesity decreases exercise capacity

Weight loss is the best method to reverse the effects of obesity. One method that can help people lose weight is increased physical activity. Unfortunately, exercise capacity is known to decline with obesity, and therefore losing weight through exercise becomes more difficult, creating a vicious cycle. Exercise capacity is defined as the maximal amount of physical exertion that a person can sustain, and it is frequently defined clinically using a treadmill test. A study of people with pre-existing diabetes showed that BMI and waist circumference negatively correlate with exercise capacity, demonstrating the link between obesity and impaired exercise capacity [4]. Mechanisms that might help improve exercise capacity could help to treat obesity and improve quality of life for obese people.

Exercise capacity is extremely important to overall health, as a study of patients referred for clinical exercise testing showed that level of physical fitness inversely correlated with death from all causes. In fact, exercise capacity was a stronger predictor of death than established cardiovascular risk factors such as hypertension, smoking, and diabetes [184]. A second study of exercise capacity in diabetic men showed that reduced exercise capacity was predictive of mortality in a manner that was independent of BMI [185]. These results suggest that fitness in the form of exercise capacity may be more important in preventing deaths than previously identified factors such as hypertension, smoking, obesity, and diabetes. Mechanisms that might improve exercise capacity even in the context of obesity are therefore important to study.

Exercise capacity depends on muscle substrate oxidation

Exercise capacity depends on multiple different factors throughout the body, but all are related to the ability of muscle mitochondria to efficiently oxidize substrate to produce ATP. Exercise capacity depends both on central factors such as the ability of the lungs to extract oxygen and the ability of the heart to pump oxygenated blood to muscle tissue [186, 187]. Additionally, peripheral factors such as the ability of oxygen to diffuse from blood vessels to muscle, the density of capillaries in the muscle, and the ability of mitochondria to effectively perform oxidative phosphorylation all contribute to exercise capacity [188, 189]. In addition to delivery of oxygen, exercise capacity also depends on oxidation of substrate. At lower levels of exercise intensity (less than 50% of maximal capacity), the majority of substrate used for exercise is lipid. At higher levels of exercise intensity, carbohydrate becomes the preferred substrate. A study of men

running on a treadmill at a fast versus slow pace showed that nearly 100% of the energy expenditure was from carbohydrate in the fast group compared to 80% in the slower-paced group[190]. Therefore, mechanisms that improve muscle glucose oxidative capacity might help to improve exercise capacity, as improved ability to utilize glucose in muscle might lead to increased tolerance for higher intensity exercise.

Studies have suggested that bile acid signaling through Gpbar1 increases muscle glucose utilization by improving mitochondrial oxidative capacity. Since CETP is known to promote RCT and bile acid production, I propose that CETP expression might increase exercise capacity by improving muscle mitochondrial oxidative capacity.

Summary

Obesity is a serious health challenge, as it correlates with risk of CHD and diabetes. Mechanisms that improve metabolic health in obese people might help to delay or prevent the development of these diseases. Cholesteryl ester transfer protein may be a previously underappreciated factor that could promote healthy metabolism in obesity. CETP was previously thought to be harmful due to its effects on lipid profile, but inhibition of CETP has not proven to be an effective strategy to prevent CHD. Further study of CETP is therefore required to fully understand its role in metabolism. Studies in humans have suggested a sexual dimorphism related to CETP and have shown that CETP might be a neutral factor in men but have a positive effect on RCT and glucose homeostasis in women. Recent research in animal models has demonstrated that CETP can promote RCT and secretion of cholesterol into the gut in the form of bile acids. Bile acids have been shown to activate signaling pathways in gut, liver, adipose

tissue, and muscle that lead to improved glucose homeostasis (Fig. 1.4). Bile acid signaling also leads to improved mitochondrial function in muscle, which is an important factor in exercise capacity. Therefore, I propose that CETP is a positive factor that might protect against the development of insulin sensitivity and exercise intolerance with obesity.

CHAPTER II

MATERIALS AND METHODS

Animals and diets

CETP transgenic mice on a C57BL/6 background were purchased from Jackson Laboratories (C57BL/6-Tg(CETP)1Pnu/J, Stock Number: 001929). This strain expresses a simian CETP gene under control of the metallothionine promoter [15, 16]. All mice were maintained on a standard chow diet until placed on a sucrose-free high fat diet (HFD) with carbohydrate content comprised of cornstarch (60% fat, 20% carbohydrate, 20% protein, Research Diets D08060104). Mice were 12-14 weeks old at onset of diet and were fed HFD for 4 weeks. Body composition was determined on the day of study using an mq10 NMR analyzer (Bruker Optics). All procedures were performed in accordance with National Institutes of Health Guidelines for the Care and Use of Animals and approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

Surgical catheterization

Five to seven days prior to the clamp study, mice received catheters in the jugular vein and carotid artery at the Vanderbilt Mouse Metabolic Phenotyping Center. Surgeries were performed using aseptic technique, and all surgical instruments and other materials were sterilized prior to use. Mice were anesthetized with isoflurane using

a vaporization system. To catheterize the carotid artery, a small vertical midline incision was made 5mm cephalic to the sternum. The left sternomastoid muscle was exposed using forceps, and the carotid artery was separated from connective tissue. Once isolated, the cephalic end of the artery was ligated with silk suture, and a second piece of suture was loosely knotted on the caudal end of the exposed artery. The blood vessel was clamped with micro-serrefine hemostats, and a small incision was made in the blood vessel just below the ligated end. Six cm pieces of silastic flexible plastic tubing (0.012" ID) were prepared for use as catheters. The catheter was inserted into the carotid artery, and both ligatures were tied to secure the catheter. A second incision was made 5 mm to the right of the midline and 2 mm caudal to the first incision. The jugular vein was exposed, and a catheter was inserted using a similar procedure to the arterial catheterization. Once the two catheters were implanted, free catheter ends were tunneled under the skin to the back of the neck, externalized, and sealed with steel plugs. All incisions were closed with silk suture. Mice were monitored for health during surgical recovery and treated with antibiotics (ceftriaxone) and analgesics (ketofen). Mice were maintained on HFD and were allowed 5-7 days for recovery after surgery. Only mice returning to within 10% of pre-surgical body weight were studied. Overall, these methods permit arterial sampling and are less stressful than cut-tail sampling.

Hyperinsulinemic-euglycemic clamp studies

To assess insulin sensitivity, a 2-hour hyperinsulinemic-euglycemic clamp study was performed on mice fasted for 5 hours. Mice were weighed and then fasted starting

between 0800 and 0900h. During the fast, glucose, donor blood, insulin, and radiolabeled glucose were prepared. Fifty percent dextrose solution was drawn into a 3mL syringe for glucose infusion. Donor blood was prepared by collecting 1mL of blood from a donor mouse. Blood was then centrifuged, and red blood cells were isolated. Red blood cells were washed with heparinized saline, centrifuged, and then resuspended in an equal volume of heparinized saline. Donor blood was infused at a rate of 2.5 $\mu\text{L}/\text{min}$ during the clamp. Insulin was prepared by diluting U-100 insulin tenfold in a 3% saline-donor plasma mixture. Insulin was then diluted based on the weight of the mouse to achieve an infusion rate of 4 mU/kg/min at a constant infusion rate of 3 $\mu\text{L}/\text{min}$. Fifty μCi of radiolabeled [$3\text{-}^3\text{H}$] glucose per mouse was dried and resuspended in 1 mL of saline. Twenty μL of this solution was saved to determine the exact dose of tracer given to each mouse. Glucose, insulin, donor blood, and radiolabeled glucose were each attached to a separate infusion pump (Harvard apparatus), and all infusions were into the jugular vein. The arterial catheter was flushed with heparinized saline prior to the clamp study and used to draw blood for all samples.

To trace whole-body glucose turnover, a 1.5- μCi bolus of [$3\text{-}^3\text{H}$] glucose was given at $t = -90$ min (90 mins prior to insulin/glucose infusion) followed by a 0.05 $\mu\text{Ci}/\text{min}$ infusion until $t=0$. The clamp was begun at $t = 0$ min with a continuous infusion of insulin ($4 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). The [$3\text{-}^3\text{H}$] glucose infusion was increased to 0.1 $\mu\text{Ci}/\text{min}$ for the remainder of the experiment to prevent changes in specific activity. More recent developments in tracer technique suggest mixing the tracer with the non-labeled glucose 50/50. Euglycemia (100-150 mg/dl) was maintained by measuring blood glucose every 10 min by glucometer starting at $t = 0$ min and infusing 50% dextrose by variable

infusion. Hematocrit was measured at t = 0 and t = 90 to determine that there was no fall in red blood cell count. Arterial samples to measure plasma hormones and glucose turnover were taken at t=60, and during the steady-state period at 80, 90, and 100 min. The steady state period is defined by a constant state of glucose infusion, indicating that the rate of glucose production equals the rate of glucose removal. At 120 min, mice were sacrificed and tissues were collected and frozen in liquid nitrogen. Insulin sensitivity index (SI) was calculated by dividing the steady-state glucose infusion rate in mg/kg*min by the steady state insulin concentration in mU/mL to yield SI in (mg*mL)/(kg*min*mU) [191].

Tracer analysis

[3-³H] glucose was infused to measure glucose rate of appearance (EndoRa) and glucose rate of disappearance (Rd). Levels of radioactivity were determined by scintillation counting on a Perkin-Elmer TriCarb 2390 scintillation counter and expressed in disintegrations per minute (DPM). Glucose turnover is calculated using the body weight of the mouse, the glucose infusion rate, the tracer infusion rate, and the specific activity of plasma glucose at each time point. The tracer infusion rate is determined by scintillation counting of the tracer infusate and the rate of tracer infusion. A 10 µL of a 1:200 dilution of the tracer infusate was dried down and counted to obtain the Chemical Standard Evaporated (CSE). The tracer infusion rate in dpm/kg/min is therefore equal to:

$$\text{Tracer infusion rate (dpm/kg/min)} = \frac{(\text{Pump rate} \cdot \text{CSE} \cdot 20)}{(\text{Body Weight} / 1000)}$$

Specific activity (SA) is defined as the ratio of tracer to tracee and is measured in

dpm/mg. To calculate plasma SA, the plasma glucose concentration and the plasma [3-³H] glucose concentration are required. Plasma samples are treated with Ba(OH₂) and Zn₂SO₄ to de-proteinate the sample. Samples are then evaporated and counted on a scintillation counter. A sample of the infusate is treated and counted in the same manner to provide the Chemical Recovery Standard (CRS). Plasma specific activity is therefore:

$$\text{specific activity (dpm/mg)} = \frac{\frac{\text{Plasma dpm}}{10} \cdot \frac{\text{CSE}}{\text{CRS}} \cdot 1000}{\text{plasma glucose}/100}$$

Glucose turnover in mg/kg/min can therefore be represented by:

$$\frac{\text{tracer infusion rate (dpm/kg/min)}}{\text{Plasma glucose specific activity (dpm/mg)}}$$

The endogenous glucose rate of appearance (Endo Ra) is equal to the glucose turnover rate minus the exogenous glucose infusion rate

Western blots

Whole cell protein extracts were performed on frozen liver and muscle tissue using a T-PER protein extraction kit (Pierce bioscience). Protein concentration in extracted samples was measured using a Pierce BCA protein assay kit. Western blotting was performed using precast 4-12% bis-tris gels (Life Technologies) in an Invitrogen Novex mini-cell chamber. Twenty µg of protein was loaded per sample. Protein was transferred from the gel onto nitrocellulose membrane and then blocked using Odyssey blocking buffer. Following blocking, membranes were treated with primary antibody, washed with tris buffered saline, treated with secondary antibody, washed, and then imaged. Primary antibodies for Akt, P-Akt (Ser473), Erk1/2, and P-

Erk1/2 (Thr202/Tyr204) were purchased from Cell Signaling Technology. Antibodies for actin and ER α were purchased from Santa Cruz Biotechnology. IR-Dye 800 anti-rabbit secondary antibody (LI-COR Biotechnology) was used for band visualization on an Odyssey imaging system.

Serum analysis

Serum insulin values were determined using a commercial ELISA kit (Millipore). CETP activity was measured using a commercially available kit (Roar Biochemical RB-CETP). Serum TG and cholesterol levels were measured using commercially available kits (Raichem). Serum estradiol was quantified using a commercially available kit (Calbiotech). Serum leptin, adiponectin, and IL-6 were quantified by luminex assay at the Vanderbilt Hormone Assay & Analytical Services Core.

Gene expression

RT-PCR was used to measure gene expression. RNA was extracted from tissues (RNeasy Mini, Qiagen) and cDNAs were synthesized using 1 μ g RNA template (iScript cDNA synthesis kit, BioRad). qPCR was conducted using SYBR Green JumpStart Taq ReadyMix (Sigma) in a 20 μ l reaction with 400nM final primer concentration. Reactions were carried out for 30 cycles of 95°C for 10s, 58°C for 45s, and 72°C for 60s (MyIQ, Bio Rad). Ct values were analyzed using the efficiency corrected Pfaffl method and were normalized to cyclophilin A. Fold change was determined relative to fasted WT littermates. Primer sequences are provided in Table 2.1.

Metabolite analysis

Metabolite analysis was performed by Metabolon Inc. (Durham NC). Briefly, aqueous and organic metabolites were methanol extracted and then analyzed both by LC/MS and GC/MS. Metabolites were identified by comparison to a library of known compounds and normalized to internal standards. Welch's two-sample *t*-test and an estimate of the false discovery rate (*q*-value) were used to take into account the multiple comparisons that normally occur in metabolomic-based studies. One animal was excluded as an outlier for hepatic bile acid data, as the gallbladder was ruptured during tissue collection.

Gut bile acid analysis

Bile acids were extracted from ileum tissue by ethanol extraction using a dounce homogenizer. Bile acids were quantified using a commercially available enzymatic assay (Crystal Chem).

Indirect calorimetry

Indirect calorimetry was performed on HFD-fed female CETP and WT littermates using a Promethion system in the Vanderbilt MMPC (Sable Systems International). The system allows for measurement of VO₂, VCO₂, food intake, feeding behavior, heat generation and activity level. Mice were individually housed in the system for a period of 4 days during which measurements were taken. The facility uses a standard 12 h light/dark cycle, and measurements for light and dark phases are reported separately.

Exercise studies

Exercise studies were performed in 12-week old female CETP transgenic mice and their non-transgenic littermates. Mice were fed a standard chow diet until the date of the first exercise study, after which they were fed a sucrose-free HFD with carbohydrate content comprised of cornstarch (60% fat, 20% carbohydrate, 20% protein, Research Diets D08060104). Animals were subjected to an exercise tolerance test every 2 weeks after the beginning of HFD. One week before the first exercise study, animals were acclimated to treadmill running. In the exercise tolerance test, mice were individually placed on an enclosed treadmill attached to an oxymax oxygen analyzer and run at an initial speed of 10 m/min at a 0% grade. Every 3 minutes, speed was increased by 4 m/min. The study was continued until the animal was exhausted, as indicated by remaining on the shock pad at the rear of the treadmill. A total of 4 exercise tolerance tests were performed at 0, 2, 4, and 6 weeks of HFD.

After 10 total weeks on HFD, mice were subjected to a single bout exercise test. Mice were run at a constant speed of 10 m/min for 15 minutes. Immediately before the study, a bolus of 2-deoxyglucose was administered to trace glucose uptake by muscle tissue. Following the exercise study, animals were sacrificed and tissues were collected and flash frozen.

Statistics

Data are presented as mean \pm SEM. Data were analyzed by student's t-test, 1-way ANOVA using Tukey's post-test, or 2-way ANOVA using Bonferoni post-test as appropriate. $p < 0.05$ is considered statistically significant.

Sample sizes were chosen to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. Power calculations were performed using PS Power and Sample Size Calculations, Version 2.1.30

Primer	Sequence
cpt1b_F	TCTAGGCAATGCCGTTTAC
cpt1b_R	GAGCACATGGGCACCATAC
dio2_F	CTTCCTCCTAGATGCCTACAAAC
dio2_R	GGCATAATTGTTACCTGATTCAGG
fxr_F	TCCGGACATTCAACCATCAC
fxr_R	TCACTGCACATCCCAGATCTC
g6pc_F	CTGCAAGGGAGAACTCAGCAA
g6pc_R	GAGGACCAAGGAAGCCACAAT
hk2_F	GGAACCCAGCTGTTTGACCA
hk2_R	CAGGGGAACGAGAAGGTGAAA
hnf4a_F	ACCAAGAGGTCCATGGTGTTT
hnf4a_R	GTGCCGAGGGACGATGTAG
lrh1_F	TGGGAAGGAAGGGACAATCTT
lrh1_R	CGAGACTCAGGAGGTTGTTGAA
ppia_F	CGATGACGAGCCCTTGG
ppia_R	TCTGCTGTCTTTGGAACCTTGTGTC
shp_F	CGATCCTCTTCAACCCAGATG
shp_R	AGGGCTCCAAGACTTCACACA

Table 2.1: RT-PCR Primer sequences

CHAPTER III

CHOLESTERYL ESTER TRANSFER PROTEIN PROTECTS AGAINST INSULIN RESISTANCE IN OBESE FEMALE MICE

Introduction

Insulin resistance associated with obesity leads to metabolic syndrome, diabetes, and increased risk of developing CHD. These risk factors are reduced by weight loss; however, weight reduction regimens rarely result in long-term maintenance of reduced body weight [1, 2]. Activation of pathways that improve insulin sensitivity would be an attractive alternative to weight loss as a way to prevent complications of obesity. One important pathway that has recently been linked to insulin sensitivity is bile acid signaling to liver and muscle [15, 16, 183]. Activation of pathways that promote bile production and secretion may improve insulin sensitivity in the setting of obesity. CETP is a lipid transfer protein that allows for the exchange of lipids between lipoproteins, ultimately resulting in delivery of cholesterol esters to the liver, where they are converted into bile acid [192, 193]. Activation of CETP, therefore, may contribute to improved insulin sensitivity with obesity.

In addition to their role in digestion, bile acids are signaling agents that regulate pathways important for glucose metabolism. Treatment of obese humans with the bile acid tauroursodeoxycholic acid improves liver and muscle insulin sensitivity [183]. Bile acids signal through three pathways to alter metabolism in multiple tissues. The effect of bile acids on hepatic insulin sensitivity is in part mediated by Fgf15, which signals

through Fgf4r in the liver to promote glycogen storage and reduce gluconeogenesis [14, 15]. Bile acids are ligands for the nuclear transcription factor Fxr, which induces expression of Shp [180, 181]. Fxr and Shp regulate multiple genes involved in liver glucose metabolism [182, 194]. Additionally, bile acids signal to muscle through Gpbar1 to activate type-2 thyroid deiodinase (Dio2), which leads to an increase in muscle glucose oxidation by mitochondria [16]. Since bile acids are produced from cholesterol in the liver, mechanisms that increase cholesterol delivery to the liver might promote insulin sensitivity.

CETP has been shown to increase RCT in mice [10, 11]. Since mice do not naturally express CETP, transgenic mice expressing CETP have been studied [176, 177]. Because of its lipid transfer capacity, CETP alters lipid uptake and metabolism by tissues. CETP has the net effect of increasing delivery of CE to the liver by promoting the movement of CE from HDL to VLDL or LDL, which can be taken up by the liver through LDLR and VLDLR [10, 192, 193, 195]. CETP also facilitates uptake of CE from serum macrophages into HDL, which is then delivered to the liver by scavenger receptor B1 (SR-B1)[11, 196]. CE delivered to the liver is converted into bile acids, which are stored in the gallbladder and then secreted into the intestine to facilitate absorption of lipophilic nutrients. Taken together, these results suggest that increased cholesterol delivery to the liver mediated by CETP increases bile acids in the gut and their related signaling to promote insulin sensitivity in liver and muscle. To test this hypothesis, we assessed insulin sensitivity in HFD fed male and female CETP transgenic mice and their non-transgenic littermates. We observed increased gut bile acids in female but not male CETP-expressing mice. This increase in bile acid levels

corresponded with improved insulin sensitivity and increased bile acid related signaling in liver and muscle in CETP-expressing female mice. Overall, we show that CETP expression protects female mice from the metabolic complications of obesity.

Results

CETP expression does not alter high fat diet-induced weight or adiposity gain in males or females

Age- and weight-matched male and female CETP transgenic mice and their wild-type littermates (WT) lacking CETP expression were placed on HFD for 4 weeks to test their metabolic adaptation to obesity. As expected, CETP activity was significantly higher in CETP mice compared to WT in both males and females (Males Fig. 3.1A, Females Fig. 3.1E). There was no difference in CETP activity between transgenic males and females. Both male and female WT and CETP mice gained similar weight and had similar adiposity on HFD (Males Fig. 3.1B, Females Fig. 3.1F Body weight: Table 3.1).

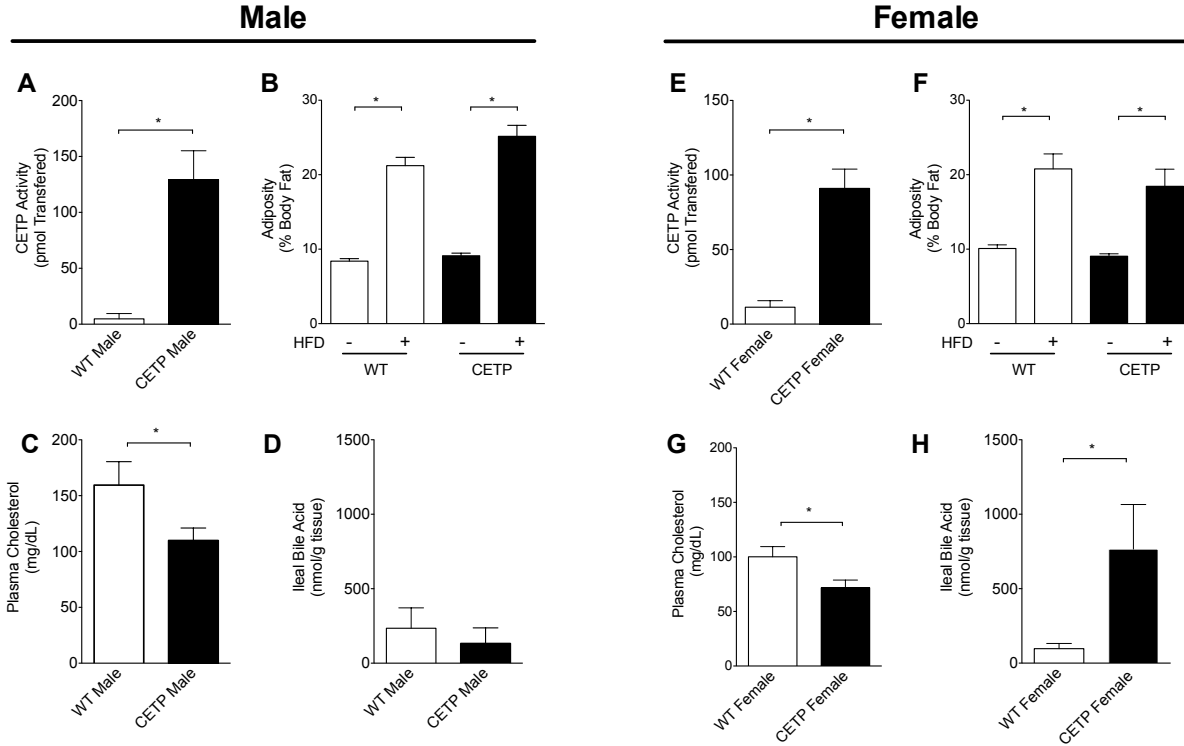


Figure 3.1: CETP expression alters serum cholesterol and ileal bile acids in obese mice. A) CETP activity in WT littermates and CETP-transgenic males. B) Adiposity before (-) and after (+) HFD in male mice. C) Serum cholesterol in HFD-fed male mice. D) Total fasted ileal bile acids in HFD-fed male mice. E) CETP activity in WT littermates and CETP-transgenic females. F) Adiposity before (-) and after (+) HFD in female mice. G) Serum cholesterol in HFD-fed female mice. H) Total fasting ileal bile acids in HFD female mice. Data represent mean \pm SEM from n = 6-8 animals per group. *p < 0.05.

	WT Female	CETP Female	WT Male	CETP Male
Initial Body Weight (g)	20.5 ± 2.3	21.0 ± 1.8	24.0 ± 2.1	25.4 ± 3.6
Post-HFD body weight (g)	26.5 ± 5.1†	24.1 ± 3.6†	28.4 ± 3.2†	32.1 ± 5.0*†
HFD Fasting Blood Glucose (mg/dL)	152 ± 35.8	134 ± 19.6	148 ± 24.1	151 ± 38.1
HFD Clamp Insulin (ng/mL)	4.8 ± 1.6	2.6 ± 1.1	2.8 ± 0.6	3.7 ± 1.0
Chow Clamp Insulin (ng/mL)	1.7 ± 0.4	2.1 ± 0.3	ND	ND
HFD Plasma Free Fatty Acid (mmol/L)	1.44 ± 0.96	1.62 ± 0.50	1.57 ± 0.77	1.44 ± 0.96
HFD Plasma LDL (mg/dL pooled)	33.8	14.6	39.1	21.7
HFD Plasma HDL (mg/dL pooled)	110.3	50.0	79.5	52.1
HFD Basal Glucose EndoRa (mg·kg ⁻¹ ·min ⁻¹)	17.3 ± 7.4	19.6 ± 5.6	10.3 ± 4.4	14.6 ± 10.1
HFD Clamp Glucose EndoRa (mg·kg ⁻¹ ·min ⁻¹)	2.2 ± 2.1‡	3.9 ± 2.8‡	3.7 ± 4.8	2.9 ± 5.7‡
HFD Clamp Glucose Rd (mg·kg ⁻¹ ·min ⁻¹)	17.1 ± 3.4	45.0 ± 6.1*	16.9 ± 4.4	17.19 ± 4.8
HFD Clamp AUC GIR/Glucose (mg ² ·min·kg ⁻¹ ·dL ⁻¹)	0.10 ± 0.03	0.25 ± 0.05*	0.09 ± 0.03	0.11 ± 0.02
HFD Fasting Plasma Triglyceride (mg/dL)	73.8 ± 22.6	53.5 ± 21.0	46.5 ± 13.0	65.0 ± 15.0
HFD Clamp Plasma Triglyceride (mg/dl)	51.7 ± 23.1	36.6 ± 5.9 ‡	43.8 ± 18.6	50.0 ± 11.3
HFD Liver Cholesterol (mg/g tissue)	2.75 ± 1.5	4.27 ± 2.1	2.47 ± 0.8	3.86 ± 1.6
HFD Liver Triglyceride (mg/g tissue)	54.8 ± 21.7	75.1 ± 9.4	79.2 ± 20.5	53.8 ± 14.2
HFD Liver Diacylglycerol (mg/g tissue)	2.67 ± 0.63	2.67 ± 0.61	3.61 ± 1.53	2.53 ± 1.02
HFD Plasma Interleukin 6 (pg/mL)	77.6 ± 65.7	76.1 ± 31.9	54.3 ± 23.5	113.1 ± 64.2
HFD Plasma Leptin (ng/mL)	12.9 ± 5.2	10.6 ± 5.8	12.7 ± 0.1	18.6 ± 5.0
HFD Plasma Adiponectin (µg/mL)	13.1 ± 1.8	15.7 ± 1.8	15.1 ± 1.6	17.6 ± 3.6
HFD Serum Estradiol (pg/mL)	6.8 ± 3.1	6.9 ± 2.0	ND	ND

Table 3.1: Additional physiological parameters. Values represent mean ± standard deviation. *p < 0.05 versus WT, † p < 0.05 versus pre-HFD, ‡ p < 0.05 versus clamp baseline. n = 4-8 animals per group

CETP expression reduces serum cholesterol in males and females and increases gut bile acid content in females only

To determine the effect of CETP expression on lipid and bile metabolism, we measured fasted serum lipids and ileal bile acids. We observed that CETP expression reduced serum cholesterol in both male and female mice (Males Fig. 3.1C, Females Fig. 3.1G). CETP-expressing males showed no difference in ileal bile acid content compared to WT males (Fig. 3.1D). CETP-expressing females, however, showed a significant increase in gut bile acid content compared to WT (Fig. 3.1H). We saw a trend towards increased bile acid species in the serum of CETP females (Table 3.2). Additionally we observed an increased hepatic bile acid species in the CETP females (Table 3.2, $p < 0.05$ for genotype effect by 2-way ANOVA). We did not observe a difference in plasma free fatty acid, triglyceride, or estradiol (Table 3.1). We saw a trend toward increased hepatic cholesterol content in both male and female CETP-expressing mice, but this difference was not significant (Table 3.1). Overall, we observed a reduction in serum cholesterol in both male and female CETP mice and an increase in gut bile acids in female mice only.

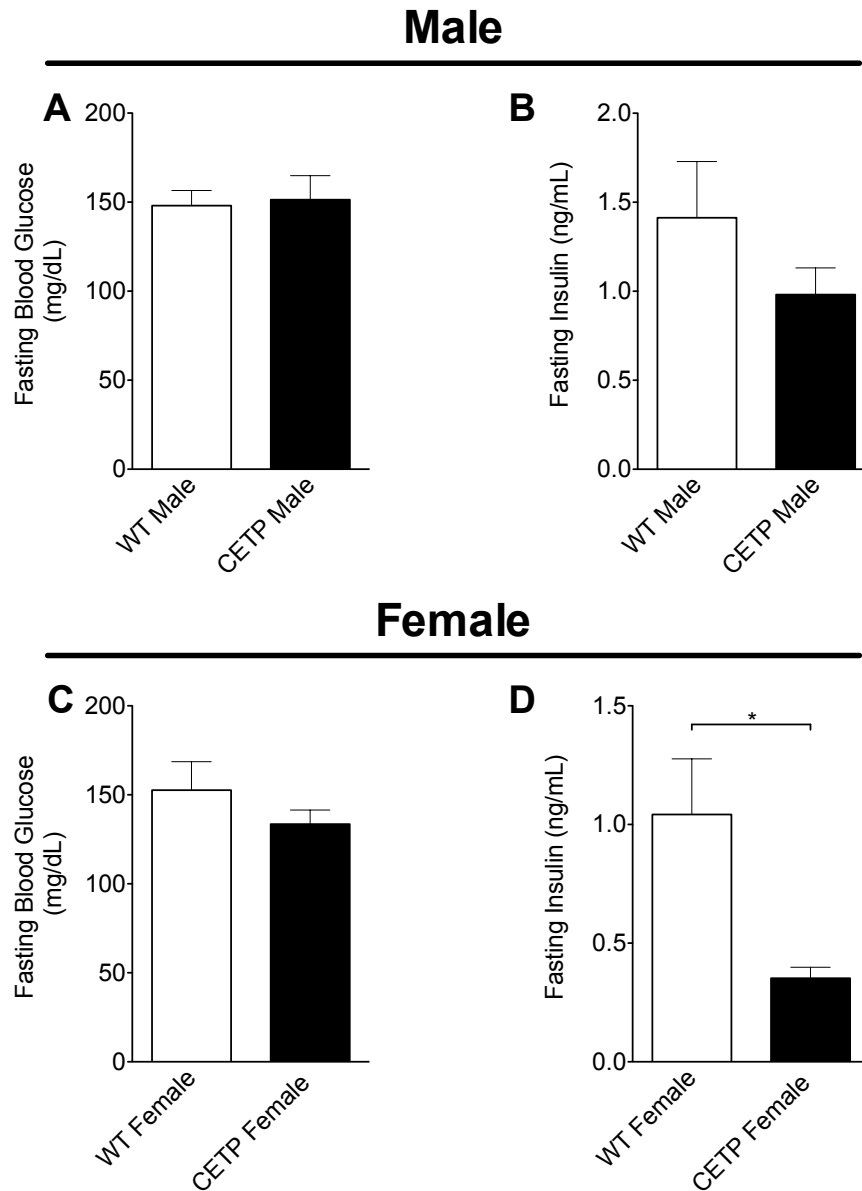


Figure 3.2: CETP expression decreases fasting plasma insulin in female mice. A) Fasting blood glucose in HFD-fed male mice. B) Fasting plasma insulin in HFD-fed male mice. C) Fasting blood glucose in HFD-fed female mice. D) Fasting plasma insulin in HFD-fed female mice. All animals were fasted for 5 hours. Data represent mean \pm SEM from n = 6-8 animals per group. * $p < 0.05$.

	WT Male	CETP Male	WT Female	CETP Female
Liver Bile Acid (nmol/g)	109.5 ± 25.6	81.5 ± 47.6	99.9 ± 20.2	74.9 ± 37.2
Liver Cholate (AU)	ND	ND	0.74 ± 0.36	1.27 ± 0.39 †
Liver Taurocholate (AU)	ND	ND	0.88 ± 0.27	1.08 ± 0.44 †
Liver Glycocholate (AU)	ND	ND	0.84 ± 0.17	1.41 ± 1.10 †
Liver Taurochenodeoxycholate (AU)	ND	ND	0.85 ± 0.32	1.15 ± 0.46 †
Liver Beta-Muricholate (AU)	ND	ND	1.00 ± 0.04	1.07 ± 0.37 †
Liver Tauro-Beta-Muricholate (AU)	ND	ND	1.23 ± 0.36	1.11 ± 0.69 †
Liver Taurosodeoxycholate (AU)	ND	ND	1.03 ± 0.14	1.32 ± 0.85 †
Serum Bile acid (nmol/mL)	10.72 ± 5.80	21.9 ± 25.3	12.6 ± 12.2	37.7 ± 48.6
Serum Cholate (AU)	ND	ND	1.26 ± 1.08	2.37 ± 2.49
Serum 1,2 Dehydrocholate (AU)	ND	ND	0.68 ± 0.41	1.48 ± 1.41
Serum Beta-Muricholate (AU)	ND	ND	1.21 ± 0.87	2.01 ± 1.91

Table 3.2: Liver and serum bile acid species. Values represent mean ± standard. † indicates significance as a group versus WT by 2-way anova for all marked species. n = 4 animals per group

CETP expression reduces fasting insulin in female mice

Since bile acids are known to affect insulin sensitivity, we measured fasting glucose and insulin levels in the CETP mice and WT littermates. After 4 weeks of HFD-feeding, there was no difference in fasting blood glucose between CETP and WT mice (Males Fig. 3.2A, Females Fig. 3.2C). While there was a small but not significant reduction in fasting insulin in CETP males compared to WT (Fig. 3.2B), CETP female mice had significantly lower fasting insulin levels than WT (Fig. 3.2D). Thus, despite weight and adiposity gain on HFD, female CETP mice had lower fasting insulin levels than WT, suggesting that they may be protected from HFD-induced insulin resistance.

Expression of CETP protects against diet-induced insulin resistance in female mice

Since the changes in fasting insulin suggested improved insulin sensitivity in the CETP female mice, we measured insulin sensitivity using a hyperinsulinemic-euglycemic clamp in mice fed HFD for 4 weeks (Fig. 3.3A). The hyperinsulinemic-euglycemic clamp technique assesses insulin sensitivity by determining the glucose infusion rate (GIR) required to maintain euglycemia in response to a physiologic increase in serum insulin; increased GIR corresponds to greater insulin sensitivity. There was modest but non-significant increase in GIR between the CETP and WT males during the clamp (Fig. 3.3B-C). Male CETP mice showed no change in insulin sensitivity index (SI) compared to WT (Fig 3.3D), which is defined by the GIR divided by the average insulin level in the steady-state clamp period.

In contrast, CETP females showed a markedly increased GIR compared to WT (Fig. 3.3E-F). Additionally, SI was increased by approximately 3-fold in the CETP females compared to WT (Fig. 3.3G). Insulin suppressed endogenous glucose production (EndoRa) in all groups. The dose of insulin used in the clamp study, however, was optimized to study muscle metabolism by maintaining insulin at a concentration similar to post-prandial levels (Table 3.1). Glucose rate of disappearance (Rd), an index of muscle insulin action, was significantly increased in CETP females compared to WT. (Table 3.1). Rd in CETP-expressing females was significantly higher than both WT females and CETP-expressing males. Overall, the clamp results show that CETP expression significantly improves insulin sensitivity in obese female mice fed HFD.

To determine if CETP-mediated effects on insulin sensitivity are dependent on diet composition, we performed a parallel hyperinsulinemic-euglycemic clamp study on CETP and WT females fed a standard chow diet (Fig. 3.3A). We focused on the female mice because of the large phenotype observed in the HFD-fed females. In contrast to the HFD-fed group, there was no difference in GIR or SI between the chow-fed CETP and WT mice, suggesting that the improvement in insulin sensitivity observed with CETP expression depends on HFD-feeding (Fig. 3.3H-J). Thus, CETP protects against the effect of HFD on insulin sensitivity in females; it has no effect on insulin sensitivity in lean chow-fed females. This interaction between CETP and diet in females is consistent with a mechanism mediated by bile acid signaling, which would be increased on HFD.

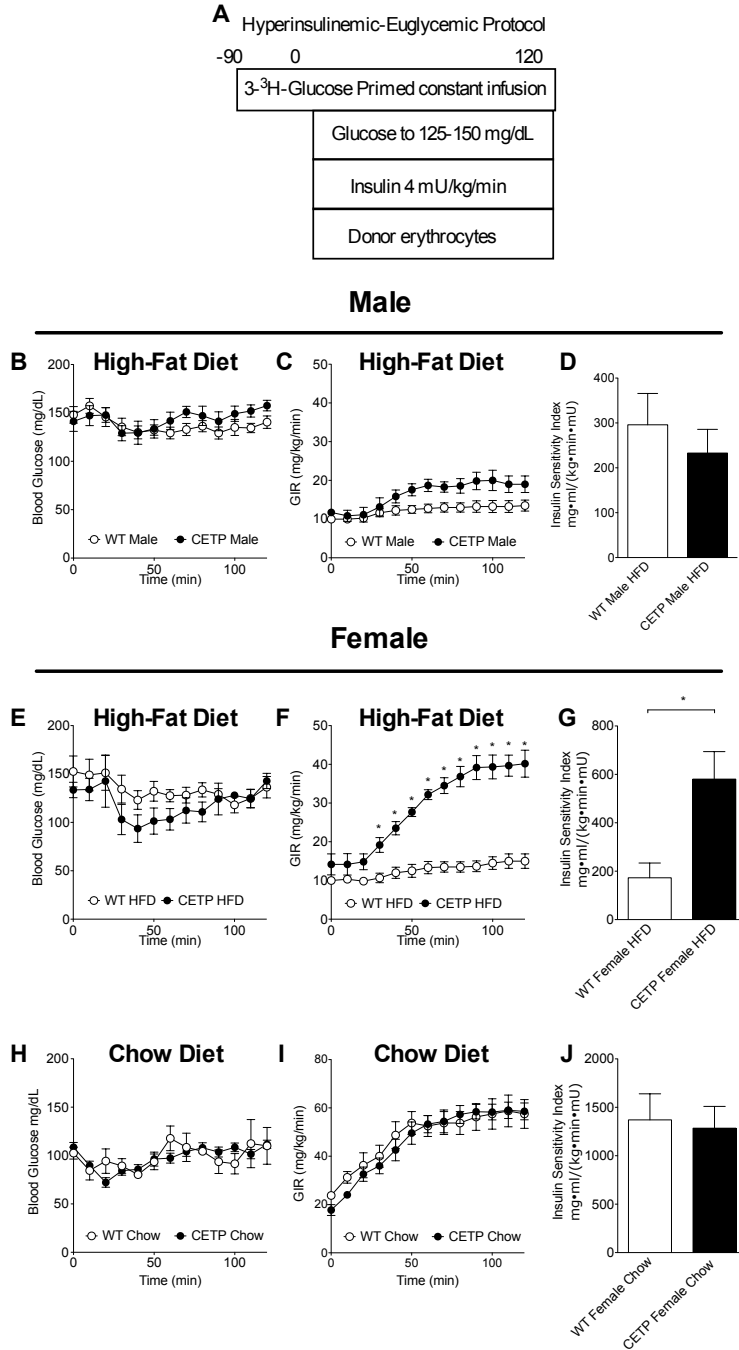


Figure 3.3: CETP expression protects female mice against diet-induced insulin resistance. A) Hyperinsulinemic-euglycemic clamp study design. B) Clamp blood glucose in HFD-fed males. C) Glucose infusion rate (GIR) required to maintain euglycemia during hyperinsulinemic clamp in HFD-fed males. D) Insulin sensitivity index based on steady-state GIR/insulin for HFD-fed males. E) Clamp blood glucose in HFD-fed females F) GIR for HFD-fed females. G) Insulin sensitivity index for HFD-fed females. H) Blood glucose during clamp in chow-fed females. I) GIR for chow-fed females. J) Insulin sensitivity index for chow-fed females. Data represent mean \pm SEM from $n = 6-8$ animals per group. * $p < 0.05$.

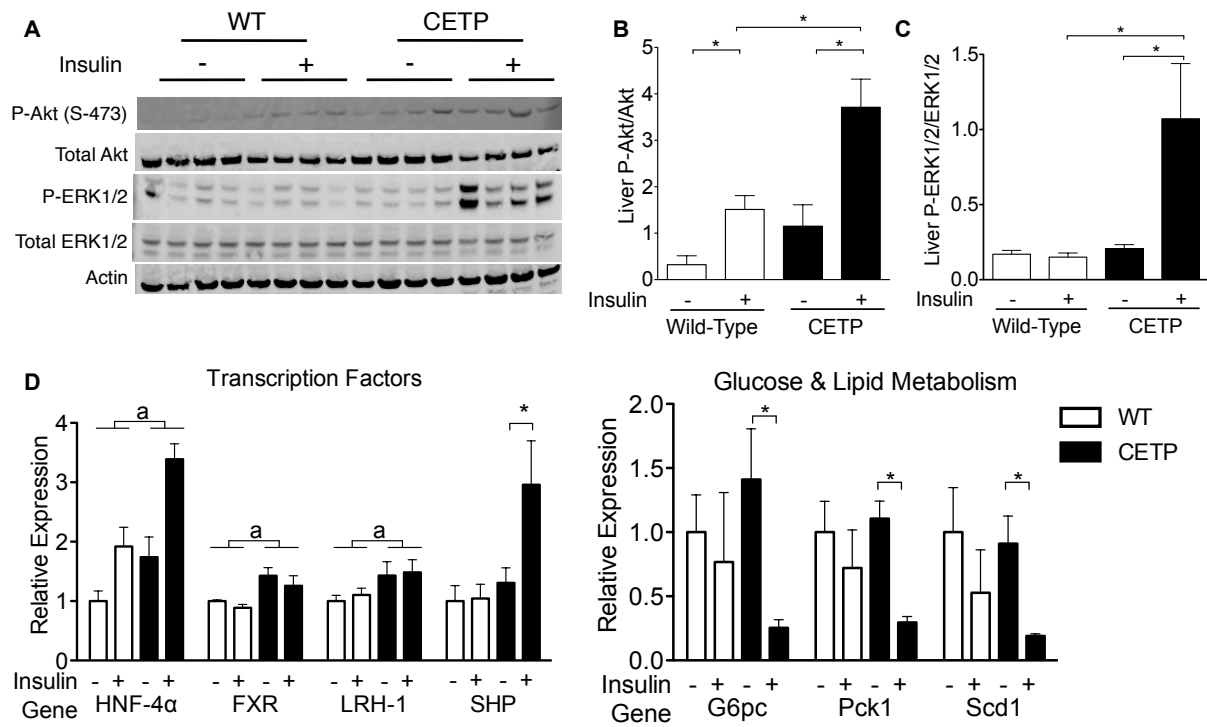


Figure 3.4: CETP expression alters hepatic insulin signaling and bile acid signal related gene expression. A) Immunoblot analysis of Akt, P-Akt (S-473), Erk1/2, P-Erk1/2, and Actin in liver whole-cell extract from fasted and clamped CETP and WT female mice on a HFD. B) Quantification of P-Akt to Akt ratio C) Quantification of P-Erk1/2 to Erk1/2 ratio D) Gene expression for genes the products of which control glucose metabolism, bile acid metabolism, and gene transcription determined by qRT-PCR from hepatic RNA Data represents the mean \pm SEM from n=4 animals per group. *p < 0.05 versus fasted, brackets (a) indicate statistical significance for genotype effect by 2-way ANOVA.

CETP expression increases insulin-stimulated phosphorylation of hepatic Akt and Erk1/2 in HFD-fed mice

To define changes in insulin signaling compared to the fasted state, we added an additional non-insulin treated cohort of CETP and WT female mice that were HFD-fed for 4 weeks and then fasted for 5 hours with no insulin treatment prior to sacrifice. Insulin-stimulated Akt phosphorylation was greater in clamped female CETP mice compared to WT (Fig. 3.4A-B). We did see a trend towards increased hepatic AKT phosphorylation in the CETP males, although this result was not statistically significant (Fig. 3.5).

We performed western blot analysis for phosphorylation of liver Erk1/2, which occurs via multiple pathways including the hepatic estrogen, bile acid, and insulin signaling pathways [14, 15, 197]. Erk1/2 phosphorylation was increased in the insulin-clamped CETP females compared to clamped WT females (Fig. 3.4A, C). Fasting Erk1/2 phosphorylation was similar between fasted CETP and WT females, suggesting that insulin is required for increased Erk1/2 phosphorylation in CETP female mice (Fig. 3.4A, C). Erk1/2 phosphorylation was not increased in CETP males (Fig. 3.5). To further investigate the sex difference, we performed western blots for estrogen receptor alpha (ER α) in liver and muscle of female CETP and WT mice. We did not observe a difference in ER α expression between CETP and WT in either liver or muscle (Fig. 3.6). Overall, we observed that CETP expression improves the insulin signal to Akt in the context of HFD-feeding. Female CETP mice had an additional robust signal to increase signaling to Erk1/2, likely contributing to the marked sex-difference seen in our clamp study.

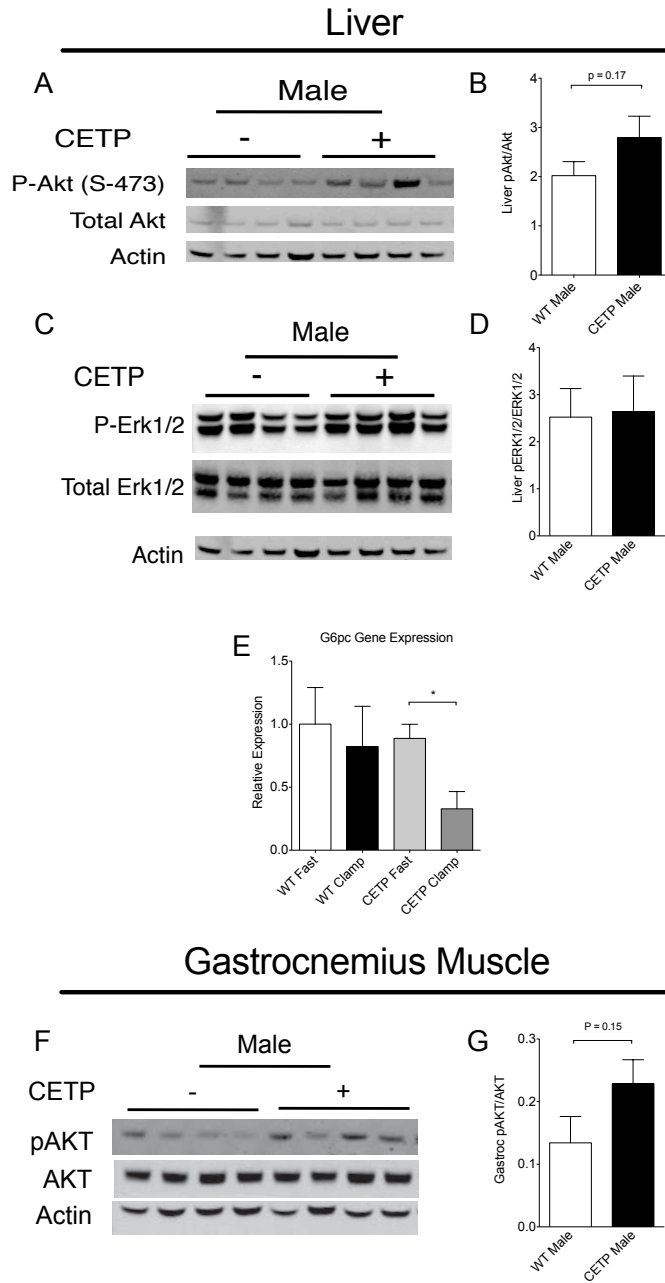


Figure 3.5: CETP has a modest effect on insulin signaling in male mice on a high fat diet. A) Immunoblot analysis of Akt and P-Akt (S-473) from livers of insulin-clamped males. B) Quantification of P-Akt to Akt ratio. C) Immunoblot analysis of Erk1/2 and p-Erk1/2 from liver of insulin clamped males. D) Quantification of P-Erk1/2 to Erk1/2 ratio. E) Relative gene expression of *G6pc* in fasted and clamped males. F) Immunoblot analysis of Akt and P-Akt (S-473) from gastrocnemius muscle of insulin-clamped males G) Quantification of P-Akt/Akt ratio. Data represent mean \pm SEM from n = 6-8 animals per group.

CETP expression alters expression of genes involved in liver glucose metabolism

As bile acids were only altered in the female CETP mice, we focused our further mechanistic studies of bile acid signaling on female CETP mice *versus* female WT. To determine the effect of CETP expression on hepatic gene expression, we performed qRT-PCR on hepatic mRNA from fasted and insulin-clamped CETP and WT female mice. We determined mRNA levels for genes the products of which are involved in glucose and lipid metabolism and regulate gene transcription (Fig. 3.4D). After HFD-feeding, insulin failed to suppress glucose-6-phosphatase (*G6pc*) and phosphoenol pyruvate carboxylase (*Pck1*) expression in the WT mice. These genes products are central to control of hepatic gluconeogenesis. Female CETP mice showed improved insulin-suppression of mRNA for *G6pc* and *Pck1* compared to WT (Fig. 3.4D). Male CETP mice also showed improved insulin suppression of mRNA for *G6pc* compared to WT (Fig. 3.5E) Additionally, insulin-mediated suppression of RNA for sterol CoA desaturase expression, a gene the product of which is involved in *de-novo* lipogenesis, was improved in the female CETP mice compared to WT. Female CETP mice had increased expression of *Hnf4α*, *Fxr*, and *Lrh-1*, as well as increased *Shp* under insulin treatment. These transcription factors regulate genes the products of which control bile, glucose, and lipid metabolism. Based on our observed changes in ileal bile acids, liver protein, and mRNA, CETP expression may improve insulin action on control points of hepatic glucose metabolism, potentially through bile acid signaling.

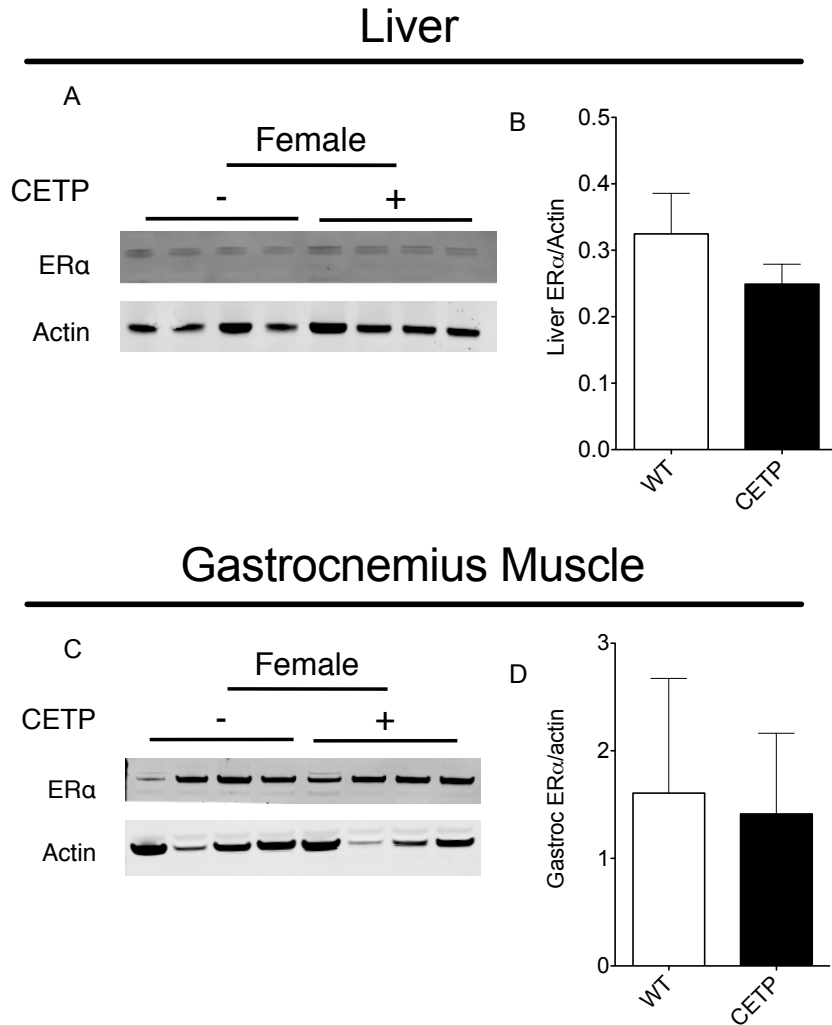


Figure 3.6: CETP expression does not alter hepatic or muscle levels of ERα. A) Immunoblot analysis of ERα in liver of WT and CETP female mice. B) Quantification of hepatic ERα protein normalized to actin. C) Immunoblot analysis of ERα in gastrocnemius muscle of WT and CETP female mice. D) Quantification of muscle ERα protein normalized to actin. Data represent mean ± SEM from n = 4 animals per group.

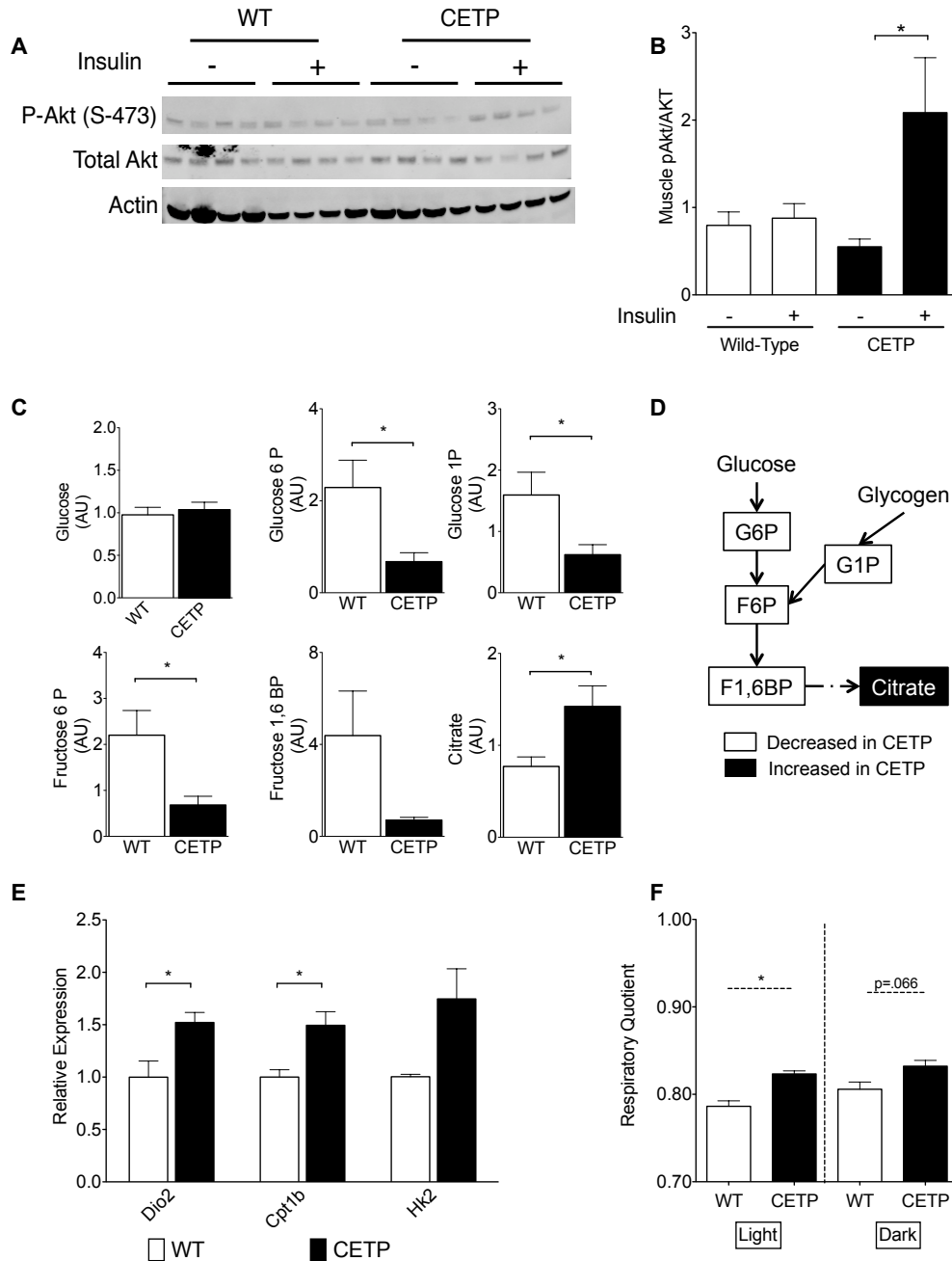


Figure 3.7: CETP-expressing mice on a high fat diet display improved muscle insulin signaling and altered glucose metabolism A) Immunoblot analysis of Akt, P-Akt (S-473), and Actin in muscle whole-cell extract from fasted and clamped CETP and WT female mice. B) Quantification of P-Akt to Akt ratio. C) Metabolite analysis of glycolytic and TCA cycle intermediates in muscle of fasted CETP and WT female mice. D) Schematic representation of changes in glycolytic and TCA cycle intermediates E) Gene expression for *Dio2*, *Cpt1b*, and *Hk2* determined by qRT-PCR from muscle RNA extracts. F) Respiratory quotient determined by indirect calorimetry for HFD fed CETP and WT female mice Data represent mean \pm SEM from n=4 animals per group except for (F) which is n=3 animals per group. *p < 0.05.

CETP expression increases insulin-stimulated phosphorylation of muscle Akt in HFD-fed mice

The large increase in GIR and Rd in CETP females was likely due to increases in muscle insulin sensitivity, since muscle is the largest depot for insulin-stimulated glucose uptake. We measured phosphorylated Akt in gastrocnemius muscle from fasted and insulin-clamped CETP and WT females. We observed an increase in Akt phosphorylation in response to the insulin in CETP but not WT females (Fig. 3.7 A-B). We saw a trend toward increased Akt phosphorylation in muscle of CETP males, but this result was not significant (Fig. 3.5 F-G). Western blotting for ERK1/2 phosphorylation was attempted in muscle tissue from these mice, but the results showed significant variability from animal to animal. This result suggests that CETP expression ameliorates the HFD-induced decrease in muscle insulin signaling to Akt in female mice.

CETP expression reduces muscle glycolytic intermediates

Having observed improved muscle insulin signaling and increased glucose disposal, we measured glycolytic intermediates in muscle tissue from fasted CETP and WT females (Fig. 3.7C). CETP animals had significantly decreased levels of glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P). We also saw a trend towards decreased fructose 1,6 bisphosphate (F1,6BP) in the CETP females ($p=0.11$). Glycogen-derived glucose-1-phosphate (G1P) was also significantly decreased. Muscle glucose was not altered. Levels of citrate were increased in the CETP mice, an indicator of increased glucose and/or lipid oxidation. The reduced glycolytic intermediates despite

increased citrate (an inhibitor of glycolysis) suggest that CETP expression promotes muscle TCA cycle flux in the fasting state. We examined soleus muscle mRNA for genes the products of which promote TCA cycling, and found significantly increased gene expression for *Dio2*, carnitine palmitoyltransferase 1b (*Cpt1b*), and a trend towards increased hexokinase 2 (*Hk2*) gene expression in CETP mice (Fig. 3.7E: $p=0.058$ for *Hk2*). Taken together, the reduction in muscle glycolytic intermediates along with increased gene expression for *Dio2* and *Cpt1b* suggests that CETP expression causes increased muscle substrate oxidation. These changes likely contribute to improved insulin sensitivity in CETP females

Having observed a significant difference in insulin sensitivity in HFD-fed CETP females as well as altered muscle glucose metabolism, we performed indirect calorimetry on HFD-fed CETP and WT females to measure energy expenditure. Food intake and energy expenditure were similar between CETP and WT, although CETP females ate more frequently than WT (Fig. 3.8). Additionally, the increase in respiratory quotient showed that CETP females had increased carbohydrate oxidation compared to WT (Fig. 3.7F). The finding that CETP expression increases the relative utilization of carbohydrate compared to lipid is consistent with our observation that CETP alters muscle glucose oxidation. Activation of glycolysis and *Dio2* are known effects of bile acid signaling in muscle, and these observations are consistent with our model that bile signaling is responsible for improved insulin sensitivity in the CETP females

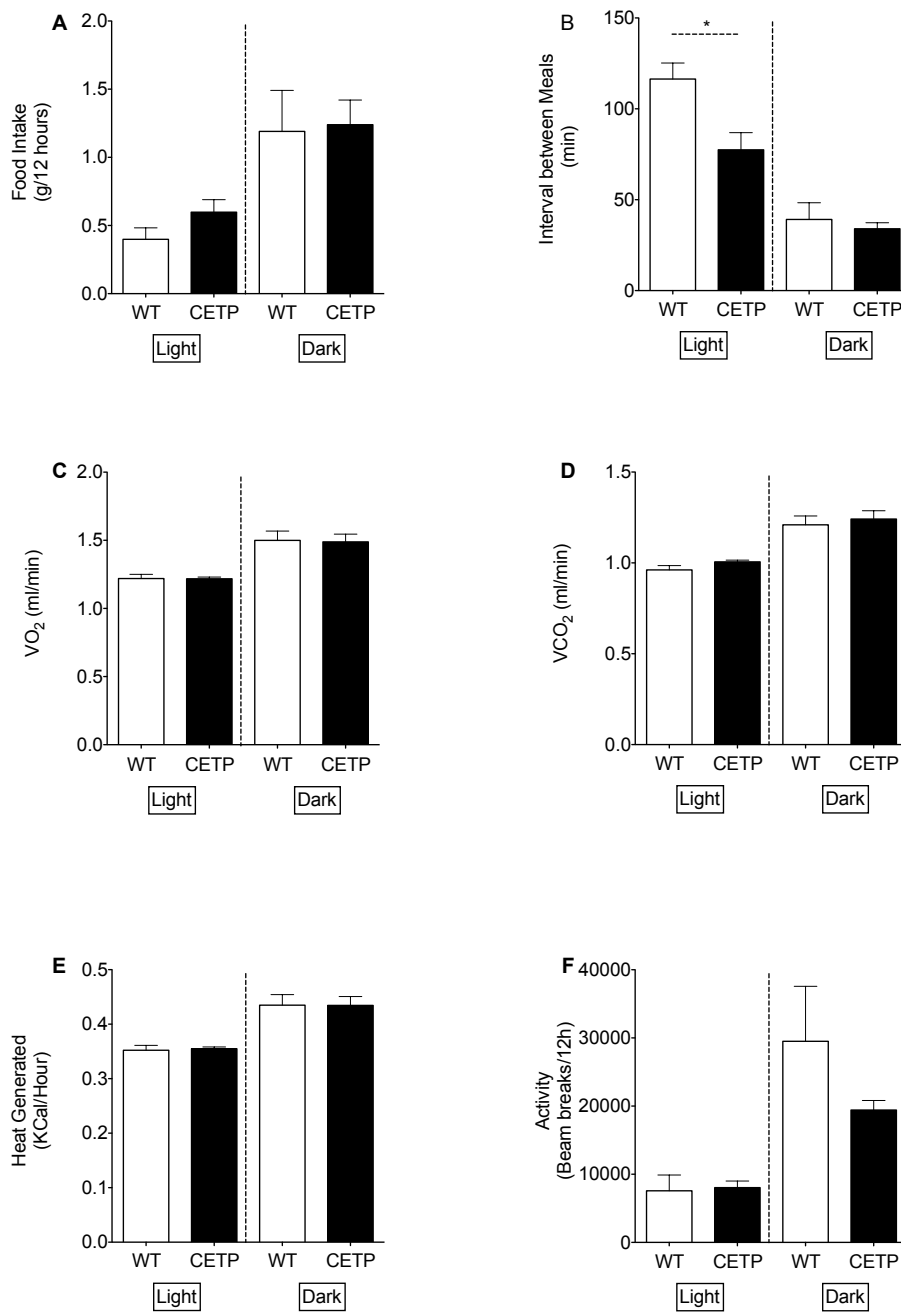


Figure 3.8: CETP expression does not alter food intake or energy expenditure in HFD-fed female mice. A) Food intake over 12 hour periods. B) Feeding behavior based on frequency of feeding events. C) Rate of oxygen consumption. D) Rate of carbon dioxide production. E) Heat generation. F) activity level measured by beam breaks. Animals were housed in a facility with 12 hour light-dark cycles. The light or dark period is indicated below the graph. Data represent the mean ± SEM from n = 3 animals per group.

Discussion

Our studies demonstrate a novel role for CETP expression to ameliorate insulin resistance in obese female mice. We propose that the increase in bile acids in the CETP females improves insulin sensitivity. Our data is consistent with the growing body of evidence that bile acids can promote insulin sensitivity [15, 16, 183]. CETP expression did not alter bile acid levels in male mice, and, correspondingly, there was no alteration in insulin sensitivity in the male CETP mice. Additionally, we did not observe a difference in insulin sensitivity between female CETP mice and female WT littermates fed chow, suggesting that CETP may have metabolic benefits that promote insulin sensitivity only in the context of a lipid-rich diet.

We propose that the metabolic effect of CETP in female mice is due to enhancement of insulin signaling due to the effects of bile acids. We observed induction of the bile-sensitive Fxr and Fgf15 signaling pathways in the liver that corresponded with alterations of gene expression of *Shp*, *G6pc*, and *Pck1*. We also observed increased Erk1/2 phosphorylation in the livers of the CETP female mice, which is a known effect of Fgf15 signaling. We also observed effects consistent with activation of bile-sensitive pathways in muscle, which likely had a large effect on the phenotype. We saw a large increase in the rate of glucose disappearance during our clamp studies, an index of muscle glucose disposal. The increase in Rd corresponded with decreased glycolytic intermediate metabolites and an increase in the TCA cycle intermediate citrate. These effects are consistent with bile acid signaling through Gpbar1 to induce *Dio2*, which was upregulated in the CETP female animals. Impairments in muscle glucose disposal are believed to be the first major defect in metabolism that leads to

development of diabetes [198]. Therefore, mechanisms that promote muscle glucose disposal such as activation of Gpbar1 signaling can protect against development of insulin resistance. I propose that the observed increase in muscle glucose disposal in the CETP mice is the major mechanism by which they are protected from HFD-induced insulin resistance. Collectively, our results show a strong correlation between CETP expression in females and increased bile acid signaling in liver and muscle that likely results in increased glucose disposal and improved insulin sensitivity. Further study is required to determine if bile acid signaling has a truly causative role in this improvement.

There was no change in insulin sensitivity in the chow-fed CETP-expressing mice compared to WT. This result suggests either that HFD feeding is required to induce bile acid production or that the chow-fed mice are already insulin sensitive and that CETP does not have an additive effect on insulin sensitivity in an already insulin sensitive animal. Bile acids are known to be increased by HFD and particularly diets that contain high levels of saturated fat such as our 60% lard HFD [199, 200]. We did not measure gut bile acids in chow-fed animals and can therefore only speculate that they would have lower bile acid levels than the HFD mice. Determining the levels of bile acids in these animals will be performed in a future study.

The role of CETP in human health has been a topic of considerable controversy, and there are significant unresolved questions about its effects on lipid and glucose homeostasis. CETP promotes the movement of cholesterol esters and triglycerides between lipoproteins, resulting in a net flux of cholesterol from HDL to LDL. Since reduced HDL is a well-known risk factor for CHD, CETP inhibitor drugs were developed with the goal of increasing HDL and reducing CHD. While the inhibitors successfully

raised HDL in humans, the clinical benefit of these drugs on CHD has not yet been established [9, 153]. These findings, along with our data, suggest that CETP may have important additional effects on metabolism.

Studies in humans have also suggested that CETP may have sex-specific roles in glucose metabolism [13, 163, 201]. We observed a pronounced sexual dimorphism in insulin sensitivity between the male and female CETP mice, which is consistent with differences observed between men and women in human studies. In our studies, CETP expression did not significantly improve insulin sensitivity in males. Although the GIR was higher in CETP males in our clamp study, mice in the CETP group also had higher blood glucose levels in the clamp period. Two measures of insulin sensitivity, SI and the AUC of the GIR/glucose, were not different in male CETP vs. WT.

Reciprocally, studies in females suggest beneficial effects of CETP on cholesterol efflux and glucose metabolism. Higher CETP activity is associated with increased transfer of CE from macrophages to HDL in women but not men [12, 131]. This improved cholesterol transport capacity with increased CETP was independent of HDL cholesterol [12]. In women, CETP polymorphisms that increase CETP activity correlated with reduced risk of ischemic heart disease [162]. Higher CETP activity is associated with improved glucose metabolism after roux-en-Y gastric bypass in women but not men [13, 163]. In our studies, we found that female CETP mice had 3-fold improvement in insulin sensitivity with HFD-feeding compared to WT, showing a strong protection of female CETP mice against diet-induced insulin resistance.

The observed sexual dimorphism in insulin sensitivity in our study may be due to sex-differences in bile acid signaling. CETP female mice showed a significant increase

in ileal bile acids compared to WT; CETP males showed no difference in ileal bile acid levels compared to WT littermates. These observations show that CETP expression increases ileal bile acid content in a sex-specific manner. This difference in bile content correlates with the observed increases in bile acid related signaling in the CETP-expressing females. Additionally, estrogen may augment the bile signal in female CETP mice, as both CETP and estrogen signaling through ER α increase bile secretion [202, 203]. We observed alterations in signaling pathways that were unique to the females, such as increased hepatic Erk phosphorylation and increased Shp gene expression, pathways that are shared between estrogen and bile-signaling pathways [14]. CETP has also been shown to augment estradiol delivery to tissues, enhancing estrogen signaling [204, 205]. We did not, however, observe an increase in ER α levels in liver or muscle of CETP-expressing mice, and CETP-expressing females had levels of circulating estradiol similar to WT. Since bile acids were increased in females but not males, I propose that the mechanism for the observed sex difference in insulin sensitivity lies in the alteration in gut bile acids observed in the CETP-expressing females. The ability of gut bile acids to signal to *Dio2* in muscle through Gbpar1 leads to increased muscle glucose disposal, which is a known factor to promote whole-body insulin sensitivity [198].

We observed a trend towards improved insulin sensitivity in the male CETP mice. Hepatic *G6pc* gene expression was suppressed by insulin in the CETP-expressing male mice fed HFD, suggesting improved hepatic insulin sensitivity. This result is consistent with our tracer data that show suppression of hepatic glucose production during the clamp. We also observed a trend towards increased AKT phosphorylation in both liver

and muscle in the CETP expressing males. While these results are not significant, the studies may have been underpowered to detect the moderate changes caused by CETP expression in the males. For example, based on the mean and standard deviation for liver Akt phosphorylation, 9 animals would be required to achieve 80% power whereas the study group contained only 6 animals per group. The changes observed in the CETP expressing males are not likely caused by an effect of CETP on bile acids, as there was no difference in gut bile acids between CETP and WT males. While the size of the gut bile acid pool did not change in the CETP-expressing males, it is possible that the bile acid species composition was altered by CETP expression. Changes in bile acid species independent of pool size have been shown to improve insulin sensitivity [18]. The trend towards improved insulin sensitivity in the CETP males may also relate to the intracellular function of CETP to promote storage of lipids in adipose tissue, which is a topic for future investigation.

Our finding that CETP expression promotes insulin sensitivity does not necessarily suggest that CETP inhibition would be harmful with regard to glucose homeostasis. CETP has an extracellular role in lipid transfer between lipoproteins but also functions intracellularly to promote CE uptake and direct partitioning of CE between subcellular compartments [206, 207]. If our effects were mediated by intracellular CETP, we would not expect CETP inhibition to adversely impact the phenotype [206]. Additionally, several CETP inhibitors promote RCT, culminating in increased CE delivery to the liver and bile secretion [157, 208]. The mechanism for this improvement of RCT appears to be improved lipidation of nascent HDL [157]. In contrast, CETP's role to promote RCT is dependent on flux of CE from HDL to LDL, which is then cleared by

LDLR in the liver [10, 11]. Mechanisms that promote RCT would be predicted to increase bile acid signaling and perhaps improve insulin sensitivity.

In humans, dietary weight-loss strategies are complicated by weight regain. Pharmacologic strategies to achieve weight loss often have significant side effects on the cardiovascular system or central nervous system. Strategies to improve insulin sensitivity in the setting of obesity would be an attractive alternative to weight loss. The idea of “metabolically healthy obesity” is an important goal in the prevention of diabetes and cardiovascular risk associated with obesity. We observe a metabolically-healthy obese phenotype in female CETP mice. The model shown here, though dissimilar to the human in some regards, demonstrates an important proof-of-principle that augmenting aspects of CETP function, such as bile acid signaling, may be a therapeutic approach to improve insulin sensitivity and lessen the negative metabolic impact of obesity.

CHAPTER IV

CETP EXPRESSION PROTECTS FEMALE MICE FROM OBESITY-INDUCED DECLINE IN EXERCISE CAPACITY

Introduction

Obesity carries with it a significant increase in risk of diabetes and heart disease [22, 23]. Adherence to an exercise program can help obese patients to lose weight and reduce the risk of complications of obesity. In a study of patients who had lost weight, 89% reported using a combination of diet and increased physical activity to achieve weight loss, whereas only 10% achieved weight loss by diet alone [2]. Unfortunately, obesity leads to reduced exercise tolerance, making weight loss through exercise more difficult [4]. Beyond its role in weight loss, exercise capacity is critical to overall health, as impaired exercise capacity correlates with increased risk of death from all causes [184]. Additionally, impaired exercise capacity was identified as a stronger predictor than BMI of mortality among men with diabetes [185]. Therefore, it is important to identify novel factors that might help improve exercise tolerance in obese individuals.

A crucial factor for exercise capacity is the ability of the body to properly deliver oxygen to muscle and its ability to effectively perform oxidative metabolism. Exercise capacity is often measured by VO_{2max} , which is defined as the maximal rate that oxygen can be taken up and utilized during exercise [188]. Several factors play into oxygen utilization including the ability of lungs to extract oxygen, the ability of the heart to pump oxygen through the blood, the oxygen carrying capacity of the blood, and muscle and

mitochondrial function. Pulmonary function can become a limiting factor for VO_{2max} in highly trained athletes and is not usually a limiting factor for healthy untrained individuals [186]. Cardiac output is very important to VO_{2max} , and exercise training leads to an increase in maximal cardiac output that leads to improvement in VO_{2max} [187].

The ability of skeletal muscle to oxidize fuel substrates is crucial to exercise capacity. A study of patients with congestive heart failure showed that changes in muscle metabolism correlated with decreased exercise capacity [209]. Additionally, reduced muscle oxidation has been observed in impaired glucose tolerance and diabetes and may be linked to the age-related decline in exercise capacity [210]. Endurance training leads to increased TCA cycle flux in the mitochondria [189]. We have previously shown that female mice expressing CETP are protected against the effect of HFD on insulin sensitivity [211]. This improvement in insulin sensitivity was marked by increases in muscle glycolysis. At higher levels of exercise intensity, muscle is increasingly dependent on glycolysis as fuel, as opposed to lower intensity exercise, which is primarily dependent on fatty acid oxidation [212]. Because the CETP-expressing mice showed increased muscle glycolysis, we propose that CETP-expressing female mice might have improved oxidative capacity and increased exercise tolerance when fed HFD.

Increased HDL or ApoA1 has recently been demonstrated to improve exercise capacity. Specifically, mice overexpressing ApoA1 showed improved exercise capacity and were resistant to age-related decline in exercise capacity [213]. These results suggest that either RCT mediated by HDL or the HDL particle itself acts to improve exercise capacity. We were interested in defining exercise capacity in the CETP

transgenic mouse, a mouse model that has improved RCT and a lipid profile more similar to a human than a wild-type mouse. CETP is a lipid transfer protein that facilitates the movement of cholesteryl esters and triglycerides between lipoproteins. CETP activity results in a net flux of CE from HDL into the ApoB containing lipoproteins LDL and VLDL, which are then delivered to the liver via LDL receptor in a process termed reverse cholesterol transport. This flux allows for an SR-B1 independent method for CE to be cleared by the liver. CETP expression increases RCT in SR-B1 knockout mice but not LDL receptor knockout mice [11]. HDL cholesterol is reduced in the CETP mice, but the lipid flux between lipoproteins is more humanlike. CETP-expressing mice therefore provide a model of increased RCT with more humanlike levels of HDL.

To test the hypothesis that CETP activity can improve exercise capacity, we measured exercise capacity in CETP-expressing female mice. While there was no difference in exercise capacity on chow diet, HFD-fed CETP-expressing mice were protected from the obesity-related decline in exercise capacity. This improvement in exercise capacity corresponded with an increase in mitochondrial oxidation.

Results

CETP expression does not alter exercise tolerance in chow-fed female mice

To test the effect of CETP expression on exercise tolerance in female mice, we performed an exercise study in CETP-expressing female mice and their non-transgenic female littermates. At the beginning of the study, mice were 12 weeks old, had been fed a standard chow diet, and were lean. Groups were matched by initial body weight and body composition (Fig. 4.1 A-B). Mice were subjected to an exercise tolerance test

consisting of a treadmill run with speed increasing every 3 minutes. The duration and distance run by the mice provide an index of exercise capacity. In the initial exercise tolerance test, we saw no difference in exercise capacity between lean, chow-fed CETP mice and their WT littermates. CETP mice ran for an average of 22.9 ± 2.3 minutes (535.3 ± 89.5 meters) versus 24.0 ± 1.9 minutes (586 ± 169.8 meters) for the WT mice (Fig 4.1 C-D). These results show that there is no genotype effect of CETP expression on exercise capacity in the context of a low-fat chow diet.

CETP expression protects female mice from high fat diet-induced decline in exercise capacity

Because obesity induces a decline in exercise capacity, we measured the changes in exercise capacity in CETP-expressing female mice and their non-transgenic littermates over a period of HFD feeding. Following the initial exercise test, mice were switched from a normal chow diet to a 60% HFD to induce obesity. Exercise capacity was measured every 2 weeks for 6 weeks using the same exercise tolerance test protocol used in the initial measurement. HFD feeding effectively induced weight gain and increased adiposity in the mice. By 4 weeks on HFD, both groups had gained over 15% of their initial weight and showed a significant increase in fat mass (Fig 4.1 A-B). This increase in weight and adiposity corresponded with a decrease in exercise capacity in both groups. The CETP-expressing mice, however, had a smaller reduction in exercise capacity compared to WT. The CETP mice decreased from 22.9 ± 2.3 minutes (535.3 ± 89.5 meters) to 14.8 ± 3.6 minutes (271.5 ± 99.4 meters), while the WT mice decreased from 24.0 ± 1.9 minutes (586 ± 169.8 meters) to 10.4 ± 1.8 minutes

(157.6 ± 38.7 meters) (Fig. 4.1 C-E). While the CETP-expressing mice showed a 49% decrease in run distance over 6 weeks of HFD, the WT mice showed a much larger 73% decrease in run distance over the same 6 weeks of HFD feeding. These results indicate that the CETP-expressing mice are protected from the deleterious effect of obesity on exercise capacity.

CETP-expressing mice show increased muscle oxidative capacity

To define muscle function after a matched exercise period, mice were kept on HFD for a total of 12 weeks, after which they were subjected to a single 15-minute bout of exercise at a constant speed of 16 m/min. Immediately following exercise, mice were sacrificed and tissues were collected. Muscle fibers from the red gastrocnemius muscle were isolated immediately following sacrifice, and mitochondrial oxidative capacity in these muscles was determined *ex-vivo* using an oxygraph-2k system. The muscle fibers were treated with either glutamate-malate or palmitoylcarnitine to measure the response to TCA cycle intermediates or ATP production from fatty acid oxidation. We observed a significant increase in oxygen consumption in the CETP-derived muscle fibers when treated with the glutamate/malate mixture but not the palmitoylcarnitine mixture (Fig 4.2). This result indicates that the CETP-expressing mice have higher oxidative capacity in muscle mitochondria but have a similar level of fatty acid oxidation. This is consistent with our prior observations that CETP-expressing female mice show increased glycolysis and TCA cycle intermediates. These results suggest that the mechanism for

the observed improvement in exercise capacity is an increased ability of muscle to oxidize carbohydrate in the CETP-expressing mice.

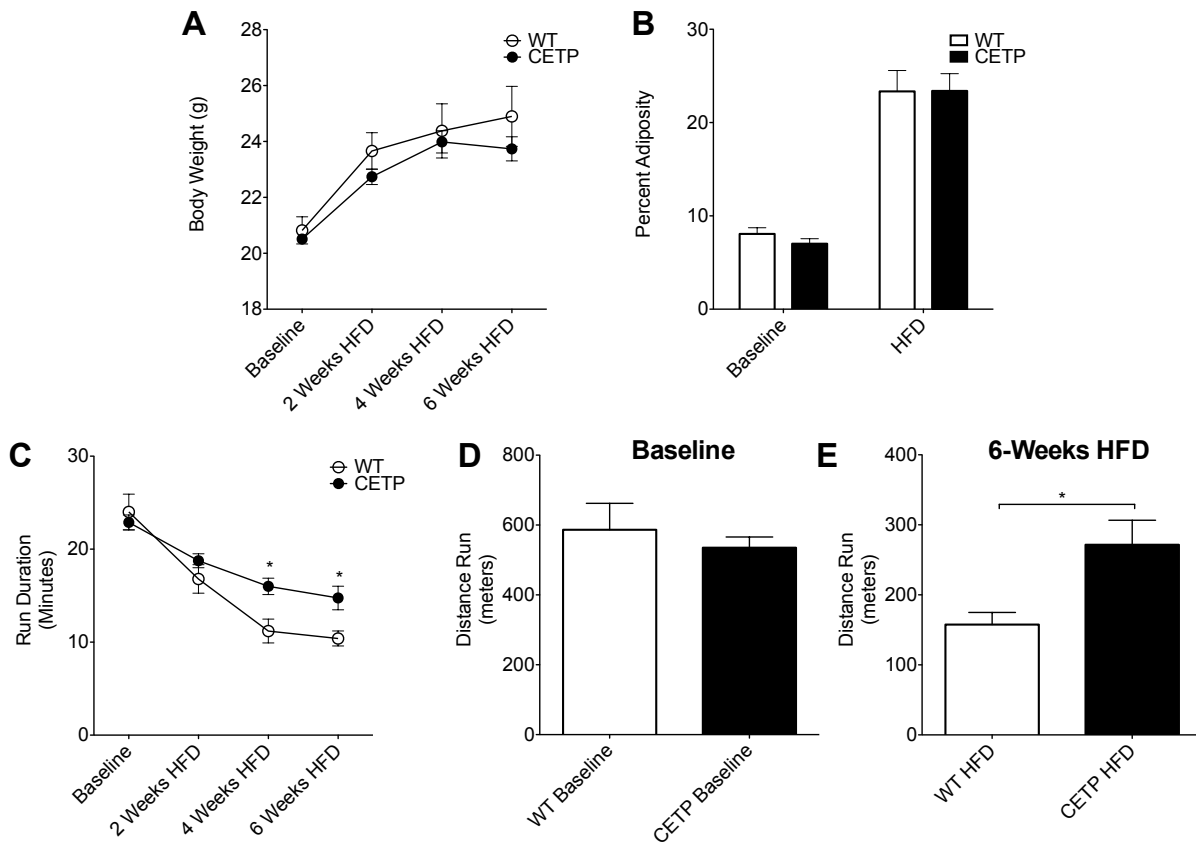


Figure 4.1: CETP expression protects female mice against HFD-induced decline in exercise capacity. A) Body weight over the course of HFD feeding. B) Body composition at baseline and 4-weeks post HFD. C) Run duration in each exercise test. D) Total distance run per mouse in the initial exercise test. E) Total distance run per mouse in the 6-week post HFD exercise study. Error bars represent mean \pm SEM. n= 5-8 mice per group.

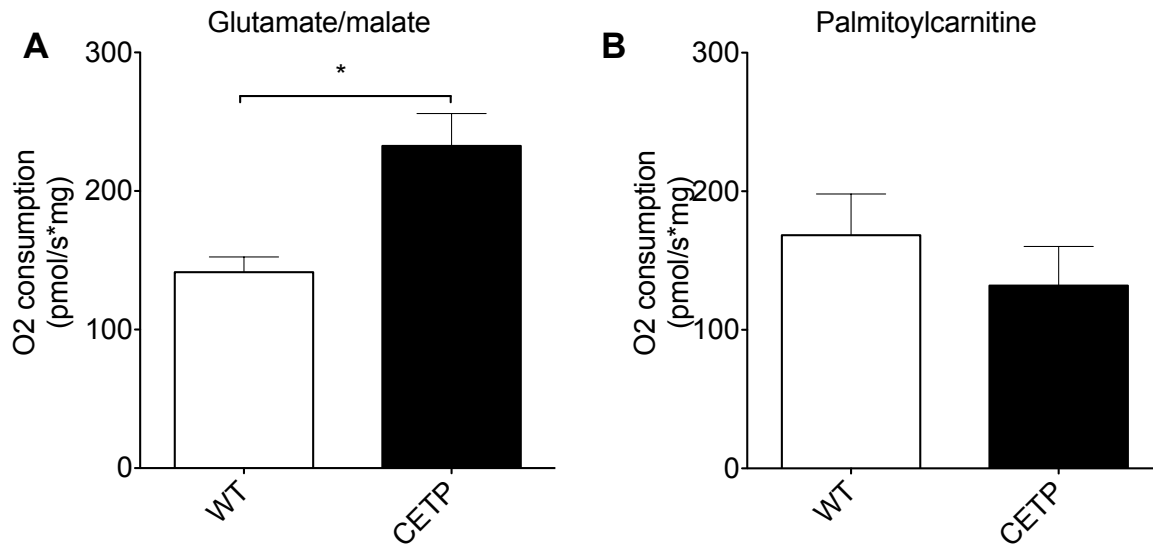


Figure 4.2: CETP expression increases mitochondrial oxidation in female mice. A) Oxygen consumption in isolated muscle fibers treated with glutamate/malate mixture to measure total substrate oxidation. B) Oxygen consumption in isolated muscle fibers treated with palmitoylcarnitine to measure fatty acid oxidation. Data represent mean \pm SEM. n = 8-14 muscle fibers per group.

Discussion

A decline in exercise capacity with obesity creates a vicious cycle of worsened insulin resistance and further impairment in exercise capacity. I aimed to determine if CETP expression has an effect on exercise capacity in female mice. Overall, I observed that CETP-expressing female mice are relatively protected against HFD-induced exercise intolerance compared to WT. The improvement in exercise capacity on HFD corresponds with increased mitochondrial oxidative capacity in the CETP female mice compared to WT littermates. These results are consistent with our previous observation that CETP-expressing female mice have increased muscle glucose flux to the TCA cycle and a preferential oxidation of carbohydrate compared to fatty acid. These results suggest that mechanisms that improve RCT may help to improve mitochondrial function and exercise capacity in people with obesity.

We used CETP-expressing mice to test the hypothesis that increasing RCT independently of HDL would lead to improved exercise capacity. Recent studies have linked HDL levels and ApoA1 to improved exercise capacity, although the mechanism by which HDL improves exercise capacity has not been fully explored [213]. Our results suggest that high levels of HDL are not required for improved exercise capacity, but rather that increasing RCT leads to improved exercise capacity in the context of high-fat feeding. Overexpression of ApoA1 corresponded with improved exercise capacity and increased mitochondrial oxidation [213]. We also observe an increase in mitochondrial oxidative capacity in the CETP-expressing female mice compared to WT mice fed HFD. Decline in mitochondrial oxidative capacity has been observed with obesity and aging,

and is likely responsible for the impairments in exercise capacity observed in these conditions [214, 215].

A potential link between the lipid transfer activity of CETP and the observed improvement in mitochondrial oxidation is bile acid signaling to muscle through Gpbar1. CETP expression increases RCT and efflux of cholesterol to feces in the form of bile acids [11]. GPBAR1 signaling has been shown to increase energy expenditure in brown fat and skeletal muscle [16, 17]. We have observed increased gut bile acids in the CETP-expressing mice and increased transcription of *Dio2*, which is a target of GPBAR1 signaling [211]. Additionally, the fact that the CETP and WT mice did not show a difference in exercise capacity on the chow diet suggests that increased bile acids may be necessary for an effect on exercise capacity. Bile acids are known to be increased by high-fat diets, particularly those that contain high amounts of saturated fat [199, 200]. When placed on HFD, the CETP female mice display increased gut bile acids (Fig 3.1H). The changes in RCT that are mediated by CETP may therefore account for the improvement in exercise capacity observed in the CETP-expressing female mice. Alternatively, the lack of a difference in exercise capacity between chow-fed CETP and WT mice may indicate that there is not an additive effect of CETP activity on exercise capacity in metabolically healthy animals but rather that CETP protects against the deleterious effect of obesity on exercise capacity.

Improving exercise capacity in the context of obesity represents an attractive target for treating obesity. Impaired exercise capacity has been correlated with increased risk of death from all causes, and obesity causes a reduction in exercise capacity. We identify a novel role for CETP to protect female mice against the obesity-

related decline in exercise capacity. This improvement in exercise capacity correlated with improved muscle glucose oxidation, which is indicative of bile acid signaling to muscle. Mechanisms that promote RCT may therefore promote healthy muscle function in the context of obesity.

CHAPTER V

SUMMARY AND CONCLUSIONS

Promoting metabolic health in the context of obesity

Obesity has become a serious health issue throughout the world, being declared a global epidemic by the WHO. Obesity has a deleterious effect on human health by increasing risk of CHD and type-2 diabetes. The deleterious effects of obesity can sometimes be prevented by weight loss; however, maintaining weight loss is difficult, and recent studies have suggested that weight loss may not be enough to reverse the effects of obesity. In the Look AHEAD trial, obese people with type-2 diabetes were treated with an intensive lifestyle intervention based on reduced caloric intake and increased physical activity or a control treatment of diabetes education and support. The lifestyle intervention was successful, resulting in weight loss (8.6% of body weight), reduced waist circumference, reduced hemoglobin a1c, improved exercise capacity, and improvement in markers for cardiovascular risk such as reduced blood pressure and increased HDL cholesterol [216]. Surprisingly, despite the improvement in multiple risk factors for CHD, there was no difference in the incidence of cardiovascular events between the lifestyle intervention group and the control group [216]. These results suggest that weight loss may not be enough to reverse the effects of obesity on CHD in the context of type-2 diabetes. Therefore, mechanisms that can improve metabolic health in the context of obesity are attractive targets to study with the goal of treating the deleterious effects of obesity.

A novel role for CETP expression to promote healthy obesity in mice

I have shown that CETP is a previously unrecognized factor that can promote healthy obesity. My studies have demonstrated that CETP-expressing female mice are protected against the deleterious effects of obesity on insulin sensitivity and exercise capacity. I propose that CETP's ability to promote RCT leads to an increase in bile acid signaling that ultimately results in improved muscle glucose disposal that improves systemic insulin sensitivity (Fig 5.1). I have observed increased activation of bile acid signaling pathways and physiological effects are reflective of bile acid signaling. These effects correspond with improved insulin sensitivity and increased insulin signaling in liver and muscle. While CETP was previously thought to have a negative effect on risk of CHD, my studies suggest that it promotes metabolic health in the context of obesity, particularly in females. The observed sexual dimorphism is also supported by studies of CETP in humans. Additionally, with the future of CETP inhibitor drugs in question, my research has provided additional insight into the role of CETP and may help contribute to a better understanding of the appropriate usage of CETP inhibitor drugs. On the whole, my research has demonstrated a novel role for CETP in protecting against the negative metabolic effects of obesity.

Clinical Relevance: CETP expression increases bile acid signaling

Once thought to function mainly in digestion, recent studies have demonstrated that bile acids have significant signaling roles. I have demonstrated that CETP expression leads to a significant increase in bile acids in the gut. This increase in bile

acids corresponds with increased *Dio2* transcription in gastrocnemius muscle, which is known to be upregulated by the activity of the bile acid receptor Gpbar1. This corresponds with improved muscle glucose disposal, resulting in improved insulin sensitivity. I also observed increased bile acid associated signaling in the liver through the Fxr and Fgf15/19 pathways, although these effects were only seen in the presence of insulin.

I propose that bile acid signaling, particularly the activation of Gpbar1 in muscle, is the mechanism by which CETP protects against diet-induced insulin resistance. Gpbar1 activity is known to increase energy expenditure and glucose oxidation in brown adipose tissue and muscle. I observed increased muscle glycolysis, TCA cycling, and glucose disposal in the CETP-expressing female mice, which is consistent with the effect of Gpbar1 signaling on muscle. A Gpbar1 agonist, INT777, is currently in development. Study of INT777 in mice has shown that activating Gpbar1 improves insulin sensitivity in mice fed HFD [16] and also reduces atherosclerotic lesions in a mouse model of atherosclerosis [217]. These results suggest that specifically targeting Gpbar1 signaling can lead to improved metabolic health.

Bile acids have shown promise in humans as a treatment for obesity-induced insulin resistance. A 4-week oral course of treatment with the bile acid tauroursodeoxycholic acid (TUDCA) in obese humans caused an increase in hepatic and muscle insulin sensitivity as determined by hyperinsulinemic clamp [183]. Additionally, muscle Akt phosphorylation was increased by treatment with the bile acid. Since the gut is easily accessible by oral treatment, these results suggest that bile acids could be a possible drug to treat the metabolic complications of obesity. The study of

TUDCA treatment showed an improvement in insulin sensitivity comparable to treatment with traditional anti-diabetes drugs such as thiazolidiones and metformin [218, 219]. Bile acid treatment could potentially provide an alternative anti-diabetic treatment, especially given the concerns regarding possible linkages between thiazolidiones and elevated risk of heart disease [220].

The composition of bile acids in the gut and serum may be more important than the total amount of bile acids. A study of the bile acid sequestrant colesevelam demonstrated that colesevelam treatment reduces the bile acid pool size but has the same effect on improving glucose tolerance, insulin sensitivity, and energy expenditure as treatment with bile acids [18]. Both the bile acid-treated mice and the colesevelam-treated mice were resistant to the effects of HFD on insulin sensitivity. While the bile acid-treated mice had 2.6-fold more bile acids in the gut and nearly twice in the serum, the treatments appeared to have the same effect on metabolism. Interestingly, both the bile acid-treated mice and the colesevelam-treated group showed a significant increase in the proportion of the bile acid pool consisting of taurocholic acid, which is known to be a strong agonist for Gpbar1 [221], so increasing the amount of a specific bile acid that activates Gpbar1 may be sufficient to promote insulin sensitivity.

Overall, my dissertation project has shown an important role for bile acid signaling in promoting insulin sensitivity in obese mice. Bile acids and their related signaling pathways are a strong drug target as the gut is easily accessible through oral delivery. Bile acids are also naturally occurring in the gut, which should likely reduce the possibility of side effects. I predict that bile acid signaling will become a pathway of great interest clinically as a method of promoting metabolic health in obesity.

Clinical Relevance: Sexual dimorphism in the effect of CETP on metabolism

I observed a significant sexual dimorphism in insulin sensitivity in the CETP-expressing female mice. While there was a modest improvement in GIR and some evidence for improved insulin sensitivity in the male mice, the female CETP-expressing mice showed a large difference in insulin sensitivity. Therefore, I focused the exercise studies on female mice only. The mechanism by which this sexual dimorphism occurs is yet to be determined. Evidence from human and mouse studies, however, suggest that CETP has a beneficial role in RCT and glucose metabolism in females. CETP may have a significant effect on estrogen signaling due to its role in production and trafficking of esterified estrogens. Additionally, female sex hormones may interact with CETP to promote RCT and increase bile acid production through their effect on hepatic LDL receptor. Overall, I propose that the sexual dimorphism is most likely caused by increased RCT in CETP female mice that leads to increased bile acid production, increased muscle glucose disposal, and therefore improved insulin sensitivity.

The sexual dimorphism that I observed with regard to CETP is supported by recent findings from studies in humans. CETP has a stronger effect on RCT in women, as studies of *ex-vivo* RCT showed that serum from women with higher CETP activity was better able to promote efflux of cholesterol from macrophages to serum. Additionally, clinical studies of surgical weight loss by gastric bypass surgery have shown that CETP mass correlated with reduced glycemia at a 12-month follow-up [13]. These results, combined with the strong sexual dimorphism observed in my studies suggest an interaction between CETP and female sex hormones that promotes RCT and improves metabolic health in the context of obesity. Interestingly, I did not observe

a difference in circulating estrogen levels or estrogen signaling to ER α in either liver or muscle, suggesting that absolute levels of estrogen or signaling through ER α do not account for the observed phenotype.

CETP expression may increase the amount of esterified estrogen in the serum, which may have an effect on estrogen signaling. Because of their hydrophobicity, estrogen esters must be carried through the circulation in lipoproteins. Estrogens can be esterified by LCAT in HDL and then transferred into LDL through the activity of CETP [222]. Esterified estrogen has been demonstrated to prevent LDL oxidation. Since oxidized LDL can contribute to formation of atherogenic plaques, estrogen esters likely have an anti-atherogenic role. Additionally, estrogen esters, which reside in serum lipoproteins, may be able to target estrogen signaling to specific tissues. I propose that CETP may not only increase the levels of esterified estrogen in LDL but also increase delivery of estrogen esters to the liver. In the liver, estrogen is known to have important signaling roles in promoting insulin sensitivity. Hepatic knockout of ER α , one of the main targets of estrogen signaling, results in hepatic insulin resistance [36]. Therefore, CETP may have insulin sensitizing effect in females through its effect on estrogen ester formation and trafficking.

Alternatively, CETP may not change estrogen signaling, but estrogen may instead have an effect on hepatic cholesterol uptake that promotes CETP-mediated RCT. The ability of CETP to promote RCT is dependent on hepatic LDL receptor [11]. Interestingly, estrogen is one of the most potent activators of LDL receptor expression in the liver [223, 224]. In fact, treatment of men with estrogen also increases hepatic LDL receptor expression and increases the rate of LDL clearance from the serum [225].

Additionally, our lab has observed that LDLR protein level is reduced in the livers of obese mice that lack ovarian hormones in a model of surgical menopause [226]. Since bile acid signaling appears to be central to the insulin sensitivity and exercise tolerance phenotypes observed in the CETP female mice, the increase in LDL receptor by estrogen may allow for CETP to promote RCT and increase bile secretion. The increase in bile acids leads to increased activation of *Dio2* in muscle through the action of Gpbar1, resulting in increased muscle glucose oxidation. This hypothesis is supported by my observation that only female CETP mice showed increased gut bile acids. Therefore, induction of hepatic LDL receptor by estrogen signaling is likely central to both the sexual dimorphism and the overall protection of female CETP mice from the metabolic effects of HFD.

CETP likely has a positive effect on RCT and glucose metabolism in women. My results from CETP-expressing female mice further contribute evidence to this hypothesis. The mechanism by which female sex interacts with CETP has not been fully established, but I propose that estrogen regulation of LDL receptor is central to the observed phenotype. CETP's ability to promote RCT depends on LDLR. Therefore, increased LDLR expression by estrogen could help CETP promote RCT and increase gut bile acids. Additional study of this mechanism could contribute to a better understanding of lipoprotein metabolism in women.

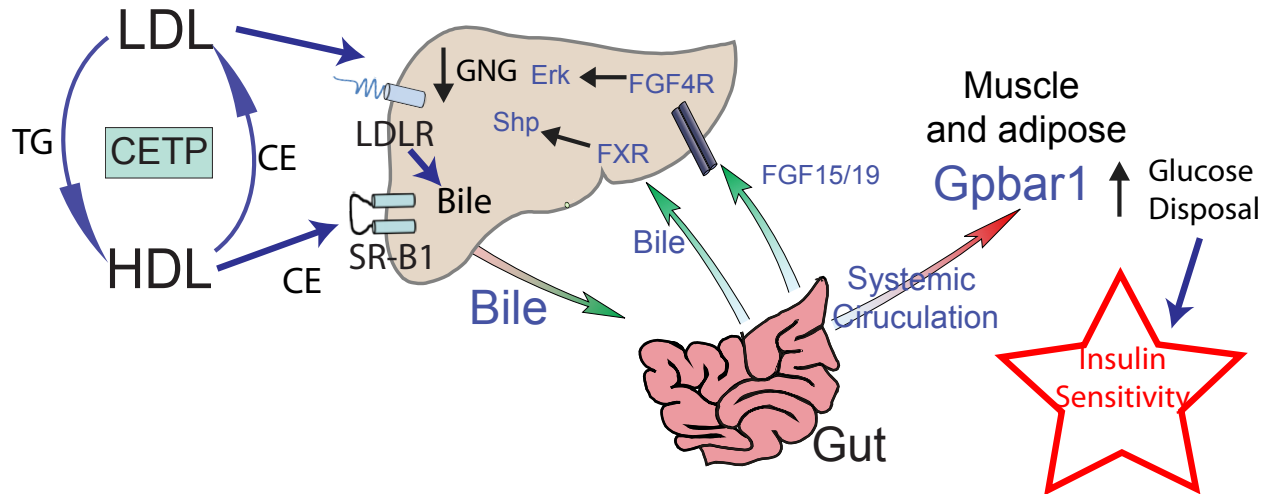


Figure 5.1: Overall model for the effect of CETP activity on bile acid signaling and hepatic and muscle insulin sensitivity. I propose that CETP mediates increased delivery of CE to the liver through LDLR. Once in the liver, CE is converted to bile and secreted into the gut. Gut bile acids can: 1) recycle to the liver and activate the Fxr signaling pathway, resulting in reduced gluconeogenesis, 2) Activate FGF15/19 production which signals to Fgf4r in the liver to promote glycogen production and suppress gluconeogenesis, or 3) Escape the enter-hepatic circulation into the systemic circulation, where they can activate Gpbar1 to promote glucose oxidation and energy expenditure in brown adipose tissue and muscle. This increase in glucose disposal leads to an improvement in insulin sensitivity in both liver and muscle tissue.

Clinical Relevance: Future of CETP inhibition

CETP inhibitor drugs have been controversial in the medical and pharmaceutical community because their failure to protect against CHD goes against well-established models of cardiovascular risk. Inhibition of CETP leads to a significant increase in HDL levels, which, according to the current model of cardiovascular risk, should lead to a significant reduction in incidence of cardiovascular events. Surprisingly, clinical trials of CETP inhibitor drugs have not demonstrated clinical efficacy in terms of reducing death from cardiovascular events. The CETP inhibitor Torcetrapib was associated with a higher incidence of death from cardiovascular causes, while the CETP inhibitor Dalcetrapib did not show a significant effect on reducing incidence of cardiovascular events. Clinical trials of Anacetrapib, a third CETP inhibitor drug, are currently ongoing. While the CETP inhibitor class of drugs has not yet demonstrated clinical efficacy in reducing risk of CHD, there may still be some hope for these drugs in promoting RCT.

My dissertation work on CETP suggests that improving RCT through the action of CETP overexpression improves insulin sensitivity. Interestingly, there is seemingly paradoxical evidence that inhibition of CETP leads to improved RCT. Treatment of model organisms with Torcetrapib [227], Dalcetrapib [157], and Anacetrapib [208] corresponds with improved RCT. If increased RCT promotes insulin sensitivity in the context of obesity, inhibition of CETP should cause improved insulin sensitivity. In a *post-hoc* study of the clinical trials of Torcetrapib, a correlation between CETP inhibition by Torcetrapib and a modest improvement in insulin sensitivity was observed [201]. This study, however, was performed mainly in men (approximately 70% of the study population), and the women in the study were post-menopausal, so effects of female

sex were likely not observed in this study. Studying CETP inhibition in younger women could help provide a further understanding of the intersection between CETP inhibition and female sex.

The ability of CETP inhibitor drugs to promote RCT depends on HDL-mediated flux of cholesterol to the liver, as CETP inhibition increases HDL cholesterol and reduces LDL cholesterol. CETP inhibitors are proposed to improve RCT by promoting lipidation of nascent HDL [157]. In contrast, CETP promotes RCT by increasing LDL cholesterol and its clearance by LDL receptor[10, 11]. The pathway by which HDL and LDL promote RCT may be central to the phenotype that I observed in the CETP-expressing female mice. Since LDLR is upregulated by the activity of estrogen signaling [223, 224], women may have a larger capacity for RCT by LDL. Also, a number of studies in humans have suggested a correlation between CETP activity and RCT in women [12, 131]. Considering these outcomes, it appears that CETP inhibition might have a deleterious effect on RCT in women.

Overall, these results suggest that improving RCT by CETP inhibition could improve insulin sensitivity by increasing HDL-mediated delivery of cholesterol to the liver. CETP promotes RCT through LDL. The significance of cholesterol delivery to the liver by LDLR versus SR-B1 has not been fully investigated. Since CETP inhibitor drugs increase RCT but do not have a large effect on insulin sensitivity, I speculate that RCT by LDLR may have a more significant effect than RCT mediated by HDL/SR-B1 on bile acid production and insulin sensitivity.

Conclusions

Obesity is an increasingly prevalent health problem that increases the risk of developing diabetes and CHD. While weight loss can reverse the negative effects of obesity, long-term weight loss maintenance is difficult. Additionally, weight loss may not reverse the effects of obesity on CHD in people with type-2 diabetes. Therefore, methods to improve metabolic health in obesity must be identified. My dissertation project has identified CETP as a factor that protects females against the negative effects of obesity on insulin sensitivity and exercise capacity. I propose that this protection is mediated by the signaling effects of bile acids, which are increased in only female mice. These results both provide insight into the relative protection of women from CHD and suggest that targeting bile acid signaling is a potential therapeutic method for protecting against insulin resistance and exercise intolerance in obesity. These findings could help to lessen the burden of obesity on both individuals and healthcare systems by spurring the development of treatments that promote metabolically healthy obesity.

CHAPTER VI

FUTURE DIRECTIONS

Insulin sensitivity in CETP mice

Bile acid signaling

My dissertation work has demonstrated that CETP protects against obesity induced insulin resistance in female mice. I propose that the ability of CETP to promote RCT leads to an increase in gut bile acids, which have been associated with improved insulin sensitivity in liver and muscle and increased energy expenditure in muscle and brown adipose tissue. Bile acids signal through three major pathways: 1) Fxr in the liver, 2) Fgf15 signaling to FGFR4 in liver, and 3) Gpbar1 in muscle. I propose the following future directions that will further investigate the relative contributions of each pathway to the observed phenotype of improved insulin sensitivity. The long-term goal of this work has been to define pathways that can convert obesity from an unhealthy state to a metabolically healthy state. These aims will define the relative contribution of three different bile acid signaling pathways to the metabolically healthy obesity phenotype observed in the CETP-expressing mice.

1: Evaluate insulin sensitivity in HFD-fed CETP-expressing mice treated with a Shp siRNA.

Bile acid signaling and estrogen signaling in the liver converge on the induction of Shp, a transcriptional co-repressor that downregulates genes the products of which

control gluconeogenesis. Shp is induced by both bile acid signaling through Fxr and estrogen signaling through ER α . Knocking down Shp with an anti-sense oligo (ASO) will allow for the assessment of the role of hepatic Fxr and ER α signaling to Shp in the observed improvement in insulin sensitivity mediated by transgenic expression of CETP in female mice. I propose assessing insulin sensitivity in HFD-fed CETP-expressing female mice treated with the Shp ASO or scrambled ASO. I expect that the knockdown of Shp will result in increased transcription of *G6pc* and *Pck1* in the liver, as Shp is known to repress their transcription. I do not expect the Shp knockdown to have a significant effect on muscle, as the ASO will be localized to the liver. I expect that knockdown of Shp will not alter the insulin sensitivity phenotype observed in CETP expressing mice, as I predict that the phenotype of improved muscle glucose disposal will still promote insulin sensitivity in the context of Shp knockdown. The insulin signal to the liver will likely still be strong enough to suppress *G6pc* and *Pck1* gene expression even in the context of Shp knockdown. Determining the relative contribution that hepatic bile and estrogen signaling to Shp makes towards the insulin sensitivity phenotype will help provide a better understanding of the mechanism by which CETP expression improves insulin sensitivity in HFD-fed female mice.

2: Evaluate insulin sensitivity in HFD-fed CETP-expressing mice crossed with a Fgf4r knockout mouse.

Bile acids in the gut promote the production of the enterokine Fgf15/19, which signals to the liver through Fgf4r. Fgf4r signaling leads to decreased bile acid production and suppression of genes the products of which control gluconeogenesis.

Knocking out Fgf4r will allow us to assess the role of Fgf15/19 signaling to the liver in promoting insulin sensitivity in the CETP-expressing female mice. I propose to evaluate insulin sensitivity in the in CETP-expressing female mice that have Fgf4r knocked out or control CETP-expressing female mice fed HFD. I expect that the knockout of Fgf4r will result in decreased ERK1/2 phosphorylation in the liver and a reversal of the effects of CETP on *G6pc* and *Pck1* signaling. I expect that the Fgf4r knockout mice will show reduced hepatic glycogen storage capacity and increased hepatic gluconeogenesis, as FGF15/19 signaling through Fgf4r is known to promote glycogen storage and suppress gluconeogenesis in the liver. I expect that the Fgf4r knockouts, therefore, will result in a relative reduction in the insulin sensitivity phenotype observed in the CETP-expressing females. I also expect that *cyp7a1* will be increased in the Fgf4r knockout mice, as Fgf4r signaling downregulates *cyp7a1*. This effect may prove confounding, as increased *cyp7a1* promotes bile acid signaling and may increase the effect of bile acid signaling on muscle [228]. This study will allow us to determine if Fgf4r signaling to the liver is required for the observed changes in insulin sensitivity in the CETP-expressing mice.

3: Evaluate insulin sensitivity in HFD-fed CETP transgenic mice that are deficient in Gpbar1.

A portion of bile acids (approximately 5%) escape the entero-hepatic circulation and enter into the serum. These bile acids activate a third bile acid receptor known as g-protein coupled bile acid receptor (Gpbar1). Gpbar1 signaling increases glucose oxidation in brown fat and skeletal muscle and may have an important role in the improved insulin sensitivity in the CETP-expressing female mice. Gpbar1 also acts in

the gut to promote secretion of Glp1, which is known to promote insulin secretion and has a protective effect on pancreatic beta cells. Crossing the CETP transgenic mouse line into a mouse model that lacks Gpbar1 expression will define if bile acid signaling through Gpbar1 is necessary for the resistance to diet-induced insulin resistance observed in the CETP-expressing female mice. I propose to evaluate insulin sensitivity in the in CETP/Gpbar1ko mice or control CETP-expressing female mice fed HFD.

I expect that knocking out Gpbar1 will reverse the protective effects of CETP expression on insulin sensitivity in the context of HFD. In my previous studies, I observed changes in the muscle of the CETP-expressing mice that are consistent with Gpbar1 signaling such as increased glycolysis, increased TCA cycle intermediates, and increased expression of *Dio2*, which is a known target of Gpbar1. I therefore expect that the CETP/GPBAR1ko mice would not show increased glycolysis or increased muscle glucose disposal and would therefore have impaired whole-body insulin sensitivity compared to the CETP-expressing mice. This experiment is critical to understanding the involvement of bile acid signaling in the CETP-expressing mice, as our previous observations suggest that Gpbar1 signaling is central to the observed improvement in insulin sensitivity. If Gpbar1 knockout does not alter whole-body insulin sensitivity, I propose that effects of bile acids on hepatic signaling to Shp and Fgf4r might be the main link between bile acids and the observed improvement in insulin sensitivity in CETP female mice. Additionally, bile acids promote secretion of Glp1 in the gut, which has an insulin sensitizing effect. Overall, this study will determine the contribution of bile acid signaling to muscle through Gpbar1.

4: Quantify the levels of bile acid species in the CETP-expressing female mice

Recent studies of the role of bile acid signaling in metabolism have suggested that the composition of bile acid species may be more important than the size of the bile acid pool itself. In fact, bile acid sequestrants that decrease the bile acid pool size had a similar effect on insulin sensitivity to bile acid treatment [18]. Increased levels of taurocholic acid, which is a strong ligand for Gpbar1, were correlated with improved insulin sensitivity. Different bile acid species have variable degrees of potency in activating bile acid signaling pathways. For example, chenodeoxycholic acid is the strongest natural agonist for Fxr [229], while lithocholic acid is the strongest Gpbar1 agonist [230]. In my studies I measured bile acid species in liver, gut, and serum using a metabolomic approach that was optimized for more hydrophilic biochemicals. By performing a more targeted measurement of bile acid species, we can determine the effect of CETP expression on bile acid species, which could potentially contribute to the improvement in insulin sensitivity observed in the CETP-expressing female mice by differential activation of different bile acid signaling pathways.

Sexual dimorphism

I observed a significant sexual dimorphism in insulin sensitivity in the CETP-expressing mice, with CETP expression having a much greater effect on insulin sensitivity in the female mice compared to the males. I propose that this sexual dimorphism is related signaling by female sex hormones, particularly estrogen. Estrogen signaling interacts with multiple signaling pathways critical to insulin

sensitivity, especially AKT and ERK signaling in the liver. Additionally, estrogen signaling is known to increase expression of LDLR in the liver, which is likely critical to CETP's ability to promote RCT. Investigating the role of estrogen signaling in CETP-expressing mice will allow for greater understanding of the observed sexual dimorphism in insulin sensitivity. I propose the following aims to investigate the mechanism of the sexual dimorphism in insulin sensitivity observed in the CETP-expressing mice. Understanding mechanisms that contribute to sex differences in the effect of CETP could help to provide further insight into the sexual dimorphism observed in CETP function in humans. CETP appears to have a beneficial role to promote RCT and improve glycemic control in women [12, 13, 131]. Additionally, since women are relatively protected from the risk of CHD, CETP expression could potentially be contributing to the reduced relative risk in human women. Further understanding of the interaction between female sex and CETP could help provide insight into the role of CETP in CHD in women.

1: Assess insulin sensitivity in CETP mice that lack ovarian hormones

Since we observed a strong phenotype with regards to insulin sensitivity in the female CETP mice only, I propose that female sex hormone signaling contributed to the observed phenotype. To test the hypothesis that female sex hormones are required for insulin sensitivity in the CETP-expressing female mice, ovarian hormones will be eliminated by surgical removal of the ovaries by ovariectomy. Ovariectomy will globally remove the contribution of all ovarian hormones to metabolism. I propose measuring insulin sensitivity in HFD-fed CETP females and WT littermates that have been either

treated with ovariectomy or sham surgery. If the ovariectomized CETP mice have similar insulin sensitivity to the WT sham mice, it would suggest that ovarian hormones are required for the protection against HFD-induced insulin resistance observed in the CETP female mice. I expect that the ovariectomized CETP female mice will not be protected against HFD-induced insulin sensitivity, as this effect appeared to depend on the presence of female sex hormones. Additionally, I expect that the ovariectomized mice will show lower levels of hepatic LDLR, which will interfere with CETP's ability to promote RCT. This study will improve our understanding of the mechanism by which female sex influences insulin sensitivity in CETP mice.

2: Assess insulin sensitivity in CETP-expressing mice that lack hepatic ER α expression

Estrogen signaling to ER α in the liver is known to promote insulin sensitivity, as mice lacking ER α exhibit insulin resistance. To test the hypothesis that hepatic estrogen signaling is required to improve insulin sensitivity in CETP-expressing female mice, CETP mice will be bred with mice that lack ER α in the liver (Liver ER α knockout or LERKO). ER α signaling is known to alter hepatic metabolism, and liver ER α knockout mice exhibit hepatic insulin resistance. I propose measuring insulin sensitivity in female CETP mice and CETP/LERKO mice fed a HFD as well as WT littermate controls. If the CETP/LERKO mice show impaired insulin sensitivity compared to the CETP females, it would then appear that hepatic estrogen signaling is required to protect against HFD-induced insulin resistance in CETP mice. I expect that hepatic knockout of ER α in the CETP mice will reverse the phenotype of improved insulin resistance observed in the female CETP-expressing mice. Since ER α promotes expression of LDLR in the liver, I

expect that ER α knockout will interfere with CETP's ability to promote RCT, which I hypothesize is the mechanism by which CETP improves insulin sensitivity. This study will allow us to understand the specific contribution of hepatic ER α signaling to the insulin sensitivity phenotype in CETP female mice.

3: Assess insulin sensitivity in male CETP mice that have been treated with estrogen

Since the insulin sensitivity phenotype mediated by transgenic expression of CETP is only observed in females, I hypothesize that female sex hormones such as estrogen are responsible for the difference in insulin sensitivity. To test the hypothesis that elevated estrogen signaling is sufficient to protect CETP mice against HFD-induced insulin resistance, I propose treating male CETP mice and their WT littermates with estrogen. Males normally have low levels of estrogen signaling, and increasing estrogen in males by implanting an estrogen pellet will increase estrogen signaling throughout the body. There is evidence from estrogen-treated human males that pharmacological doses of estrogen in males increases hepatic LDL receptor and accelerates the clearance of LDL [225]. I propose measuring insulin sensitivity in male HFD-fed CETP mice and their WT littermates treated with either an estrogen pellet or sham surgery. If the estrogen-treated CETP-expressing males show improved insulin sensitivity on HFD compared to the control CETP mice and estrogen-treated WT mice, it would suggest that estrogen signaling interacts with CETP activity to protect against HFD-induced insulin resistance. I expect to see an increase in hepatic LDL receptor in the estrogen treated mice. Since the RCT activity of CETP is dependent on LDL receptor levels, I expect that the estrogen-treated CETP males would show improved insulin sensitivity

compared to the control CETP males. This result would suggest that estrogen signaling is sufficient to improve insulin sensitivity in HFD-fed CETP-expressing mice and would provide further insight into the observed sexual dimorphism in insulin sensitivity.

CETP mouse model

I used a mouse model of CETP that constitutively expresses CETP. Human CETP expression is regulated by diet, insulin, and ovarian hormones. Therefore, the improvement in insulin sensitivity and exercise capacity observed in the CETP transgenic mice may be a result of constitutive CETP expression. A second mouse model is available that expresses CETP under control of the human promoter. Studying HFD-induced insulin resistance in a mouse model that expresses CETP under the control of the human CETP promoter would allow us to further investigate the role of CETP in insulin sensitivity. Human CETP expression is increased by obesity [165] and estrogen [166], and its activity is acutely suppressed by insulin [167]. I propose assessing insulin sensitivity in male and female human CETP transgenic mice and their non-transgenic littermates fed 4 weeks of HFD. If the mice that express human CETP do not show an improvement in insulin resistance on HFD compared to littermates, it suggests that acute insulin suppression of CETP activity may impair the ability of CETP to improve insulin sensitivity. I expect that the female HFD-fed human CETP-expressing mice will show resistance to diet-induced insulin resistance, as obesity and estrogen increase CETP levels. The sexual dimorphism may be more pronounced in the human CETP mice, as CETP expression will likely be lower in males. This study will allow for

further testing of our hypothesis that CETP protects against HFD-induced insulin resistance to a more human-like model.

Exercise capacity in CETP mice

Bile acid signaling

I observed that CETP prevents the decline in exercise capacity associated with obesity in female mice. This improvement in exercise capacity correlated with increased muscle glycolysis and TCA cycling. Additionally, mitochondrial oxidative capacity was increased in the CETP-expressing mice compared to WT littermates. These results suggest an increase in muscle Gpbar1 signaling, which has been associated with increased energy expenditure and mitochondrial oxidation. Additionally, increased expression of *Dio2*, a target of bile acid signaling through Gpbar1 was observed in the muscle of CETP-expressing female mice. I propose that bile acid signaling through Gpbar1 in muscle leads to the improvement in exercise capacity observed in the female CETP mice. I propose the following aims to test the hypothesis that muscle Gpbar1 signaling improves exercise capacity by promoting mitochondrial glucose oxidation.

1: Determine if Gpbar1 signaling is necessary for improved exercise capacity in CETP-expressing mice

Bile acid signaling to Gpbar1 increases glucose oxidation in brown adipose tissue and skeletal muscle. I observed changes in glycolysis, TCA cycling, and mitochondrial function in the CETP-expressing female mice that were consistent with Gpbar1 signaling. I hypothesize that CETP promotes bile acid production leading to

increased activation of Gpbar1, which allows for improved exercise capacity. To test the hypothesis that bile acid signaling through Gpbar1 is the mechanism by which CETP expression improves exercise capacity in HFD fed mice, I propose performing exercise capacity tests in female HFD-fed CETP mice and CETP/GPBAR1ko mice. If the CETP/GPBAR1ko mice show reduced exercise capacity compared to the CETP mice, it would seem that bile acid signaling through Gpbar1 is required for the improvement in exercise capacity observed in the CETP female mice. Since I observed a number of effects that are consistent with Gpbar1 signaling in the muscle of CETP female mice, I expect that knocking out Gpbar1 will decrease the exercise capacity phenotype observed in the CETP female mice. This study will allow us to determine whether bile acid signaling to Gpbar1 leads to CETP improves exercise capacity and will help define pathways that may improve exercise capacity in obese people.

2: Determine if Gpbar1 activity is sufficient to improve exercise capacity

Signaling through Gpbar1 leads to increased glucose oxidation in brown adipose tissue and skeletal muscle. Since exercise capacity depends on the ability of muscle to oxidize substrate, I hypothesize that increasing Gpbar1 signaling would lead to improved exercise capacity. To test this hypothesis, I propose assessing exercise capacity in male and female HFD-fed WT mice treated with the Gpbar1 agonist INT777 or vehicle. If the INT777 mice show improved exercise capacity compared to the vehicle-treated mice it would suggest that activation of Gpbar1 is sufficient to improve exercise capacity in the context of HFD. Investigating the specific role of Gpbar1 in exercise capacity will allow for a better understanding of the role of bile acid signaling in

the CETP-expressing female mice and will determine if Gpbar1 activation alone is sufficient to improve exercise capacity.

Sexual dimorphism

Based on the strong sexual dimorphism observed in insulin sensitivity in the CETP mice, I focused the exercise studies on female mice only, as they showed significantly improved insulin resistance compared to the CETP male mice. It is possible, however, that CETP-expressing male mice might have a difference in exercise capacity despite not showing a large change in overall insulin sensitivity when fed HFD. Therefore, I propose that exercise capacity should be studied in CETP-expressing male mice. Male CETP-expressing mice and their WT littermates would be subjected to an exercise test every 2 weeks while being fed HFD. If the CETP-expressing male mice show improved exercise capacity during the course of HFD compared to the WT littermates, it would suggest that there is not a significant sexual dimorphism in CETP-expressing mice with regard to exercise capacity. Since we did not observe an improvement in insulin sensitivity or an increase in gut bile acids in the CETP-expressing male mice, I do not expect to observe an improvement in exercise capacity in the HFD-fed CETP-expressing male mice compared to WT. If the CETP-expressing males do show improved exercise capacity on HFD, it would suggest that the mechanism by which CETP improves exercise capacity is separate from the mechanism that leads to improved insulin sensitivity.

Human studies

My studies have shown that CETP expression improves exercise capacity in female mice. We are interested in expanding on the results from mice by investigating the role of CETP in exercise capacity in human females. To test the hypothesis that CETP activity improves exercise capacity in female humans, we have established a collaboration with Dr. Peter Bovary at the University of Michigan, who has studied female endurance athletes. We are planning to measure CETP activity in blood samples from trained athletes and untrained, age and BMI matched controls. If we observe a higher level of CETP activity in the highly trained athletes compared to controls it would suggest that CETP has a role in improving exercise capacity in humans. This study, however, would only establish a correlation between CETP and exercise capacity in female endurance athletes. Additionally, since both the athletes and controls are lean, this study would not investigate the effects of CETP on protecting against the obesity-induced decline in exercise capacity. In the ideal human study, CETP mass and/or activity would be correlated against exercise capacity in obese human females. If CETP expression has the same effect on improving exercise capacity in humans, I would expect that the patients with higher CETP mass/activity would show improved exercise capacity. These experiments will allow for a better understanding of the role of CETP in human exercise capacity and could potentially establish CETP as a diagnostic marker for exercise capacity.

Conclusions

My dissertation project has demonstrated a novel role for CETP to protect female mice against HFD-induced insulin resistance and exercise intolerance. I have identified multiple pathways that may promote metabolically healthy obesity and have provided insight into the mechanism of sex differences in the role of CETP. My findings have raised questions for ongoing study regarding the contributions of bile acid signaling through Fxr, Fgf15/19, and Gpbar1 to improved metabolic health with obesity. Determining which of these pathways has the strongest effect on improved metabolic health could help provide new drug targets with the goal of improving insulin sensitivity and exercise capacity in obese people. Additionally, further investigation into the mechanism behind the strong sexual dimorphism observed in the CETP-expressing mice could lead to an improved understanding of the sexual dimorphism observed in clinical studies of CETP function as well as potentially address the sex difference observed in CHD in women. Overall, my studies represent a significant contribution to the understanding of the role of CETP in metabolic health and have raised important questions for ongoing studies that could lead to better treatments for the chronic metabolic effects of obesity.

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