6-2011

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Published in *Applied Physiology, Nutrition, and Metabolism* 36:3 (June 2011), pp. 313–322;
doi: 10.1139/H11-003

Submitted July 19, 2010; accepted December 23, 2010; published online May 16, 2011.

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Abstract

Obese and diabetic humans display decreased plasma high-density lipoprotein cholesterol (HDL-C) concentrations and an increased risk for coronary heart disease. However, investigation on HDL metabolism in obesity with a particular emphasis on hepatic ATP-binding cassette transporter A1 (ABCA1), the primary factor for HDL formation, has not been well studied. Human apolipoprotein B_{100} transgenic (*hApoB*^{tg}) and brown adipose tissue deficient (*BATless*) mice were crossed to generate *hApoB*^{tg}/*BATless* mice. Male and female *hApoB*^{tg} and *hApoB*^{tg}/*BATless* mice were maintained on either a regular rodent chow diet or a diet high in fat and cholesterol until 24 weeks of age. The *hApoB*^{tg}/*BATless* mice that were fed a HF/HC diet became obese, developed hepatic steatosis, and had significantly elevated plasma insulin levels compared with their *hApoB*^{tg} counterparts, but plasma concentrations of total cholesterol, HDL-C, triglycerides, and free fatty acids and lipoprotein distribution between genotypes were not significantly different. Hepatic expression of genes encoding HDL-modifying factors (e.g., scavenger receptor, class B, type I, hepatic lipase, lecithin:cholesterol acyltransferase, and phospholipid transfer protein) was either altered significantly or showed a trend

of difference between 2 genotypes of mice. Importantly, hepatic protein levels of ABCA1 were significantly lowered by ~35% in male obese *hApoB*^{tg}/*BATless* mice with no difference in mRNA levels compared with *hApoB*^{tg} counterparts. Despite reduced hepatic ABCA1 protein levels, plasma HDL-C concentrations were not altered in male obese *hApoB*^{tg}/*BATless* mice. The result suggests that hepatic ABCA1 may not be a primary contributing factor for perturbations in HDL metabolism in obesity-induced hyperinsulinemia.

Keywords: high density lipoprotein cholesterol, ATP-binding cassette transporter A-I, *hApoB*^{tg}/*BATless* mice, insulin resistance, obesity

Introduction

Coronary heart disease (CHD) is one of the major metabolic diseases highly associated with the development of obesity, and it is strongly influenced by lifestyle and diet (De Lorgeril 2007). Atherosclerosis, a major underlying condition for CHD, is characterized by the buildup of atherosclerotic plaque in the arteries. This process may progress without any clinical symptoms but may manifest into a heart attack or stroke as plaque growth restricts blood flow. In humans, approximately 20% of circulating cholesterol is normally transported in high-density lipoprotein (HDL) (Attie 2007). Plasma HDL cholesterol (HDL-C) concentrations have an inverse relationship with the risk of CHD (Sasahara et al. 1997; Ooi et al. 2005; Van Gaal et al. 2006). Reduced plasma HDL-C concentrations by ~15%–30% compared with normal subjects are associated with obesity and obesity-related disorders such as insulin resistance, metabolic syndrome, and type 2 diabetes (Gordon et al. 1977, 1989; Krauss 2004; Ooi et al. 2005; Perségol et al. 2007), which may contribute in 1 way to the increased risk for CHD in the obese and diabetic population.

The major athero-protective mechanism of HDL is reverse cholesterol transport (RCT), in which cholesterol is removed from peripheral tissues and carried to the liver for ultimate excretion from the body (Sasahara et al. 1998; Lee and Parks 2005; Lee et al. 2005). For this process, nascent HDL is necessary to take up excess cholesterol from the extrahepatic tissues. Nascent HDL formation begins as a discoidal structure known as nascent or pre-β HDL, consisting of the major protein constituent apolipoprotein A-I (apoA-I), phospholipids, and free cholesterol (Ooi et al. 2005; Singaraja et al. 2006), consequent to the interaction of apoA-I and ATP-binding cassette transporter A-1 (ABCA1). ABCA1 mediates the efflux of cellular phospholipids and free cholesterol to extracellular acceptors, namely lipid-free or lipid-poor apoA-I, producing pre-β HDL particles (Francis et al. 1995; Rogler et al. 1995; Brewer et al. 2004; Lee and Parks 2005; Lee et al. 2005). The significant role of ABCA1 in HDL formation is underscored by the finding that mutations in *Abca1* lead to near absence of plasma HDL-C concentrations in patients with Tangier disease (Oram 2000) and *Abca1* knockout mice (Schreyer et al. 1994; McNeish et al. 2000; Francone et al. 2003). Interaction of apoA-I with ABCA1 produces heterogeneous-sized, pre-β migrating nascent HDL subpopulations (pre-β_1 to pre-β_4) that vary in size, lipid, and apoA-I content in vitro and have different metabolic fates with less lipidated pre-β HDL being rapidly removed from the circulation without participating in RCT (Mulya et al. 2007, 2008). The nascent HDL

undergo maturation to become spherical mature particles by several plasma enzymes, including lecithin:cholesterol acyltransferase (LCAT) (Brewer et al. 2004) and phospholipid transfer protein (PLTP) (Huuskonen et al. 2001). Mature HDL deliver the cholesterol mainly to the liver and steroidogenic tissues such as the adrenal glands, testis, and ovaries by interacting with the surface HDL receptor, scavenger receptor class B type I (SR-BI) (Acton et al. 1996; Krieger 1998, 1999). Hepatic lipase (HL) is another important enzyme whose action affects the rate of apoA-I catabolism by primarily hydrolyzing triglycerides in HDL particles. HL activity is increased when HDL particles are triglyceride-rich (Lewis and Rader 2005).

Although reduced plasma HDL-C concentrations have been recognized in obese individuals, mechanisms underlying this phenomenon are not clearly understood. High plasma triglyceride levels and triglyceride enrichment in HDL particles have been suggested as a major reason for apoA-I and HDL hypercatabolism due to enhanced lipolysis by lipases, lowering plasma HDL-C levels in individuals with obesity and type 2 diabetes (Biesbroeck et al. 1982; Schaefer et al. 1982; Briones et al. 1984; Le and Ginsberg 1988; Frénais et al. 1997). However, the apoA-I fractional catabolic rate (FCR) did not differ in the individuals with low HDL-C regardless of their plasma triglyceride concentration, supporting the presence of alternative mechanisms (Brinton et al. 1991). As mentioned, ABCA1 plays a pivotal role in nascent HDL formation. Importantly, deletion of *Abca1* in the liver and intestine reduced plasma HDL-C concentrations by ~80% and ~30% in mice, respectively, indicating that ABCA1 in these tissues is quantitatively most important for maintaining plasma HDL-C levels (Timmins et al. 2005; Brunham et al. 2006). In addition to its role in nascent HDL formation, hepatic ABCA1 has been implicated in HDL catabolism. When ^{125}I-radiolabeled mature HDL particles were injected into mice, plasma die-away of the radiolabel was faster in mice with liver-specific deletion of *Abca1* compared with wild-type counterparts (Timmins et al. 2005). Whether low plasma HDL-C concentrations in obese and diabetic subjects are attributed to any alterations in hepatic ABCA1 expression is not known. In this study, we intended to examine if the factors important in HDL formation, maturation, and catabolism are altered in obese mice compared with lean control animals. We chose brown adipose tissue (BAT) deficient (BATless) mice crossed with human apoB$_{100}$ transgenic mice (*hApoB^{tg}*) to generate *hApoB^{tg}/BATless* mice. The *hApoB^{tg}/BATless* mice on a high-fat–high-cholesterol diet showed dyslipidemia similar to that of humans with development of obesity and insulin resistance (Siri et al. 2001).

Materials and methods

Animal care and diet

hApoB^{tg} mice with low-density lipoprotein receptor knockout (*hApoB^{tg}ldlr$^{-/-}$*) were obtained from Dr. Larry Rudel at Wake Forest University School of Medicine (Winston-Salem, North Carolina, USA) (Callow et al. 1994). To restore the expression of low-density lipoprotein receptor in *hApoB^{tg}* mice, they were crossed with C57BL/6J mice and *hApoB^{tg}ldlr$^{+/+}$* mice were selected (*hApoB^{tg}ldlr$^{+/+}$* mice are referred to as *hApoB^{tg}* hereafter). *BATless* mice were purchased from the Jackson Laboratory (Bar Harbor, Maine, USA). The *BATless* mice were generated using the promoter of a BAT-specific uncoupling protein-1 (UCP-1) to drive the

expression of diphtheria toxin A, resulting in the loss of brown adipose tissue and weight gain (Lowell et al. 1993). Breeding pairs were set up with a *hApoB*^{tg} and a *BATless* mouse, producing an *hApoB*^{tg} control and *hApoB*^{tg}*/BATless* mice. At 21 days of age, mice were weaned and tail biopsy (~3–5 mm) was performed for DNA extraction and genotyping. Each DNA sample was tested for presence of the human apoB$_{100}$ transgene as well as the wild-type and the BATless toxigene alleles by DNA amplification using the ExTaq Polymerase system (TaKaRa, Otsu, Shiga, Japan), after which they were subjected to 1% agarose gel electrophoresis at 100 V for 70 min. Gels were visualized using Chemidoc XRS (Bio-Rad, Hercules, California, USA) and QuantityOne Software (Bio-Rad) with 2-log DNA ladder (New England BioLabs, Ipswich, Massachusetts, USA) for size identification. PCR primers used to identify human apoB$_{100}$ were reported by Callow et al. (1994), and those for wild-type allele and BATless toxigen were provided by the Jackson laboratory. The primer sequences are listed in Table 1.

Table 1. Primers for PCR genotyping and qPCR analysis

Target	Forward	Reverse
hApoB^{tg}	5′-AGAAGGTTCCAGATGTCTATGAGG-3′	5′-TCCAAGTATCTGTCTTCAAGAAACC-3′
Wild-type allele	5′-CAAATGTTGCTTGTCTGTG-3′	5′-GTCAGTCGAGTGCACAGTTT-3′
BATless toxigene allele	5′-GCGCTGATGATGTTGTTGAT-3′	5′-CATCCCCGAACCTTTTGATA-3′
GAPDH	5′-TGTGTCCGTCGTGGATCTGA-3′	5′-CCTGCTTCACCACCTTCTTGAT-3′
apoA-I	5′-GACAGCGGCAGAGACTATGTGT-3′	5′-CAGCTGTTGGCCCAAGGA-3′
SR-BI	5′-GATGTGGGCACCCTTCATG-3′	5′-CCGGGCTGAAGAATTCCA-3′
LCAT	5′-GCTGGCCTGGTAGAGGAGATG-3′	5′-GGCAGGATCACTGGTCTGGAT-3′
PLTP	5′-TGGGACGGTGTTGCTCAA-3′	5′-CCCACGAGATCATCCACAGA-3′
HL	5′-GGCAGGATCACTGGTCTGGAT-3′	5′-TGTTGATGTCCACCCCTTCA-3′
ABCA1	5′-CGTTTCCGGGAAGTGTCCTA-3′	5′-GCTAGAGATGACAAGGAGGATGGA-3′

At weaning, mice were fed a Western-style diet that was high in fat and cholesterol (HF–HC) (no. TD.88137 Adjusted Calories Diet; Harlan Teklad, Madison, Wisconsin, USA), containing 17.3% protein, 48.5% carbohydrate, and 21.1% milk fat by weight and 4.5 kcal·g^{-1} (1 kcal = 4.186 kJ). The HF–HC diet also contained 0.2% cholesterol by weight. Body weights were recorded at 4, 8, 12, 16, 20, and 24 weeks of age, and mice were sacrificed at 24 weeks of age after an injection of a mixture of ketamine (50 mg·kg^{-1}, Ketaject; Phoenix Pharmaceuticals, St. Joseph, Missouri, USA) and xylazine (10 mg·kg^{-1}, Xyla-Ject; Phoenix Pharmaceuticals). Terminal blood was collected via cardiac puncture into a 2 mL EDTA-coated BD Vacutainers (BD, Franklin Lakes, New Jersey, USA) and centrifuged at 5000*g* for 20 min at 4°C to remove red blood cells. Liver samples were quick frozen in liquid nitrogen and stored at –80°C until time of analysis. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska–Lincoln.

Plasma and liver lipid measurements

Plasma concentrations and liver contents of total cholesterol (Roche Diagnostics, Indianapolis, Indiana, USA), triglycerides (Roche Diagnostics), and free cholesterol (Free Cholesterol E, Wako Chemicals, Richmond, Virginia, USA) were determined by enzymatic

analysis as previously described (Rasmussen et al. 2009). HDL-C levels were measured using 10 μL plasma in 10 μL HDL-Precipitating Reagent (Thermo, Melbourne, Australia) according to the manufacturer's instruction. Plasma levels of free fatty acids were measured using the NEFA-HR(2) kit (Wako, Richmond, Virginia, USA) as described in the manufacturer's protocol.

Fast performance liquid chromatography (FPLC) analysis using 2 Superose 6 columns in series at a flow rate of 0.5 mL·min^{-1} was used to monitor lipoprotein distribution. Plasma from 4 mice of each HF–HC experimental group was pooled for a total of 200 μL for analysis and 100 fractions were collected and 50 μL of each fraction was used for total cholesterol and triglyceride concentrations with enzymatic analysis as described above.

Plasma insulin levels

Plasma levels of insulin were measured by enzyme immunoassay using the Insulin (Mouse) Ultrasensitive EIA kit (Alpco Diagnostics, Salem, New Hampshire, USA) and following instructions for 5 μL of standard and sample. The optical density of the plate was measured at 450 nm with a reference wavelength of 620 nm. Data were presented as plasma concentration at ng · mL^{-1}.

Plasma LCAT and PLTP activities

LCAT enzyme activity was measured according to the manufacturer's protocol for the LCAT Activity Assay Kit, Fluorometric (Calbiochem, San Diego, California, USA). Fluorescence intensity of each sample was measured with an FLx800 fluorometer (BioTek, Woburn, Massachusetts, USA), using Gen5 software (BioTek) at an excitation of 340 nm and emissions of both 390 nm and 470 nm. The emission intensity at 390 nm represents the fluorescent emission of the hydrolyzed LCAT substrate while emission intensity at 470 nm represents the fluorescent emission of the nonhydrolyzed LCAT substrate. Data were reported as a ratio of the 2 emission intensities (390/470). PLTP enzyme activity was measured using the PLTP Activity Assay Kit (BioVision, Mountain View, California, USA) according to the manufacturer's protocol. Fluorescence intensity of each sample was measured with an FLx800 fluorometer using Gen5 software with an excitation of 465 nm and emission of 535 nm.

Quantitative realtime PCR (qPCR) and Western blot analysis

Total RNA was isolated from liver samples using TRIzol reagent (Invitrogen, Carlsbad, California, USA) and qPCR analysis was performed as previously described (Rasmussen et al. 2009). Primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH), ABCA1, LCAT, PLTP, HL, apoA-1, and SRB1 were designed according to GenBank database using the Primer Express software provided by ABI and are listed in Table 1.

Protein was isolated from liver samples using radioimmune precipitation (RIPA) buffer (50 mmol · L^{-1} Tris-HCl at pH 7.4, 150 mmol · L^{-1} NaCl, 0.25% sodium deoxycholate, 1% Nonidet P-40, 1 mmol · L^{-1} EDTA) containing Protease Inhibitor Cocktail III (Calbiochem, San Diego, California, USA) and used for Western blot analysis as described before (Park et al. 2008; Rasmussen et al. 2008). Rabbit antimouse ABCA1 antibody was provided as a

courtesy from Dr. John Parks at Wake Forest University School of Medicine (Winston-Salem, North Carolina, USA). β-Actin was used as a loading control to normalize the data.

Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's pairwise comparison or unpaired *t* test was used to identify significant differences between genotypes of each gender. *p* values of < 0.05 were considered significant by GraphPad InStat 3 (GraphPad Software, Inc., San Diego, California, USA). Data are expressed as means ± SD.

Results

General observations

Body weights of mice on a chow or an HF–HC diet were recorded at 4, 8, 12, 16, 20, and 24 weeks of age. There was no statistically significant difference in body weight between *hApoB*^{tg} and *hApoB*^{tg}*/BATless* mice on a chow diet through 24 weeks irrespective of gender (33.4 ± 1.7 g vs. 32.7 ± 2.4 g for male; 25.1 ± 0.76 g vs. 24.2 ± 0.56 g for female). However, in mice fed an HF–HC diet, the body weights of *hApoB*^{tg}*/BATless* mice were significantly higher than control *hApoB*^{tg} mice starting at 8 weeks and continuing through the end of the study for both male and female animals (Fig. 1). For male and female mice, body weights of *hApoB*^{tg}*/BATless* mice were ~25% and ~38% over *ApoB*^{tg} control mice, respectively, confirming that *hApoB*^{tg}*/BATless* mice developed diet-induced obesity.

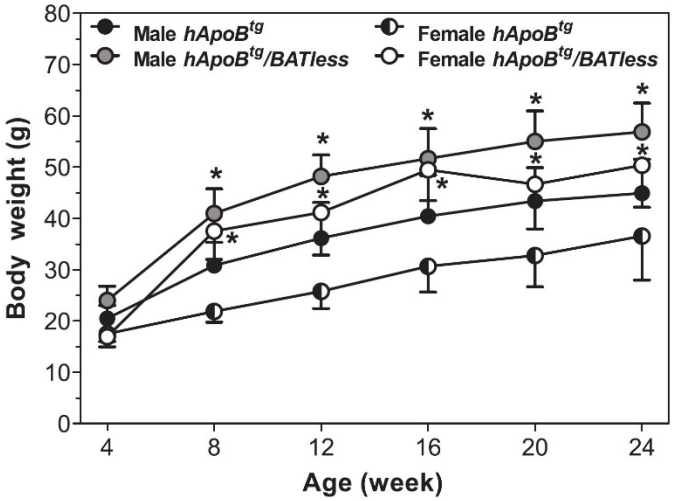

Figure 1. Body weight change of male and female *hApoB*^{tg} and *hApoB*^{tg}*/BATless* mice fed an HF–HC diet over the course of the 24-week study. Values represent means ± SD. Data were analyzed using 1-way ANOVA and Tukey's pairwise comparison at each week. Means at the same weeks of age having different superscripts are significantly different ($p < 0.05$). Male *hApoB*^{tg}, $n = 12$; male *hApoB*^{tg}*/BATless*, $n = 11$; female *hApoB*^{tg}, $n = 11$; female *hApoB*^{tg}*/BATless*, $n = 7$.

Plasma lipids and insulin

Plasma total cholesterol, triglyceride, and free fatty acid concentrations were not significantly different between *hApoB*^{tg} control and *hApoB*^{tg}/*BATless* obese mice on an HF–HC diet in both genders (Table 2). Plasma samples from male mice of each genotype were subjected to fractionation by using 2 Superose 6 columns to monitor lipoprotein distribution. Peaks corresponding with very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and HDL were identified based upon total cholesterol as well as triglyceride concentrations of each fraction and labeled as such with the VLDL peak centered at fraction 35, the LDL peak at fraction 50, and the HDL peak at fraction 65 (Fig. 2). As expected based on no significant difference in plasma total cholesterol concentrations, lipoprotein distribution was similar between the 2 genotypes of mice with a minor increase in cholesterol in the LDL fraction in *hApoB*^{tg}/*BATless* mice. Triglyceride in VLDL and LDL fraction showed a moderate increase in *hApoB*^{tg}/*BATless* mice compared with *hApoB*^{tg} mice but there was no significant difference in plasma triglyceride concentrations between 2 genotypes of mice. Additionally, triglyceride enrichment in HDL fraction was not observed with *hApoB*^{tg}/*BATless* mice.

Table 2. Plasma chemistry of *hApoB*^{tg} and *hApoB*^{tg}/*BATless* mice fed a high-fat–high-cholesterol diet until 24 weeks of age

	Gender	*n*	TC (mg·dL^{-1})	HDL-C (mg·dL^{-1})	TG (mg·dL^{-1})	FFA (mEq·L^{-1})	Insulin (ng·mL^{-1})	LCAT activity (OD_{390}/OD_{470})	PLTP activity (fluorescence)
hApoB^{tg}	Male	12	391±114	110±26	156±63	0.32±0.08	0.5±0.2	2.2±0.3	9947±992
hApoB^{tg}/*BATless*	Male	11	494±171	122±33	205±100	0.30±0.11	5.6±3.7*	2.3±0.4	10901±1196*
hApoB^{tg}	Female	11	328±99	93±27	122±42	0.38±0.14	0.4±0.1	2.2±0.6	8043±1117
hApoB^{tg}/*BATless*	Female	7	322±73	66±30	155±36	0.35±0.23	1.6±1.9*	2.0±0.3	8016±1797

Note: Values represent means ± SD. TC, total cholesterol; HDL-C, HDL cholesterol; TG, triglyceride; FFA, free fatty acids; LCAT, lecithin:cholesterol acyltransferase; PLTP, phospholipid transfer protein.

*Significantly different from *hApoB*^{tg} within the same gender ($p < 0.05$).

Fasting plasma insulin levels of *hApoB*^{tg}/*BATless* obese mice were several-fold higher than *hApoB*^{tg} control mice for both genders of animals (Table 2). Body weight of mice was significantly correlated with fasting insulin levels ($r = 0.54$, $p = 0.0003$), suggesting fasting plasma insulin increases with body weight in these mice.

As important factors for HDL maturation and remodeling, plasma LCAT and PLTP activities were measured. Plasma LCAT activity did not significantly differ between genotypes of each gender, but plasma PLTP activity was significantly higher only in male *hApoB*^{tg}/*BATless* obese mice compared with male control mice (Table 2).

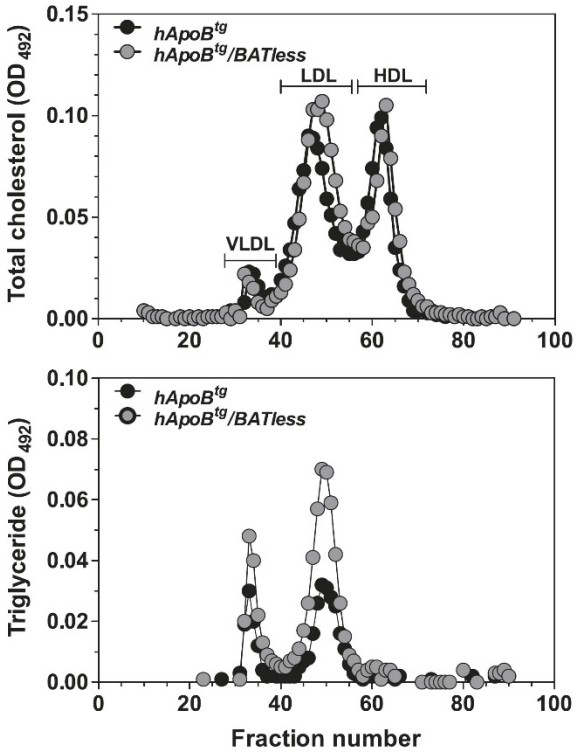

Figure 2. Plasma lipoprotein distribution by fast performance liquid chromatography (FPLC) analysis. Pooled plasma samples (200 μL) from 4 male mice of each genotype fed a high-fat–high-cholesterol diet were fractionated by 2 Superpose 6 columns in series at a flow rate of 0.5 · mL·min^{-1}. Each fraction (50 μL) was enzymatically analyzed for total cholesterol and triglyceride and peaks for very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were identified. A representative FPLC profile from 2 separate analyses is shown.

Liver lipids and gene analysis

Obesity-induced insulin resistance is commonly linked with steatosis in the liver. Liver weights of male and female *hApoB^{tg}/BATless* mice on an HF–HC diet were ~2-fold more than those of *hApoB^{tg}* control mice with significantly increased triglyceride, esterified cholesterol, and free cholesterol content (Table 3).

Table 3. Liver lipid contents of *hApoB*^{tg} and *hApoB*^{tg}/*BATless* mice fed a high-fat–high-cholesterol diet until 24 weeks of age

	Gender	*n*	Liver weight (G)	TG (mg·g^{-1} wet weight)	FC (mg·g^{-1} wet weight)	EC (mg·g^{-1} wet weight)	PL (mg·g^{-1} wet weight)
hApoB^{tg}	Male	12	2.3±0.9	120±90	2.5±0.5	7.3±4.4	16.9±2.7
hApoB^{tg}/*BATless*	Male	11	5.5±1.3*	272±60*	3.4±0.8*	20.0±5.0*	13.1±2.0*
hApoB^{tg}	Female	11	1.7±0.6	90±46	2.4±0.2	9.5±1.8	17.9±1.8
hApoB^{tg}/*BATless*	Female	7	3.4±0.9*	161±95*	2.6±0.3*	11.9±5.5	15.6±2.9*

Note: Values represent means ± SD. *hApoB*^{tg}, human apolipoprotein B$_{100}$ transgenic; BATless, brown adipose tissue deficient; TG, triglyceride; FC, free cholesterol; EC, esterified cholesterol; PL, phospholipid.
*Significantly different from *hApoB*^{tg} within the same gender ($p < 0.05$).

qPCR analysis was performed to measure mRNA abundance of lipogenic and HDL-modulating genes in mice fed an HF–HC diet (Table 4). In male *hApoB*^{tg}/*BATless* obese mice, a lipogenic gene stearoyl coA desaturease 1 (SCD-1) ($p < 0.05$) showed an increase, whereas acyl-CoA oxidase (AOX), an enzyme involving fatty acid oxidation in peroxisomes, was significantly lowered ($p < 0.05$). Fatty acid synthase (FAS) showed a trend toward increase in the male *hApoB*^{tg}/*BATless* obese mice ($p = 0.08$). Although we did not detect statistically significant differences, a similar trend was found for SCD-1 and AOX mRNA levels in the livers of female mice. Despite no differences in plasma LCAT activity, hepatic LCAT mRNA levels were significantly lower in *hApoB*^{tg}/*BATless* mice than control mice in both genders. Expression of HL ($p = 0.059$) and SR-BI ($p < 0.05$) was lowered in *hApoB*^{tg}/*BATless* male obese mice compared with control animals, and a similar trend was observed with female mice. A trend toward an increase was observed with PLTP mRNA levels in the livers of male ($p = 0.085$) and female ($p = 0.058$) *hApoB*^{tg}/*BATless* mice.

Table 4. Hepatic gene expression of *hApoB*^{tg} and *hApoB*^{tg}/*BATless* mice fed a high-fat–high-cholesterol diet at 24 weeks

	Male		Female	
Enzyme	*hApoB*^{tg}	*hApoB*^{tg}/*BATless*	*hApoB*^{tg}	*hApoB*^{tg}/*BATless*
FAS	0.42±0.31	0.81±0.65	1.07±0.50	0.97±0.76
AOX	1.25±0.36	0.89±0.41*	1.28±0.51	1.00±0.18
LDLR	1.02±0.45	1.06±0.59	0.95±0.29	0.62±0.23*
HMGR	1.25±0.53	1.29±1.05	0.74±0.25	0.71±0.42
SCD-1	0.56±0.44	0.95±0.39*	0.71±0.48	1.12±0.48
SREBP-1c	1.00±0.20	0.80±0.26	0.80±0.43	0.68±0.20
SREBP-2	0.89±0.31	0.88±0.32	1.12±0.38	0.80±0.13
ChREBP	0.89±0.31	0.47±0.27*	0.99±0.37	0.94±0.62
LCAT	1.38±0.39	0.68±0.28*	1.04±0.56	0.47±0.13*
PLTP	1.18±0.51	1.67±0.73	1.49±0.53	2.62±1.74
HL	1.28±0.46	0.91±0.43	0.91±0.37	0.50±0.21*
apoA-I	0.79±0.31	0.62±0.27	0.76±0.51	0.53±0.14
SR-BI	0.99±0.52	0.56±0.30*	0.94±0.66	0.50±0.19

Note: Values represent means ± SD. Means were analyzed using unpaired *t* test within the same gender. $n = 7–12$.
*Significantly different from *hApoB*^{tg} within the same gender ($p < 0.05$).

As hepatic ABCA1 is the primary factor for HDL formation, hepatic expression of ABCA1 was measured in mice. No significant difference was detected in the hepatic ABCA1 mRNA abundance between male *hApoB*^{tg} and *hApoB*^{tg}/*BATless* mice (Fig. 3). However, Western blot analysis showed that hepatic ABCA1 protein was significantly lower by ~35% in *hApoB*^{tg}/*BATless* mice compared with *hApoB*^{tg} mice. No significant differences in ABCA1 mRNA and protein levels were observed in both genotypes of female mice (data not shown).

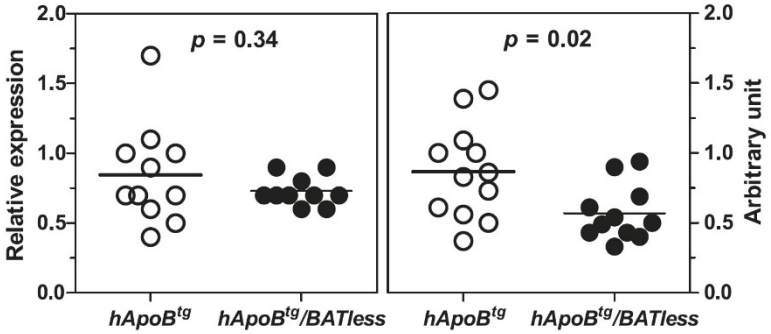

Figure 3. Hepatic mRNA and protein levels of ABCA1. Liver samples of male *hApoB*^{tg} and *hApoB*^{tg}/*BATless* mice fed an HF–HC diet were analyzed by qPCR for mRNA abundance (A) and by Western blot for protein levels (B). Data were analyzed using unpaired *t* test. Dots represent each animal and horizontal lines are for means.

Discussion

Obesity and obesity-associated metabolic diseases are currently prominent health problems in the United States and westernized countries. Among various complications of obesity, accelerated CHD has emerged as a leading cause of morbidity and mortality with the incidence being 2- to 8-fold higher for the subjects with insulin resistance and type 2 diabetes (Grundy et al. 2002; Howard et al. 2002; Wendt et al. 2002). Mechanistic understanding for this phenomenon, however, is very limited. We utilized *hApoB*^{tg}/*BATless* mice on an HF–HC diet to investigate alterations in factors affecting HDL metabolism in obesity-induced hyperinsulinemia.

While humans carry the majority of their circulating cholesterol in LDL, mice carry the majority of their cholesterol in HDL (Salmon and Hems 1973). However, mice with human *apoB*$_{100}$ transgene have cholesterol accumulation in the LDL fraction with similar lipoprotein distribution to that of humans (Linton et al. 1993). Common obesity or type 2 diabetes mouse models, such as *ob*/*ob*, *db*/*db*, *fat*/*fat* and *tub*/*tub* mice, exhibit elevated plasma HDL-C levels along with weight gain in contrast to obese humans (Nishina et al. 1994). *BATless* mice generated using a BAT-specific UCP-1 promoter to drive the expression of diphtheria toxin A become obese on a high-fat diet consequent to loss of BAT (Lowell et al. 1993). To generate a mouse model of obesity-induced dyslipidemia, *hApoB*^{tg} mice were crossed with *BATless* mice. The resulting *hApoB*^{tg}/*BATless* mice on an HF–HC diet have previously been

shown to be susceptible to diet-induced obesity, hypercholesterolemia, hypertriglyceridemia, and hyperinsulinemia (Siri et al. 2001). In addition, the plasma lipid profile of male *hApoB*^{tg}/*BATless* mice on an HF–HC diet has been shown to be similar to that of obese and diabetic humans in regards to lipoprotein distribution and size with reduced plasma HDL-C levels compared with *hApoB*^{tg} control mice (Siri et al. 2001). Our initial intention was to utilize this mouse model of obesity-induced insulin resistance and dyslipidemia for elucidation of altered HDL metabolism. Male *hApoB*$_{tg}$/*BATless* mice on an HF–HC diet indeed developed hyperinsulinemia with weight gain compared with control mice. However, inconsistent with the previous report by Siri et al. (2001) that plasma HDL-C levels were reduced in male *hApoB*^{tg}/*BATless* mice, we did not observe the reduction in our mice. The reason for this discrepancy is not known. However, we speculate that it may be related to difference in the genetic background of the mice. *hApoB*^{tg} mice used in the study of Siri et al. (2001) had C57BL/6J background whereas ours had a mixed background. An additional noticeable difference between these 2 studies is plasma triglyceride concentrations. Compared with male *hApoB*^{tg} control mice, plasma triglyceride levels were significantly increased in the study by Siri et al. (2001) but there was no significance difference in our mice (p = 0.18). It is possible that because of the disparate genetic background of *hApoB*^{tg} mice, apoB$_{100}$-containing lipoprotein metabolism could differ between 2 studies. Particularly, it is noteworthy that a reduction in plasma HDL-C levels was observed only in the presence of significantly elevated plasma triglyceride levels in *hApoB*^{tg}/*BATless* mice shown in Siri et al.'s study given an inverse relationship between plasma HDL-C and triglyceride concentrations. In the obese male *hApoB*^{tg}/*BATless* mice, hepatic SR-BI mRNA levels were significantly lowered. As SR-BI in the liver provides a major HDL particle clearance pathway, the reduction may contribute to absence of HDL-C lowering in our obese mice. However, as hepatic SR-BI expression was not measured in Siri et al.'s study, it remains speculative.

Absence of functional ABCA1 reduced plasma HDL-C concentrations by ~95% in humans and mice (Oram 2000; Brunham et al. 2006), suggesting ABCA1 is quantitatively the most important factor in maintaining plasma HDL-C levels. In particular, as ABCA1 in the liver accounts for ~80% of plasma HDL-C levels (Timmins et al. 2005), hepatic expression of ABCA1 was evaluated to gain an insight into a potential contribution of hepatic ABCA1 to the HDL metabolism in obesity. There was no significant difference in hepatic ABCA1 mRNA transcript levels between male *hApoB*^{tg} control and *hApoB*^{tg}/*BATless* obese mice. However, ABCA1 protein levels in the livers of male *hApoB*^{tg}/*BATless* mice were significantly reduced by ~35% compared with those of *hApoB*^{tg} control mice. This is the first study, to our knowledge, to demonstrate a post-transcriptional repression of hepatic ABCA1 expression in vivo in obesity-induced insulin resistance. Studies have shown that unsaturated fatty acids lower ABCA1 protein levels in macrophages without altering mRNA levels by facilitating ABCA1 protein degradation via the activation of phospholipase D2/protein kinase C δ pathway (Wang and Oram 2002, 2005, 2007). Therefore, it can be postulated that increased fat contents in the livers of *hApoB*^{tg}/*BATless* obese mice could facilitate ABCA1 protein degradation. It should be noted that in spite of significant reduction in hepatic ABCA1 protein in *hApoB*^{tg}/*BATless* mice by ~35% compared with *hApoB*^{tg} mice, plasma HDL-C concentrations were not different between 2 genotypes. Reduction in hepatic ABCA1 by ~45%–50% either by heterozygous gene deletion (Lee et al. 2005) or

using small interfering RNA (Ragozin et al. 2005) resulted in ~45%–50% decrease in plasma HDL-C concentrations. Absence of altered plasma HDL-C levels in our *hApoB*^{tg}/*BATless* mice even with ~35% reduction in hepatic ABCA1 protein could indicate that the extent of reduction may not be enough to alter plasma HDL-C levels in a state of obesity-induced insulin resistance. Alternatively, triglyceride enrichment in HDL particles, which was absent in our study, could be a primary factor to lower plasma HDL-C levels in obesity.

Factors that could modulate HDL formation and catabolism, including apoA-I, SR-BI, HL, LCAT, and PLTP, were investigated for their participation in HDL metabolism in obesity-related insulin resistance. ApoA-I accounts for ~70% of total proteins in HDL particles (Lewis and Rader 2005) and its absence in mice leads to extremely low levels of plasma HDL-C levels along with increased atherosclerosis (Lewis and Rader 2005; Moore et al. 2005). Hepatic SR-BI mediates the selective uptake of cholesteryl ester from the HDL particles and thus increased expression of SR-BI in mice increases HDL uptake into the liver, lowering plasma HDL-C levels (Kozarsky et al. 1997). HL may affect rates of apoA-I catabolism in HDL by increasing the hydrolysis of HDL triglycerides (Rader 2006). Two plasma enzymes, LCAT and PLTP, are responsible for maturation of HDL particles. The formation of cholesteryl esters in the core of HDL particles from free cholesterol on the lipoprotein surface is mediated by LCAT (Rader 2006). Decreased LCAT has been shown to increase catabolism of apoA-I and apoA-II, resulting in lower plasma levels of HDL-C (Rader et al. 1994). As a mediator of phospholipid transfer from apoB-containing lipoproteins to HDL particles, increased PLTP is associated with increased plasma HDL-C levels, whereas reduced PLTP leads to decreased plasma HDL-C concentrations (Jiang et al. 1996, 1999). In our study, we observed that the hepatic expression of HDL-modulating receptor and enzymes, such as SR-BI, HL, LCAT and PLTP, was either significantly altered or showed at least a trend of difference between *hApoB*^{tg} control and *hApoB*^{tg}/*BATless* obese mice. However, plasma HDL-C levels were not significantly different between 2 genotypes of mice. It would be possible that their protein levels and (or) activity might not be altered as shown in plasma LCAT activity. It can be alternatively postulated that the factors minimally contribute to plasma HDL-C levels in *hApoB*^{tg}/*BATless* obese mice.

Etiology for perturbed HDL metabolism in obesity and obesity-related abnormal metabolic conditions is not clear. In obesity-induced hyperinsulinemia, altered expression of HDL-modulating factors, hepatic ABCA1 in particular, was not able to lower plasma HDL-C concentrations in our study. Plasma HDL-C levels were lowered concomitantly with elevated plasma triglycerides in *hApoB*^{tg}/*BATless* obese mice (Siri et al. 2001). However, we observed that the mice did not exhibit a reduction in plasma HDL-C when plasma triglyceride levels remained unaltered. It is likely that triglyceride enrichment in HDL particles and resulting hypercatabolism of the particles could be a major contributor to perturbed HDL metabolism in obesity and obesity-related hyperinsulinemia. Fat overflow in obesity potentially outweighs the effects of other HDL modifying factors that otherwise have a large impact on HDL metabolism.

Acknowledgments – J.-Y. Lee designed research; S. J. Ehlers, S. M. Larson, H. E. Rasmussen, and Y.-K. Park conducted research; S. J. Ehlers and J.-Y. Lee analyzed data and wrote the paper. All authors read and approved the final manuscript. This work was supported by National Science Foundation–EPSCoR grant EPS-0346476 to J.-Y. Lee.

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