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Plasma Lipid Profiles of Transgenic mice expressing the *Human* ApoB100XCETP are altered differentially by Diets enriched with defined Fatty Acids

WAEL HATAHET[1], ABDUL SOOFI[2], OYONUMO E. NTEKIM[3] and THOMAS V. FUNGWE[4]

[1]Faculty of Pharmacy , Arab International University, Damascus, Syria.
[2]Department of Surgery, University of Michigan, Ann Arbor, Michigan.
[3]College of Nursing and Allied Health Sciences,
Department of Nutritional Sciences, Howard University, Washington, DC 20059
[4]College of Nursing and Allied Health Sciences,
Department of Nutritional Sciences , Howard University, Washington, DC 20059.

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ABSTRACT

Dietary fat is known to modulate plasma lipid profiles. Synthesis of high density lipoproteins (HDL), which has protective effects on vascular disease is also influenced by dietary fats, but the mechanisms are unclear. The *h*apoB100XCETP transgenic mouse was used to investigate the effects of fatty acids on the metabolism of plasma lipoproteins, including the pathway leading to synthesis HDL. Male transgenic mice were fed with diets formulated to provide TG (33% energy) as tripalmitin (TP), triolein (TO), tristearin (TS) or equicaloric substitution of fat with carbohydrate (sucrose) for 4 weeks. Analysis of plasma profile showed that HDL-cholesterol were 53.7+14; 64.6+8.6; 50.2+3.3; 47.0+9.2 and 45.2+4.9 mg/dL for control, oleate, palmitate, stearate and sucrose based diets, respectively. LDL-cholesterol levels were 51.7+7.0; 23.1+7.0; 38.9+2.2; 75.1+1.8 and 46.8.1.0 mg/dl, for control, TO, TP, TS and sucrose, respectively. Hepatic Lecithin-cholesterol acyltransferase (LCAT) protein levels increased by 2-fold in mice fed TS or TO diets, compared to TP, while sucrose had no effect. The scavenger receptor class B type I (SR-B1) which plays an important role in meditating the uptake of HDL-derived cholesterol and cholesteryl ester in the liver and steroidogenic tissues increased in livers of animals fed TP and TO, while TS and sucrose did not have a similar effects. These results suggests that fatty acids can uniquely impact HDL, in addition, the ApoB100XCETP mouse is a useful model for the evaluation of how dietary components affect the risk of developing atherosclerosis and heart disease.

Key words: ApoB100, CETP, TC, HDL-C, LDL-C & TG, Transgenic.

INTRODUCTION

The main cause of cardiovascular disease (CVD) is atherosclerosis, characterized by the combination of chronic inflammation and/or hyperlipidemia. CVD is believed to be the leading cause of death in the Western world and its prevalence is increasing in Eastern Europe and developing countries (1). High density lipoprotein-cholesterol (HDL-C) protects against vascular disease by accepting free cholesterol from macrophage foam cells in the artery wall in a process, often described as reverse cholesterol transport (RCT). Reverse cholesterol transport describes the efflux of excess cellular cholesterol from peripheral tissue and its return to the liver for excretion in the bile and ultimately the feces during digestion of dietary fat. It is believed to be a critical mechanism by which HDL exerts a protective effect on the development of atherosclerosis or vascular diseases[1, 2]. In the proposed paradigm, cholesterol efflux from arterial macrophages to extracellular HDL-based acceptors occurs through the action of transporters such as ATP binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1). After efflux to HDL, cholesterol can be esterified

in the plasma by the enzyme lecithin: cholesterol acyltransferase (LCAT), and is ultimately transported from HDL to the liver, either directly via the scavenger receptor BI (SR-BI) or after transfer to apoB-containing lipoproteins by the cholesteryl ester transfer protein (CETP). The physiological activator of LCAT is apolipoprotein A-I (apoA-I), the major HDL protein. However, cholesterol removal is compromised if the activity of apoA-I is compromised such as exposure to reactive intermediates[3-5].

Abnormalities in HDL metabolism due to elevation in plasma TG have been reported, leading to suggestions that TG may inhibit cholesterol esterification and the binding of Lecithin-cholesterol acyltransferase (LCAT) to HDL[6]. Fatty acids or TG can interfere in any of the stated pathways, and can directly or indirectly mediate activities, which result in the modulation of HDL. Our previous work suggested that oleic acid (OA) rich diets exerted a positive effect on the metabolism of plasma HDL in comparison to other fatty acids. We have previously demonstrated that dietary components such as fatty acids play key roles in the regulation of this pathway. In addition, studies demonstrated that monounsaturated fatty acids increased the expression of LCAT and SR-B1[7].

Murine transgenic models expressing human genes involved in lipoprotein metabolism have increasingly served as small mammalian models, where the spectrum of both normal and pathologic human serum lipid profiles can be simulated, and in several instances demonstrated the formation of atherosclerotic lesions[8]. In the present study, the effect of fatty acids on the expression of plasma lipoproteins in general, and HDL in particular was evaluated in a transgenic animal model with a humanized lipid profile.

MATERIALS AND METHODS

Semi-synthetic fats [triolein (TO), tripalmitin (TP), tristearin (TS)] used to modify the diets were a generous donation by ABITEC Corporation (Columbus, OH). LCAT cDNA was generously provided by Dr. R. Taramelli of Milan, Italy. The SR-B1 cDNA was a gift from Dr. Alan Tall. The antisense probe template for glyceraldehyde-3 phosphate dehydrogenase (GAPDH) was obtained from Ambion (Austin, TX) and was used to correct for RNA loading in addition to normalizing LCAT mRNA abundance. The procedure for labeling probes and measurement of LCAT mRNA abundance were previously reported (7, 9, and 10). All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Mouse Model

The double transgenic male mice expressing both human CETP and apoB100 were obtained from Taconic Labs (Germantown, NY) and bred to obtain sufficient animals to conduct this study. Tail biopsy for DNA analysis and genotyping were carried out when the mice were 13 days old, followed by immersion of the tail in ice cold ethanol for 10 seconds to minimize pain. These mice are hemizygous for both transgenes and are on a mixed (C57BL/6xSJL) background. In this animal, the LDL-cholesterol is increased by three to four fold and the HDL-cholesterol is reduced approximately by 60% as compared to non-transgenic control mice. The percentages of the total cholesterol within the HDL, LDL, and VLDL fractions of the *h*ApoB100XCETP animals are approximately 30%, 65%, and 5% respectively, similar to the distribution of cholesterol in the plasma of normolipidemic human profile [non-HDL to HDL of 2:1. The non-transgenic mice have a non-HDL to HDL ratio of 1:4[11].

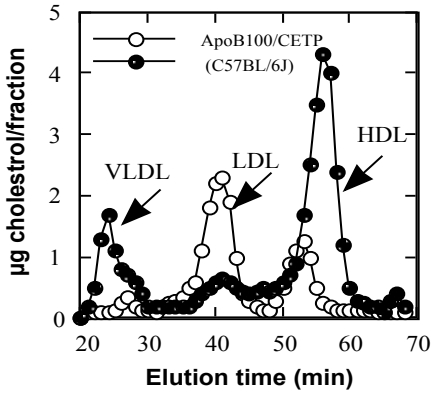
At 6 weeks of age, the mice (18 to 20 g. wt.) were placed on diet in which greater than 90% of the total fatty acids were a single fatty acid species. The control diet was a commercially available mouse chow to which the synthetic fats were added to provide 30% total calories from fat. The synthetic fats consisted of triolein (TO, oleic acid), tripalmitin (TP, palmitic acid), and tristearin (TS, stearic acid). One diet was formulated to provide the same amount of calories from sucrose (Su). Concomitant with the diet shift from normal laboratory chow to the defined fatty acid one, mice were randomly divided into 5 experimental groups according to previous treatments and fed the respective diets for 7 weeks. All the protocols for animal use were approved by Wayne State University Institutional Animal Investigation Committees and the study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services: National Research Council, 1985).

Serum Lipoprotein Analysis

Lipoprotein cholesterol distribution was determined by agarose gel electrophoresis ([Helena laboratory, Beaumont, TX], [12, 13]. This method was compared to the Fast protein liquid chromatography (FPLC) technique and the results were found to be greater than 95% correlated. Total cholesterol was quantified enzymatically (Sigma) from the individual sera as well as from 100-ul aliquots of the FPLC fractions. The relative amount of cholesterol within each peak was determined by area quantitation under the curve using the appropriate baseline modifications from the FPLC cholesterol tracings. Serum triglyceride levels were measured by enzymatic assay (Sigma). Lipoprotein cholesterol distribution was determined by agarose electrophoresis (Helena Laboratory, Beaumont, TX)[14, 15].

Immuno-analyses of hepatic scavenger receptor class B, type 1 receptor protein

Liver SR-BI receptor protein mass determination was carried out as reported previously[7]. Twenty five µg of solubilized liver membrane protein was subjected to protein electrophoresis using 4–12% pre-cast polyacrylamide gradient gels under non-reducing conditions (Novex, San Diego, CA). Proteins were transferred onto nitrocellulose membranes and incubated overnight with a 1:2000 dilution of mouse SR-B1 antibody (Novus Biologicals, Littleton, CO) and visualized by chemiluminescence anti rabbit antibody (developed in-house) and visualized by chemiluminescence detection (Alpha Innotech, San Leandro, CA). Quantification of the



*Note reduction in the HDL peak for apoB100xCETP

Fig. 1: FPLC cholesterol elution profile of transgenic for the human apoB100xCETP mice and control (wildtype C57BL/6) mice, indicating the relative positions of VLDL LDL and HDL

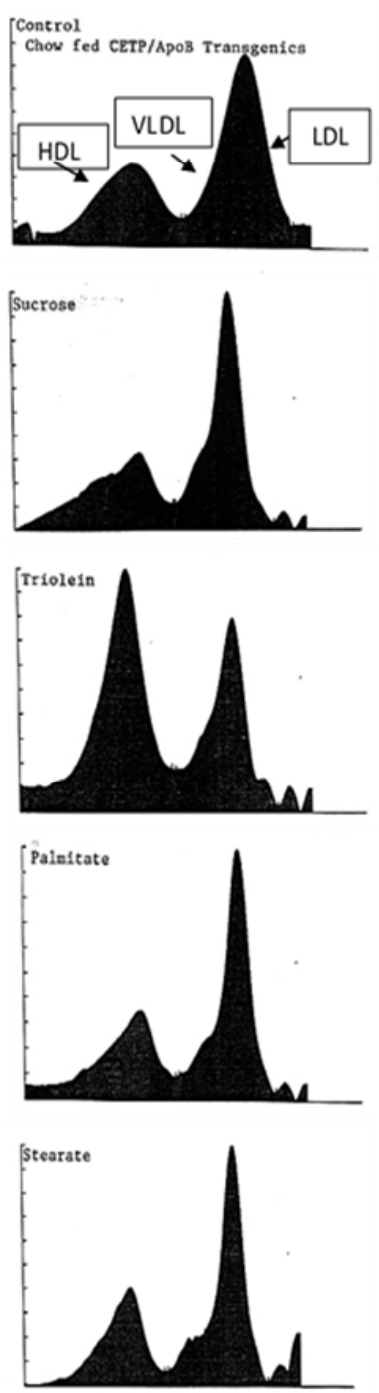


Fig. 2: Representative lipoprotein-cholesterol profile of male hApoB100xCETP transgenic mice fed different fatty acids

Cholesterol Fractions (%)		MG/DL
HDL	39.3	43.5
Lpa	0.3	0.7
VLDL	15.6	16.6
LDL	44.8	47.7
Total Chol	106.5	

Cholesterol Fractions (%)		MG/DL
HDL	36.3	38.1
Lpa	0.4	0.4
VLDL	10.7	11.3
LDL	52.6	55.2
Total Chol	105.0	

Cholesterol Fractions (%)		MG/DL
HDL	38.4	43.5
Lpa	0.6	0.7
VLDL	8.7	9.9
LDL	52.3	59.3
Total Chol	100.5	

Cholesterol Fractions (%)		MG/DL
HDL	39.8	47.5
Lpa	0.6	0.7
VLDL	13.0	15.5
LDL	46.6	55.6
Total Chol	119.3	

Cholesterol Fractions (%)		MG/DL
HDL	61.5	43.5
Lpa	0.3	0.3
VLDL	13.1	16.0
LDL	24.8	30.3
Total Chol	122.2	

detection (Alpha Innotech, San Leandro, CA). Quantification of the specific luminescent protein bands was performed with Image-Quant software (Molecular Dynamics, Sunnyvale, CA).

Immuno-analyses of hepatic Lecithin: cholesterol acyltransferase

Mouse liver was prepared by homogenizing 0.2 g of liver in 5mL of [150 mmol/L NaCl, 1 mmol/L $CaCl_2$, 10 mmol/L Tris- HCl (pH 7.5); and the protease inhibitors, pH 7.5] (Lyses buffer). The supernatant was prepared by centrifuging the homogenate at 8000 x *g* for 15 min. Supernatant protein concentrations were determined by the Bio-Rad dye-binding protein assay.

For LCAT protein immunoblotting, 25 µg of solubilized liver protein was subjected to protein electrophoresis using 12% polyacrylamide gels under nonreducing conditions. Proteins were transferred onto nitrocellulose membranes and incubated overnight with a 1:2000 dilution of rLCAT

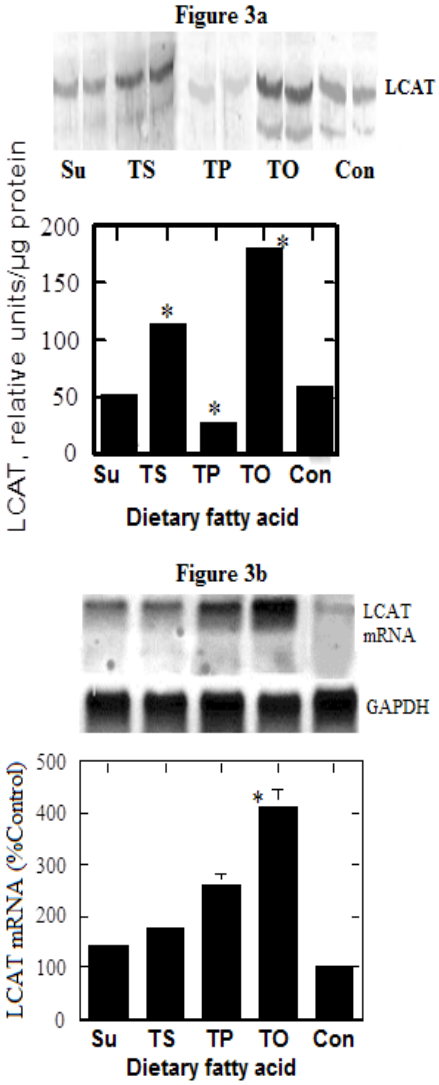


Fig. 3: Induction and synthesis of LCAT in liver tissues from male *h*apoB100xCETP transgenic mice fed sucrose (Su), tristearin (TS), tripalmitin (TP), triolein (TO) or the control diet (Con) to which the fatty acids were added

specific luminescent protein bands was performed with Image-Quant software (Molecular Dynamics, Sunnyvale, CA).

Measurement of LCAT/SRr-B1 mRNA

Poly (A^+) RNA was isolated from the liver according to the Trizol reagent protocol (Invitrogen kit; Invitrogen, Carlsbad, CA). Northern blot analysis was performed to quantitate mRNA levels of LCAT. For each sample, 5 µg of RNA was electrophoresed through formaldehyde- 1.2% agarose gels and transferred to 20x SSC-equilibrated Hybond ECL nitrocellulose membranes. Membranes were hybridized after UV crosslinking and washed at a high stringency (65°C, 0.1x SSC). Northern blots were probed with labeled ([^{32}P]dCTP, specific activity > 3000 Ci/mmol) as previously described (9). The levels of LCAT/SR-B1 mRNA in liver samples were ~~was~~ normalized to glyceraldehyde phosphate dehydrogenase (GADPH) mRNA levels for each sample.

RESULTS

Characterization of the *h*ApoB100/CETP mouse

The lipid profile of the *h*ApoB100/CETP mouse was characterized by measuring its lipid profile (Figure 1) and compared ~~it~~ to the wild type, when fed a regular chow diet. As shown in Figure 3, the LDL-C of these mice is increased three to four fold and the HDL-cholesterol reduced by approximately 60% as compared to non-transgenic control mice. The percentages of the total cholesterol within the HDL, LDL, and VLDL fractions of the ApoB100xCETP animals were confirmed and found to be similar to the distribution of cholesterol in the plasma of normolipidemic humans (11). Thus, by expressing both human apoB and human CETP, the lipoprotein cholesterol distribution in the serum of a chow-fed mouse was transformed into one that resembles a human profile (non-HDL to HDL of 2:1).

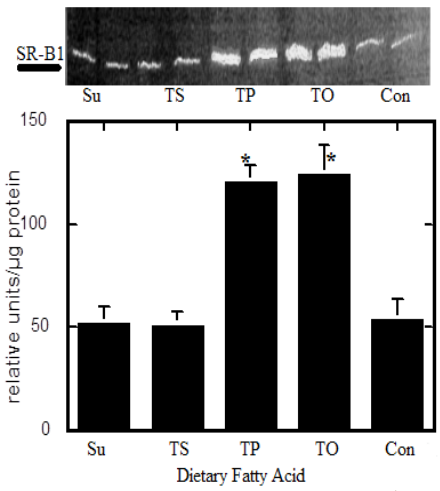


Fig. 4: Western blot of SR-B1 protein levels in liver of male human transgenic apoB100xCETP mouse fed diets containing defined fatty acids

Cholesterol profile as determined by agarose electrophoresis and confirmed by FPLC

The profiles shown (Figure 2) are representative of the distribution of cholesterol in the various lipoproteins from each dietary group. Total cholesterol did not differ between the groups. However, animals fed TO had significant elevation in HDL-C when compared to control. Tristearin fed animals on the other hand had higher, while triolein had lower, LDL-C when compared to control.

Body weights and serum lipids of mice fed the control diet and diets with different triglycerides

Final body weights of mice and relative liver weights did not statistically differ by diet or among groups. As shown in Table 1, serum LDL-C levels were significantly ($p<0.05$) lower in mice fed the triolein diet, while LDL-C levels were higher in mice fed tristearin compared to control group. As was observed in our previous rat study[7, 9], animals that were fed triolein had higher HDL-C content and reduced LDL-C levels. Although the distribution of cholesterol in HDL for the other treatments appeared similar, there was a tendency to have reduced HDL-C levels with sucrose and tristearin compared to tripalmitin. Reduced HDL-C with tripalmitin is not what is often seen in human. However, the decrease in HDL-C was also accompanied by reduced LCAT expression suggesting that tripalmitin had a negative impact on the esterification of cholesterol into HDL, in part by down-regulation of LCAT. The feeding of sucrose generated the highest TG concentration and the lowest HDL-C levels as expected, though not statistically different (Table 1). Additionally, there were differences seen in the level VLDL-C in agreement with numerous studies that FA also contribute to plasma cholesterol (other than HDL),

and contributes to the atherogenic process as well. The ratio of TC: HDL-C was higher than control in all the groups except for triolein, while LDL: HDL-C and TG: HDL-C ration were higher than control only in mice fed the tristearin and sucrose diets (P<0.05) (Table 2). Even though Apo A-I was higher in the triolein group when compared to the other diet groups, no difference was observed between the treatments for Apo A-II. In addition, hepatic lipase activity levels were not different among the groups (Table 2).

Expression of LCAT protein in hepatic tissue

The results showed that triolein increased LCAT protein levels. In addition, mice fed tristearin diets, had hepatic LCAT protein levels that were significantly higher compared to mice fed control diet (Figure 3). The elevated protein levels in the previous groups contrasted with a significant down regulation in the tripalmitin fed group. To determine if the increase in LCAT protein was a function of induction of the LCAT gene, we looked at the levels of messenger RNA in the liver. The mRNA levels correlated with the protein levels in the triolein group, however LCAT mRNA levels did not correlate with its protein levels for the tripalmitin treated animals.

Hepatic SR-B1 receptor

The hepatic scavenger receptor B1 protein concentration was increased nearly 2.5-fold in mice fed the triolein and tripalmitin diets compared to mice fed with control diet ($P < 0.05$) (Figure 4). The observed increase in SR-B1 protein levels following exposure to triolein and tripalmitin was accompanied by a similar fold induction of SR-B1 mRNA (Figure 5). No similar induction was observed with other tristearin or sucrose.

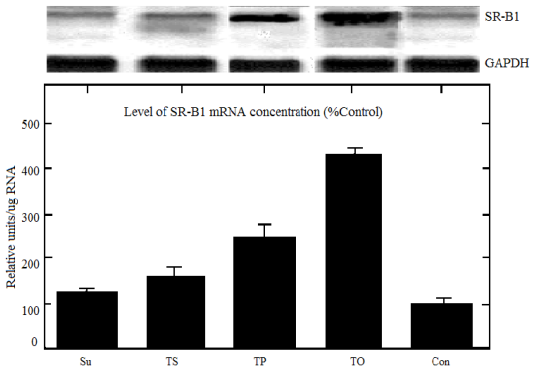


Fig. 5: Induction of mRNA and SR-B1 protein synthesis by fatty acids in liver tissues from male *h*apoB100xCETP transgenic mice

DISCUSSION

The main objective of this study was to obtain a better understanding of how DIETARY fatty acids influence HDL-C concentration using an animal model, genetically altered to incorporate human genes in the RCT pathway (human ApoB100xCETP transgenic mice with a lipid profile similar to humans). The use of this transgenic model permits a clinical evaluation of the changes at the hepatic LCAT and SR-B1 mRNA and protein levels and subsequent effects on blood lipids (TC, HDL-C and LDL-C). Low plasma concentrations of HDL-C and high VLDL/ LDLC are independent risk factors for the development of atherosclerosis (1). In addition, the ratio of VLDL/LDL to HDL is to a great extent affected by the cholesteryl CETP (50). When these animals are placed on an atherosclerotic diet, they display a significant decrease in HDL compared to wild animals. Using this approach, the effects of feeding diets enriched in different fatty acids can be evaluated. Initially, the animals were ~~was~~ characterized by measuring its lipid profile and comparing it to the wild type, when fed a regular chow diet. As shown in Figure 1, the LDL-cholesterol of these mice ~~is~~ increased three to four fold and the HDL-cholesterol decreased approximately 60% as compared to non-transgenic control mice. The distribution of total cholesterol within the HDL, LDL, and VLDL fractions of the *h*apoB100xCETP animals were similar to the distribution of cholesterol in the plasma of normolipidemic humans.

Contrary to the rat model (11), feeding these fatty acids in the genetically altered mouse did not affect total cholesterol. However, the different fatty acid treatments modulated HDL, LDL and VLDL-C differently. The triolein fed group, for example, had a significant increase in HDL-C that was accompanied by a significant decrease in LDL-C. The current observation that plasma HDL-C levels tend to increase when oleic acid is fed in the diet confirms our previous report (7), which might explain in part, observations in population studies illustrating the benefits of the Mediterranean diet that is known to be rich in olive oil (13, 14). Mice fed the tristearin diet had significant increase in plasma LDL,

VLDL-C and TG levels. This observation contradicts assertions that dietary stearic acid is neutral (14, 15) in its cholesterol raising effects. Similarly, our finding is not in agreement with the notion that palmitic acid increased total cholesterol and LDL cholesterol (16). As expected, the sucrose fed group had increased TG and VLDL-C concentration, with no changes in HDL or LDL cholesterol concentrations compared to control.

The increase in HDL-C levels observed in the triolein group was accompanied by an increase in Apo A-I concentration. Other dietary treatments failed to show any increase in Apo A-I levels. Epidemiological studies revealed an inverse relationship between coronary artery disease and the plasma Apo A-1 concentration (17, 18, 19). Thus, our results fits the general scheme of what has been described as the protective effect of diets rich in oleic acid. However, caution has to be exercised

Table 1: Serum Lipids, body weights and liver: body weight ratio of transgenic mice fed fats with defined fatty acids

Diet	Body wt.	Liver:Body wt.	TC	HDL-C	LDL-C	VLDL-C	TG
Control	30.2±0.8	0.05±0.01	113±10^{b}	54±14^{c}	52±7^{a}	7±3^{a}	131±9^{a}
Triolen	29.5±1.5	0.06±0.01	97±16^{b}	65±9^{b}	23±7^{b}	8±5^{a}	114±22^{b}
Tripalmitin	35.2±1.5	0.05±0.01	115±11^{b}	50±3^{a}	39±21^{a}	15±4^{b}	128±12^{b}
Tristearin	29.0±1.7	0.05±0.01	136±29^{b}	47±9^{a}	75±18^{c}	14±3^{b}	142±16^{c}
Sucrose	29.5±1.3	0.05±0.02	112±19^{b}	45±5^{a}	47±1^{a}	18±8^{b}	159±18^{c}

TC, HDL-C, LDL-C, VLDL-C and TG values are in mg/dL, body weight in g.

[#]Values are means ± SD, n = 8.

[abc]Means in a column without a common letter are different, $P<0.05$.

Table 2: Ratio of TC: HDL-C and TG: HDL-C, apoAI, apoAII and hepatic lipase activity levels of transgenic mice fed diets enriched with defined fatty acids or sucrose Plasma hepatic activity is reported in nmol/min/ml.

DIET (n=6)	TC:HDL-C	TG:HDL-C	Plasma HL actvity	ApoA-I	ApoA-II	ApoA-I: ApoA-II
Control	2.09^{b}	2.43^{a}	60±2	119±3	22±3	5.4
Triolein	1.49^{b}	1.77^{b}	65±5	135±10^{b}	29±6	4.7
Tripalmitin	2.28^{a}	2.55^{a}	69±4	113±9^{a}	21±2	5.3
Tristearin	2.89^{a}	3.03^{a}	67±3	111±6^{a}	23±7	4.8
Sucrose	2.48^{a}	3.51^{a}	57±6	106±7^{a}	20±4	5.3

Apo A-I and Apo A-II are reported in mg/100mg

[#]Values are means ± SD, n = 8.

[abc]Means in a column without a common letter are different, $P<0.05$.

when interpreting these data, since the increase in Apo A-1 might be accompanied by an increase in Apo A-II, leading to a decrease in Apo A-I/Apo A-II ratio. In fact, several studies in mice and in humans demonstrated an association between increased levels of apoA-II levels and increased susceptibility to atherosclerosis (20- 21), increased free fatty acid levels (20, 22) obesity, and increased metabolic syndrome (23, 24). Hedrick et al., (25), suggested that as the ratio of apoA-II to apoA-I increases, HDL particles become larger because of the inhibition of hepatic lipase (HL), leading to the loose of the anti-atherogenic properties of HDL. Taking this into consideration we measured Apo A-II and Apo A-I/ Apo A-II ration, and the results showed no statistical difference among the triolein group and the other including control group; thus, eliminating apoA-II as a confounding factor when evaluating the effects of fatty acid on RCT in particular and on the lipid profile in general.

The role of hepatic lipase (HL) in the metabolism of HDL is well established (26). HL acts on decreasing HDL core lipids, apparently making lipid-poor apoA-I available for dissociation or transfer to other particles (26-28). In addition, HL is thought to enhance hepatic HDL cholesterol uptake (29, 30). Thus, an increase in HDL-C level may be due to a decrease in hepatic lipase activity and vice versa. This notion is supported by Homanics et al. (31), who reported that HL-deficient mice have a mild dyslipidemia with increased cholesterol, phospholipid, and large HDL floating in the 1.02-1.04 g/ml density range. The results from this study showed no difference in hepatic lipase activity between the dietary treatments. This is contrary to reports by Privett and colleagues (32) using rats that were essential fatty acid deficient. In addition, their dietary treatment included ~~of~~ trans-fatty acids, which are more likely to have different metabolic pathways than fatty acids in the cis isoform.

Hepatic Scavenger receptor B1 is an important factor in modulating serum HDL levels. *In vivo* and *in vitro* studies have supported its participation in the selective uptake of HDL cholesterol (33-37) and involved in RCT. A 2-fold increase in SR-B1 protein was AB observed following exposure to triolein accompanied by a similar fold induction of SR-B1 mRNA. The amount of SR-B1 in liver protein extracts from TP fed mice where almost just as elevated as those on triolein, with corresponding increases in its mRNA. It is interesting to note that although induction of SR-B1 was observed in the mice fed both triolein and tripalmitin, no effect was observed in the tristearin fed or the sucrose fed group. Spady et al (38), reported that saturated fats tend to decrease SR-B1 expression, however tristearin and tripalmitin are both grouped as saturated fats, but both had varying effects on SR-B1 expression. In addition, the results showed that the length of the fatty acid is not the driving force behind its effect on SR-B1. The induction of SR-B1 in the triolein group make it harder to explain the high levels of HDL-C observed in this group, because higher levels of SR-BI corresponds to lower levels of circulating HDL-C (33-37). Studies using the SR-B1–null mice confirm that changes in SR-B1 activity affect the metabolism of cholesterol and when SR-B1 is overexpressed in hepatic tissue, there is a significant decrease in plasma HDL (39). These observations do not account for plasma HDL-C levels under conditions when the reverse cholesterol transport pathway (LCAT/CETP) that gives rise to plasma HDL-C is simultaneously upregulated with the SR-B1 receptor pathways. However, it can be speculated that because SR-B1 plays an important role in meditating the uptake of HDL-derived cholesterol and cholesteryl ester in the liver and steroidogenic tissues (40) the induction of SR-BI, a principal enzyme in the catabolic arm of reverse cholesterol transport, may be accompanied by a similar induction in the anabolic arm of the same pathway.

Lecithin-cholesterol acyltransferase plays a major role in RCT by esterifying free cholesterol (FC) in circulating lipoproteins to maintain a free cholesterol gradient between the peripheral cells and the HDL particle surface and, accordingly, to promote FC efflux from the cells (41). LCAT from rodents and primates has different phospholipid substrate fatty acyl specificities (42, 43), and they lack CETP responsible for the transfer of CE to other lipoproteins in humans. In the current study, *h*ApoB100xCETP mice fed the TO diet had elevated levels of hepatic LCAT mRNA, compared to mice fed the tripalmitin, tristearin, sucrose or the control diets. This suggests that oleic acid may impact the expression of LCAT at some level of transcription. The levels of hepatic

LCAT protein were similarly elevated in the triolein group, however a surprising increase was observed in hepatic LCAT mass in mice fed the tristearin diet. Despite this increase, HDL-C did not change. In fact, LDL-C, VLDL-C and TG were significantly higher in this group when compared to control. It is possible that the increase in cholesterol esterification by LCAT is offset by the increase in TG and VLDL-C. Similar to our previous observations (7) feeding tripalmitin was accompanied by a reduction in hepatic LCAT mRNA abundance and protein mass. In fact, LCAT protein in liver extracts from TP fed mice was almost 2-fold less than that of other FAs, including the control. It is not known if this effect occurs at the level of transcription. The levels of mRNA are thought to be poor predictors of protein expression, because mRNA levels can vary almost 20 times and still yield the same level of gene products (44, 45, 46).

Sucrose is an efficient triacylglycerol (VLDL)-elevating agent both in humans and in animal models (47). We used it as an endogenous control for raising triglyceride, thus comparisons were made between the effects of exogenous vs. endogenous fat on RCT. Feeding sucrose did not affect total cholesterol significantly; neither did it affect hepatic LCAT; however, TG and VLDL-C were significantly higher than control. TC: HDL-C ratio was higher in mice fed sucrose, suggesting that sucrose may have a lowering effect on HDL-C. Serum HDL originates from synthesis and secretion by the liver and via the catabolism of circulating VLDL (48, 49). Decreased levels of HDL in mice fed sucrose may be related to the decreased catabolism of VLDL, the precursor of plasma HDL, and not to the decreased LCAT production by the liver. Our data are unique in that sucrose increased serum TG but without any effect on LCAT, while it has been reported by other investigators that feeding sucrose up-regulate LCAT activity in mice and in monkeys (50, 51). This might be explained by differences in the experimental models used.

A review by Hausenloy and Yellon (53) suggested that low levels of high-density lipoprotein cholesterol (HDL-C; <1.03 mmol/l or <40 mg/dl) are an independent risk factor for CVD, and raising levels of HDL-C is a major treatment strategy for regressing atherosclerosis and enhancing CVD risk reduction. Accordingly, this can be achieved by both pharmacological and non-pharmacological lifestyle strategies that include dietary changes. In general, these studies agree with epidemiological data in humans suggesting that diets rich in monounsaturated fatty acids (MUFAs) do tend to lower the risk of coronary heart disease (12, 13). Thus, increasing the monounsaturated fatty acyl composition of plasma CE may be beneficial in preventing atherosclerosis development.

CONCLUSION

In conclusion, our observations which showed that palmitate may up-regulate SR-B1 (be it in a smaller magnitude) in opposite direction to LCAT is not only surprising, but is also new and may point to a novel pathway whereby palmitic acid may contribute to the reverse cholesterol transport. It may also explain why palmitate may not elevate LDL-C as expected of a saturated FA. Our lipid data suggest that FA may play a much more significant role in the modulation of HDL-C. However, different FA species can either improve or worsen the ratio of TC: HDL-C and TG:HDL-C, resulting in a profile that impacts the risk of atherosclerosis. The above findings make it possible to speculate that the level of TG induced by these fatty acids may have a role in the uptake or turnover rates of HDL and demonstrates the continuous call for FAs to be investigated individually rather than as groups. Finally, these results suggests that not only do fatty acids uniquely impact plasma lipid profile as shown in this model, the ApoB100XCETP mouse can be a useful model for the evaluation of how diet and dietary components affect the risk of developing atherosclerosis and heart disease.

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