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Dietary α-cyclodextrin lowers low-density lipoprotein cholesterol and alters plasma fatty acid profile in low-density lipoprotein receptor knockout mice on a high-fat diet

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Abstract

High dietary intake of saturated fat and cholesterol, and elevated low-density lipoprotein cholesterol levels are some of the modifiable risk factors for cardiovascular disease. α-Cyclodextrin (*a*-CD) when given orally has been shown in rats to increase fecal saturated fat excretion and to reduce blood total cholesterol levels in obese hypertriglyceridemic subjects with type 2 diabetes mellitus. In this study, the effects of dietary *a*-CD on lipid metabolism in low-density lipoprotein receptor knockout mice were investigated. Low-density lipoprotein receptor knockout mice were fed a "Western diet" (21% milk fat) with or without 2.1% of *a*-CD (10% of dietary fat content) for 14 weeks. At sacrifice, there was no difference in body weight; but significant decreases were observed in plasma cholesterol (15.3%), free cholesterol (20%), cholesterol esters (14%), and phospholipid (17.5%) levels in mice treated with α-CD compared with control mice. The decrease in total cholesterol was primarily in the proatherogenic apolipoprotein B–containing lipoprotein fractions, with no significant change in the high-density lipoprotein fraction. Furthermore, α-CD improved the blood fatty acid profile, reducing the saturated fatty acids (4.5%) and *trans*-isomers (11%) while increasing (2.5%) unsaturated fatty acids. In summary, the addition of α-CD improved the lipid profile by lowering proatherogenic lipoproteins and *trans*-fatty acids and by decreasing the ratio of saturated and *trans*-fatty acids to polyunsaturated fatty acids (−5.8%), thus suggesting that it may be useful as a dietary supplement for reducing cardiovascular disease.
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1. Introduction

Cardiovascular disease (CVD) is the number one cause of death in the United States, and lifestyle modifications are recommended for all patients at risk for CVD. The major modifiable risk factors for the CVD include elevated low-density lipoprotein (LDL) cholesterol, decreased high-density lipoprotein cholesterol (HDL-C), obesity, diabetes, inactivity, cigarette smoking, and a poor diet low in soluble fiber and high in saturated and *trans*-fats as well as cholesterol [1,2]. To reduce the risk of CVD, it is recommended that individuals should reduce their intake of saturated and *trans*-fats, increase dietary soluble fiber intakes, and increase the intake of omega-3 fats or fatty fish [3].

Soluble dietary fibers like psyllium and pectin have been shown to reduce the absorption of dietary fat and cholesterol, thus reducing blood levels of cholesterol and the risk of CVD [4]. α-Cyclodextrin (α-CD) is a soluble dietary fiber derived from cornstarch that is nonabsorbable. It is a polymer of 6 glucose units in a cyclic ring structure with the polar hydroxyl groups facing outward [5]. The core of the ring is hydrophobic and can bind various hydrophobic compounds, including free fatty acids. The World Health Organization has established an acceptable daily intake of "not specified," and α-CD has been granted the Generally Recognized As Safe status by the United States Department of Agriculture. α-Cyclodextrin (trade name FBCx; ArtJen Complexus

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Holdings Corp, Windsor, ON, Canada) is currently available as a dietary supplement. It has been shown to reduce weight gain in Wistar rats fed an obesity-promoting high-fat diet [6]. It also reduced blood triglyceride (TG) and leptin levels and improved the calculated insulin sensitivity in these rats, as well as showed a tendency to reduce blood total cholesterol (TC) and insulin levels [6]. In a double blind, placebo-controlled clinical trial with obese type 2 diabetes mellitus patients, those in the α-CD–treated group were able to maintain their body weight and had increased adiponectin levels, whereas patients in the placebo group gained weight [7]. Those patients who began the study with hypertriglyceridemia also had significant reductions in their TC levels. Hence, α-CD may be considered to have health benefits in obese patients with type 2 diabetes mellitus.

In the current study, we investigate the impact of α-CD feeding on the plasma lipid profile of a common mouse model of atherosclerosis [8]. Mice deficient of the LDL receptor (LDLr-KO mice) develop dyslipidemia due to impaired clearance of chylomycron remnants and hepatic proatherogenic lipoproteins, such as very low-density lipoproteins (VLDL), LDL, and intermediate-density lipoprotein (IDL) [9]. The LDLr-KO mice were fed a high-fat/high-cholesterol–containing "Western" (21% fat, 0.2% cholesterol, wt/wt) diet with or without 2.1% α-CD for 14 weeks. A significant reduction in the level of proatherogenic lipoproteins and an improvement in the fatty acid profile were observed with α-CD treatment, suggesting that supplementation with α-CD may be useful for minimizing the negative impact of high-fat diets on serum lipids.

2. Methods and materials

2.1. Animals

Twenty female LDLr-KO mice on a C57BL/6 background, 12 weeks old at the beginning of the study, were purchased from Jackson Laboratory (Bar Harbor, ME). They were housed in polycarbonate hanging cages with 5 mice per cage. All mice were fed the rodent diet NIH31 (Zeigler Bros, Gardner, PA) and watered ad libitum before the beginning of the study. All animals were treated according to *The Guide for the Care and Use of Experimental Animals*; and the experimental protocol was approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, National Institutes of Health.

2.2. Diets

The baseline diet (NIH31) had the following composition: 5% fiber, 18% protein, and 4% fat with energy content of 3.97 kcal/g. Mice were randomized to either the control diet (Harlan Teklad Western Diet TD.88137, Madison, WI) or a modified diet with 21 g/kg of α-CD (10% of dietary fat content, wt/wt) replacing an equal amount of cellulose. The control and α-CD diets had an energy content of 4.5 kcal/g and contained 17% protein, 49% carbohydrates, 0.2% cholesterol, and 21% fat (wt/wt) from dried milk fat (65% saturated fats, 31% monounsaturated fats, and 4% polyunsaturated fats). The fatty acid composition of the milk fat as analyzed by Harlan Teklad is shown in Table 1.

2.3. Procedures

Fasting blood samples were withdrawn for lipid analysis from retroorbital sinus of all the mice after anesthesia before the beginning of the study. The mice were then randomly assigned to either the control (n = 10) or the α-CD group (n = 10) and fed their respective diets ad libitum for 14 weeks. Body weights were measured weekly. Fasting blood samples were collected after 2, 5, 10, and 14 weeks of feeding. Plasma was separated into EDTA tubes and stored at −80°C until analysis. All mice were killed after 14 weeks of feeding the designated diet by cervical dislocation.

2.3.1. Food intake

The daily food intake was determined by measuring the food consumption for 5 consecutive days during week 7 of treatment. All mice were separated into individual cages with 50 g of ground diet in feeders within the cage. Data from days 4 and 5 were used to calculate the daily food consumption.

2.4. Chemical analyses

2.4.1. Plasma lipid and lipoprotein analysis

Plasma TC, cholesterol ester (CE), free cholesterol (FC), phospholipid (PL), and TG concentrations were analyzed on a Hitachi 912 Chemistry Analyzer (Indianapolis, IN) using commercial enzymatic kits (TC and TG: Roche Molecular Biochemicals, Basel, Switzerland; PL and FC: Wako Pure Chemical Industries, Richmond, VA). Lipoprotein fractions were analyzed by fast protein liquid chromatography

Table 1
Fatty acid composition of the milk fat (percentage of total fat)

Fatty acids	%
8:0	0.5
10:0	2.0
12:0	2.7
13:0	0.13
14:0	9.8
14:1	0.7
15:0	1.1
16:0	29.9
16:1	1.7
17:0	0.7
18:0	15.1
18:1 (all *cis* isomers)	26.4
18:1 (*trans*)	4.0
18:2	3.7
18:3	0.5
20:3	0.1
20:4	0.2
22:0	0.07
22:5	0.07
TC	0.2

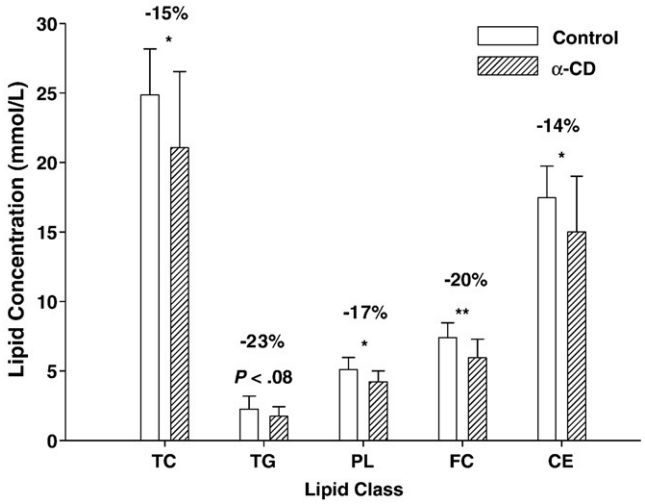

Fig. 1. Changes in plasma lipid at sacrifice (14 weeks) as compared with baseline levels. *a-Cyclodextrin group was significantly different from control group at $P < .05$; **a-CD group was significantly different from control group at $P < .01$.

(FPLC) separation and subsequent lipid analysis, as described earlier [10,11].

2.4.2. Plasma fatty acid composition

An aliquot (35-50 μL) of thawed plasma from week 10 of the study was directly saponified in 1 mL ethanol and 0.1 mL 50% KOH at 60°C for 30 minutes after the addition of an internal standard. Nonsaponifiable lipids were extracted with hexane (3 mL) and discarded. Glacial acetic acid (0.1 mL) was added to acidify the ethanol/KOH mixture, and fatty acids were extracted in hexane (3 mL). The hexane was evaporated under a stream of nitrogen, while heating the tube at 60°C. Fatty acid moieties were converted to volatile fatty acid methyl esters by the method of Metcalfe et al [12] and were separated by gas chromatography [13].

2.4.3. Statistical analysis

Mean and standard error of means (SEM) were calculated and reported. Analysis of variance with repeated measure was performed. When the treatment effects were significant, Student t tests with Bonferroni adjustment for multiple comparisons were performed. The significance level was set at $P < .05$.

3. Results

3.1. Body weight and food intake

There was no difference in body weight between the control and α-CD groups at anytime during the study. At sacrifice, the control mice had gained 4.81 ± 1.93 g compared with 4.80 ± 1.13 g in the α-CD group (not significant). No difference was observed in food intake between the control and α-CD groups (45.7 ± 3.7 mg food per day per gram of body weight for control group

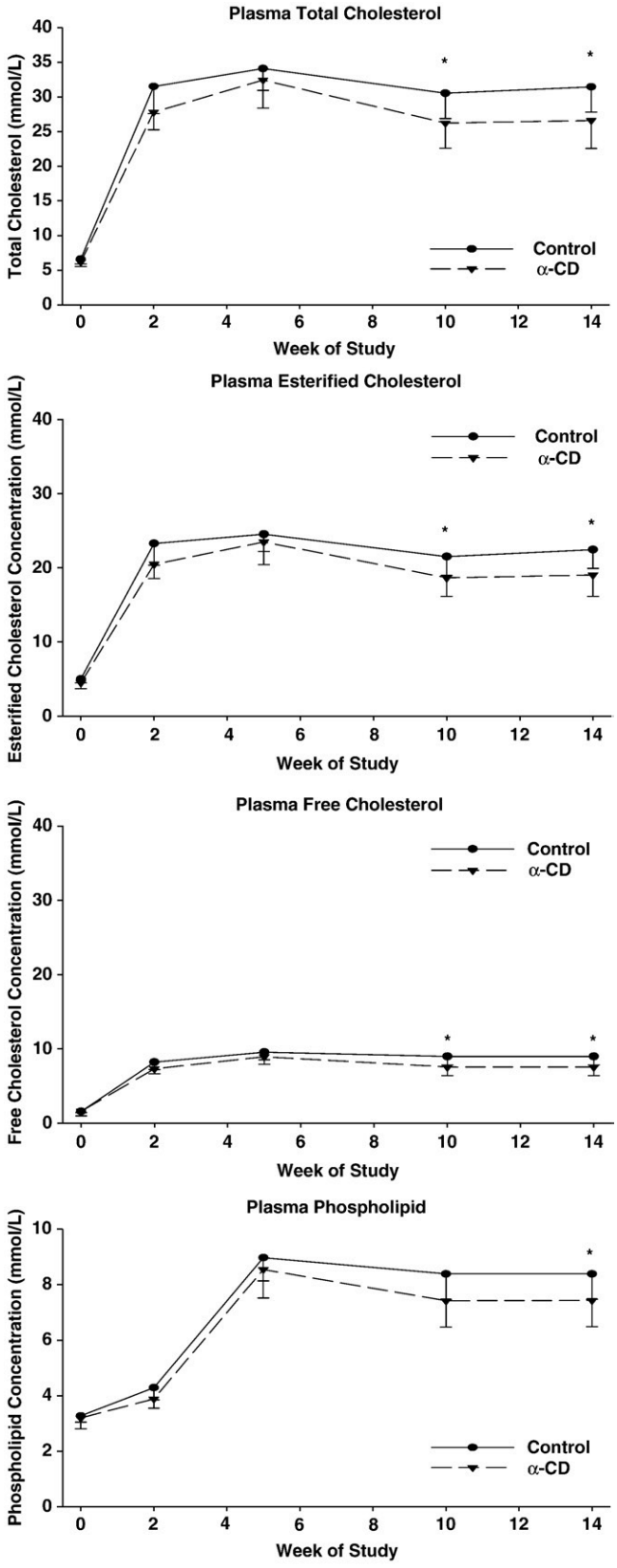

Fig. 2. Plasma lipid levels over the course of the study. *a-Cyclodextrin group was significantly different from control group at $P < .05$.

Table 2
Plasma lipid levels (in millimoles per liter) at sacrifice (14 weeks) of the 2 groups of mice

	Control	α-CD	*P*
TC	31.18 ± 3.62	26.36 ± 4.0	<.05
PL	16.16 ± 1.76	14.33 ± 1.81	<.05
FC	8.91 ± 1.13	7.49 ± 1.14	<.05
Esterified cholesterol	22.27 ± 2.51	18.88 ± 2.87	<.05
TG	8.20 ± 1.81	6.97 ± 1.58	Not significant

vs 45.0 ± 2.9 mg food per day per gram of body weight, not significant).

3.2. Total plasma lipids

Both the treatment (diet) effect ($P < .05$ or .01) and the time (weeks) effect ($P < .001$) were statistically significant for TC, CE, FC, and PL. The diet effect for plasma TG failed to reach significance ($P < .08$), although the time effect was significant ($P < .001$). As expected, we first observed a significant increase in plasma cholesterol levels after the mice were switched from the baseline diet to a milk-fat–based diet, but to a lesser extent for the α-CD–treated mice. The increases were significantly lower in the α-CD group as compared with the control group (TC by 15%, PL by 17%, FC by 20%, and CE by 14%) (Fig. 1). The increase in the TG levels of the α-CD group was also lowered by 23%, but the difference failed to reach statistical significance ($P < .08$). The reduction in plasma lipid levels first became apparent 2 weeks after the start of α-CD feeding. Changes in plasma cholesterol levels throughout the study are depicted in Fig. 2. Student *t* tests with Bonferroni adjustment for the multiple comparisons revealed that the statistical differences were observed at 10 and 14 weeks between the α-CD and control groups for TC, CE, and FC, and at 14 weeks for PL. The plasma lipid concentrations at sacrifice are presented in Table 2.

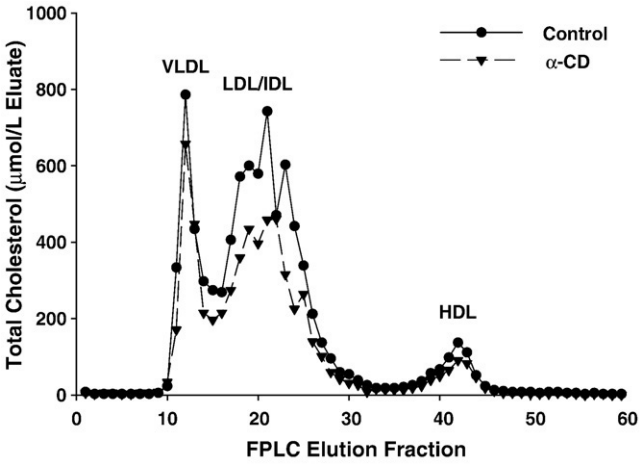

Fig. 3. The lipoprotein fractions of the cholesterol components as measured by FPLC after 10 weeks of feeding the designated diets. Results were obtained by pooling plasma from 5 mice in each group.

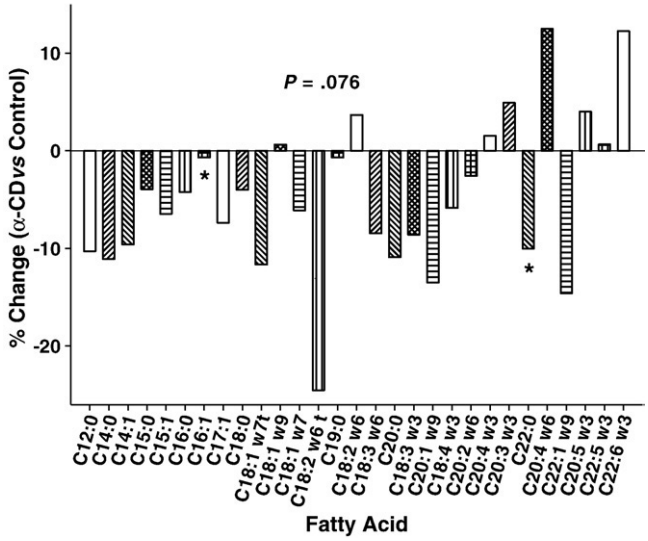

Fig. 4. Changes (percentage) in plasma fatty acid profile of *a*-CD group as compared with the control group. **a*-Cyclodextrin group was significantly different from control group at $P < .05$.

3.3. Lipoprotein fractions

Fig. 3 depicts the lipoprotein fractions of the cholesterol components, as measured by FPLC after 10 weeks of feeding the designated diets. It is apparent that the reduction observed in the α-CD group was mainly due to the reduction in the proatherogenic apolipoprotein (apo) B–containing VLDL and LDL/IDL fractions. No changes in the HDL fractions were detected. As a result, the LDL/IDL to HDL ratio was reduced from 8.3 for the control group to 7.9 for the α-CD group, a 4.8% reduction.

3.4. Fatty acid profile

Consistent with the difference in the total TG and PL levels, the plasma total fatty acid levels from the α-CD–fed mice were also lower (by 17%) than those of the control mice (α-CD group: 1137.2 ± 125.6 μg/mL; control group: 1370.2 ± 164 μg/mL; $P < .005$). After calculating each fatty acid as a percentage of total lipids in each group, significant reductions in C17:1 and C22:0 were observed in the α-CD group compared with the control group (Fig. 4). The reduction in C16:0 ($P < .055$) just missed being significant. A reduction in the concentration of C18:2w6 *trans*-isomer was also observed, but the difference failed to reach significance ($P = .076$). When the percentages of saturated and *trans*-fatty acids were added together, the α-CD group tended to have a decreased level (−4.8%, $P = .057$) relative to the control group. The ratio of *trans*- plus saturated fatty acids to unsaturated fatty acids also showed a tendency of reduction (−5.8%, $P = .055$).

4. Discussion

In this study, we have observed that α-CD, when given in the diet at a rate of 10% of the fat (wt/wt), significantly

reduces plasma TC, PL, FC, and CE, primarily in the plasma LDL fraction, while maintaining blood HDL-C levels in LDLr-KO mice as compared with that of the controls. Milk fat, high in saturated fatty acids and cholesterol content, was used as a fat source to induce elevated plasma lipid levels. This goal was achieved as demonstrated by the early onset of significant increases in plasma TC, EC, FC, and PL levels between baseline and 2 weeks of feeding (Fig. 2). α-Cyclodextrin also showed its lipid-lowering effects as early as 2 weeks of feeding, although the differences just missed the significance levels (TC, $P = .053$; PL, $P = .08$). It should be noted that both groups of mice had the same amount of fiber in the diet. The only difference was that 21 g (10% of diet fat, wt/wt) of α-CD was used to replace 21 g of cellulose in the α-CD group. The difference in the lipid profile therefore could not be attributed to the quantity of the fiber. These results demonstrated that α-CD is efficacious in lowering blood lipid levels not only in normal rats fed a high-fat diet [6], but also in LDL-r KO mice fed a moderate-fat diet.

It should be noted that α-CD has been reported to prevent weight gain in high-fat (40% wt/wt)–fed Wistar rats [6]. No difference, however, in body weight and food intake was identified in this 14-week study. In the present study, all mice were fed a milk-fat–based diet with 21% fat (wt/wt). Mice in the α-CD group also ingested 2.1% of α-CD (wt/wt) in their diet. It is possible that a higher dietary fat content, as was used in the previous rat study (40%, wt/wt) [6], is needed to show the weight loss property of α-CD. One may also speculate that mice lacking the LDL receptor may not be an ideal animal model to investigate the effect of a high-fat diet on body weight regulation because of reduced uptake of apo B–containing lipoproteins in peripheral cells. However, LDLr-KO mice have the advantage of being susceptible to insulin resistance linked to diet-induced obesity, an effect that could not be observed in apo E–deficient mice, another common murine model of atherosclerosis [14]. Further research to investigate the insulin resistance status of these LDLr-KO mice fed the α-CD–containing diet is warranted.

Soluble dietary fibers are known to reduce blood cholesterol levels; however, a meta-analysis concluded that soluble fibers reduce TC by relatively small amounts, approximately 0.045 mmol/L per gram of soluble fiber [4]. For a normal-weight subject following the recommended diet of 2000 kcal and 30% fat per day, this amounts to about 4.6% reduction in TC levels. Another meta-analysis of 8 controlled intervention trials reported a 4% reduction in TC levels after consuming 10.2 g psyllium per day in hypercholesterolemic subjects [15]. In contrast, we have reported that, in hypertriglyceridemic obese subjects with type 2 diabetes mellitus, 6 g of α-CD (2 g per meal) per day significantly reduced serum TC by 8% [7]. In a previous rat study, α-CD added to food in the amount of 10% of the fat content reduced TC by 13.2% in low-fat–fed rats, whereas a reduction of 8.5% was observed in rats fed the high-fat–containing diet. In the present study of LDL-r KO mice, the reduction in the plasma concentration of TC by α-CD was about 15%, more than 3 times the reduction in TC observed in humans treated with psyllium fibers [15]. Overall, these results consistently demonstrate that α-CD reduces TC levels by at least 8% when taken in an amount of 6 g/d (humans) or 10% of fat content of animal foods.

It has also been reported that α-CD preferentially binds and reduces the absorption of saturated fatty acids from the diet. Its ability to reduce saturated fatty acid absorption was found to be significantly higher than that of chitosan [16]. Because saturated fatty acids promote the hepatic synthesis of cholesterol and reduce its clearance [17], and reduction of saturated fat intake lowers blood cholesterol levels [18,19], the ability of α-CD to lower plasma cholesterol may be related to its effect on saturated fatty acid absorption. This is supported by the data in Fig. 4 showing that α-CD lowers the total level of saturated fatty acids in the plasma. The FPLC analysis of the plasma (Fig. 2) demonstrated that feeding of the α-CD diet preferentially lowers the proatherogenic apo B lipid fractions, leading to a major 29% decrease in LDL, IDL, and VLDL. There was no observed change, however, in the atheroprotective HDL-C fractions in the plasma of the studied mice, which is in agreement with the results of previous studies on the effect of fibers on plasma cholesterol levels [4]. The ratio of LDL/HDL was lower (−4.8%) in the α-CD–treated mice, suggesting that α-CD treatment may lower the risk for atherosclerosis. In addition, it is now understood that the antiatherogenic potential of HDL can vary significantly between individuals [20,21]. High-density lipoprotein from different subjects can vary in its antioxidant and anti-inflammatory ability [21], although the reason for this is still not fully understood. There is evidence that the consumption of saturated fat impairs the anti-inflammatory potential of HDL, whereas this beneficial property improves after consuming a polyunsaturated fatty acid (PUFA)–rich diet [22]. Hence, besides its ability to improve the LDL cholesterol to HDL ratio, α-CD may have other potential beneficial effects on lipoprotein metabolism through its ability to modulate the plasma fatty acid profile (Fig. 4).

Analysis of the plasma fatty acid profile revealed that all fatty acid concentrations were reduced in the α-CD–treated group relative to the control group. Considering the reductions in plasma TG, as well as significant reductions in CE and PL levels, this finding is not unexpected. However, when the fatty acids were expressed as percentage of the total lipids and the difference between the 2 groups were compared, an additional effect of α-CD was observed. α-Cyclodextrin reduced saturated (C16:0, C18:0, and C22:0) and *trans*-fatty acids (C18:1 *trans* and C18:2 *trans*), whereas PUFAs such as arachidonic acid (C20:4 *w*-6) and docosahexanoic acid (C22:6 *w*-3) were increased. Gallaher et al [16] have reported that α-CD preferentially bound with saturated fats and promoted their excretion into the feces. Our current finding thus is consistent with the fecal fat findings of Gallaher et al [16]. The mechanism for

this alteration would appear to be related to the higher affinity of α-CD for saturated and *trans*-dietary fat over unsaturated fats.

trans-Fatty acids are known to increase the risk of CVD and type 2 diabetes mellitus [23,24]. They also cause systemic inflammation and endothelial dysfunctions, and may alter the plasma lipoprotein fractions toward a more proatherogenic profile [24]. In addition, a study with monkeys has shown that *trans*-fats increased abdominal fat deposition and impaired glucose tolerance [25]. Abdominal obesity and reduced insulin sensitivity in turn increase the risk of developing type 2 diabetes mellitus. Not all *trans*-fatty acids, however, are equally proatherogenic. *trans*-Isomers of C18:1 and C18:2 have more detrimental effects than other *trans*-fatty acids, such as C16:1 isomers [26,27]. A recent finding from The Cardiovascular Health Study showed that elevated plasma PL 18:2 *trans* was associated with higher risk for fatal ischemic heart disease after adjusting for other risk factors [28]. Although it did not reach statistical significance, perhaps because of the relatively low content of *trans*-fat in the milk-fat diet used in this study, the α-CD–treated group had a 25% reduction in *trans* C18:2 compared with the control group. This amount of reduction may have significant clinical importance.

In summary, this study demonstrated that, in LDLr-KO mice, feeding α-CD as 10% (wt/wt) of the dietary fat not only improved blood lipid levels but also improved the fatty acid profile. This improvement was shown in reduced saturated and *trans*-fatty acid levels with concomitant increase in PUFA levels. Considering the relative safety and tolerability of α-CD [7], and the fact that both saturated fats and *trans*-fats are associated with increased risk of CVD, type 2 diabetes mellitus, abdominal obesity, and inflammation, future clinical studies assessing the benefits of the addition of α-CD to foods or as a food supplement should be performed.

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