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Dietary fat saturation and gender/hormonal status modulate plasma lipids and lipoprotein composition☆

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Abstract

Male, female and ovariectomized (to mimic menopause) guinea pigs were fed a saturated (SFA) or a polyunsaturated (PUFA) fat diet for 4 weeks to determine the effects of dietary fat saturation on lipoprotein levels and composition and to assess whether gender and hormonal status modulate the cholesterolemic response. Both diets contained 15g/100 g fat and 0.04 g/100 g cholesterol and were identical in composition except for the type of fat. The SFA diet contained 50% saturated fat (25% lauric + myristic fatty acids), 25% PUFA and 25% monounsaturated fatty acids while the PUFA diet had 50% PUFA (linoleic acid), 25% monounsaturated and 25% SFA fatty acids. Plasma LDL cholesterol (LDL-C) was an average 21% lower in guinea pigs fed PUFA compared to those fed SFA ($P < 0.05$). In addition, ovariectomized guinea pigs, both in the SFA and PUFA groups, had 20–33% higher LDL-C than either males or females ($P < 0.01$). VLDL cholesterol was 70% higher in the PUFA-fed animals ($P < 0.0001$). A gender effect was observed in plasma HDL cholesterol (HDL-C) with females and ovariectomized guinea pigs having 30–42% higher HDL-C than males ($P < 0.01$). LDL susceptibility to oxidation was not affected by dietary fat saturation or gender. In contrast, VLDL and LDL composition were significantly influenced by diet and gender. VLDL particles were larger in size in guinea pigs fed the SFA diets ($P < 0.01$) while LDL particles were larger in female guinea pigs ($P < 0.001$). Hepatic lipids were influenced by the interaction between diet and group. Hepatic cholesterol ($P < 0.01$) and TAG concentrations ($P < 0.0001$) were highest in female guinea pigs fed the PUFA diet. Since the liver is the major site of lipoprotein synthesis and catabolism, these results suggest that not only diet but also gender may play a major role in determining the composition and size of lipoproteins.

Keywords: Dietary fat saturation; LDL cholesterol; Gender; Hormonal status; Lipoprotein composition

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States. Elevated plasma LDL cholesterol (LDL-C) and triacylglycerol (TAG) concentrations are associated with increased risk for CVD [1,2]. It is known that dietary factors influence plasma lipid levels and lipoprotein metabolism, altering the atherogenicity of lipoprotein profiles [3] High intake of saturated fatty acids has been implicated as a causative factor in the high incidence of hypercholesterolemia and increased risk for atherosclerosis and CVD [4], while polyunsaturated fat intake has been associated with lower plasma cholesterol concentrations and decreased incidence of atherosclerosis in human [4] and animal studies [5].

Gender is a strong predictor of coronary heart disease susceptibility. Until recently, only male sex was considered a risk factor for the development of cardiovascular disease. However it is now well known that while men are at higher risk at a younger age, risk is increased for women after menopause [6]. Postmenopausal women have significantly higher CVD risk factors such as elevated plasma LDL-C, TAG and apo B levels [7]. The more detrimental profile in post-menopausal women could be due to the lack of estrogen, which provides a protective role in the maintenance of desirable LDL/HDL ratios and low levels of plasma TAG. [8]. In addition, LDL from postmenopausal women appears to have a high susceptibility to oxidation, increasing the

☆*Abbreviations:* HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, PUFA: polyunsaturated fatty acids, SFA: saturated fatty acids, *TAG:* Triacylglycerol; VLDL-C: very low density lipoprotein cholesterol

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possibility of lipid deposition in the arterial wall and the development of atherosclerosis [9].

Since CVD is multifactorial and associated with nonmodifiable risk factors, and since diet can influence lipoprotein levels and atherogenicity, there has been much interest in determining whether women and men respond differently to dietary interventions [10]. Many carefully controlled studies have indicated a gender-associated response to diet [11]. For example, it has been shown that women experience a significant increase in HDL-C and TAG when fed a high saturated fat (SFA) diet compared to a polyunsaturated fat (PUFA) diet, while no changes in these plasma lipids were observed for men. [12]. In addition, the effects of a psyllium supplement on plasma lipids were evaluated in men, pre- and postmenopausal women. Men lowered both their plasma cholesterol and TAG compared to the control period. In contrast, postmenopausal women increased their plasma TAG after psyllium supplementation, an indication that sex and hormonal status influence the lipidmic responses to this soluble fiber [13].

Studies conducted in our laboratory have shown that intake of PUFA results in up-regulation of hepatic LDL receptors and increased LDL turnover compared to SFA intake in male guinea pigs [14]. In addition, we have demonstrated that dietary fat saturation and chain length modulate the size and composition of lipoproteins, suggesting an effect of dietary fatty acids on the intravascular processing of lipoproteins [15].

The objective of the present studies was to evaluate whether gender and hormonal status affect the cholesterolemic response to dietary SFA and PUFA. Based on previous experiments, a diet high in short chain saturated fatty acids was used to ensure higher plasma LDL-C in the SFA compared to the PUFA group. Lauric and myristic acids are more effective in raising plasma LDL-C in guinea pigs compared to long chain fatty acids [16]. Intake of a diet high in stearic acid results in similar plasma cholesterol levels as a diet high in linoleic acid [16]. Guinea pigs were used as the animal model because they have many similarities to humans in plasma lipoprotein profile and cholesterol and lipoprotein metabolism [17]. In addition, the ovariectomized guinea pig has similar plasma lipid profiles as postmenopausal women and has been established by our laboratory as a model for menopause [18].

2. Materials and methods

2.1. Animals

Hartley guinea pigs (male, female and ovariectomized animals) were maintained in a light cycle room with constant temperature and humidity and provided water and diet *ad libitum.* Guinea pigs (250–300 g) were gradually adapted to the experimental diets by mixing the test diets with regular chow in increasing proportions during 1 week.

Table 1
Composition of experimental diets

Component	SFA g/100 g	PUFA g/100 g	Energy %
Protein	23	23	23.3
Fat mix[a]	15.1	15.1	35.4
Corn starch/sucrose[b]	40	40	41.3
Mineral mix[c]	8.2	8.2	—
Vitamin mix[c]	1.1	1.1	—
Cholesterol[d]	0.04	0.04	—
Cellulose	10	10	—
Guar Gum	2.5	2.5	—

[a] Fatty acid composition is presented in Table 2.
[b] Starch-Sucrose ratio 1:1.43.
[c] Mineral and vitamin mix adjusted to meet NRC requirements for guinea pigs. Detailed composition of the vitamin and mineral mix has been reported elsewhere [33].

Animals were fed the experimental diets during 4 weeks since previous studies have shown that this feeding time ensures constant plasma and tissue cholesterol concentrations. Based on preliminary data and calculating an $\alpha = 0.05$ and a power of 90, 8 male, 8 female and 8 ovariectomized guinea pigs were used for each dietary treatment. Non-fasted guinea pigs were killed by heart puncture after halothane anesthesia. All animal experiments were conducted in accordance with U.S. Public Health Service/U.S. Department of Agriculture guidelines Experimental protocols were approved by the University of Connecticut Animal Care and Use Committee.

2.2. Diets

Diets were prepared and pelleted by Research Diets Inc. (New Brunswick, NJ). Both diets had identical composition including fat percentage (15.1%, w/w).as indicated in Table 1. The variable was the fatty acids of the fat mix (Table 2). Fat mixes were prepared by varying the proportion of olive, palm kernel, soybean and safflower oil as reported elsewhere [19]. The fat mixes were either high in saturated short chain fatty acids lauric + myristic (SFA), or polyunsaturated linoleic acid (PUFA). Fat mixes were formulated in

Table 2
Fatty acid composition of saturated (SFA) and polyunsaturated (PUFA) fat diets

Fatty acid	Diets, g/100 g[a]	
	SFA	PUFA
Lauric (12:0)	23.8	0
Myristic (14:0)	7.8	0.5
Palmitic (16:0)	9.2	16.2
Stearic (18:0)	8.6	7.1
Oleic (18:1)	19.9	23.5
Linoleic (18:2)	26.4	52.7
Other	4.3	0

[a] Values are the mean of two determinations.

such a way that the calculated values of the fatty acids under evaluation represented 50% of total. For example the PUFA diet was postulated to contain 50% linoleic, 25% oleic and 25% saturated fatty acids. In the specific case of the SFA diet, lauric and myristic acids were formulated to represent 25% of the total fatty acids as indicated in Table 2. After the mixture of the fatty acids was analyzed by gas chromatography [15], some discrepancies were found between the analyzed and the calculated values as indicated in Table 2.

2.3. Plasma and hepatic lipids

Plasma was isolated from red blood cells by centrifugation at 2,000 x g for 20 min. A preservation cocktail of aprotinin, phenyl methyl sulfonyl fluoride and sodium azide was added to plasma samples to minimize changes in lipoprotein composition during isolation. One mL of plasma was separated for measurement of LDL susceptibility to oxidation, 500 μl of plasma from each sample was stored at 4°C for further plasma lipid analysis and the rest was used for lipoprotein isolation.

Plasma samples and liver were analyzed for total cholesterol [20] and TAG [21] by enzymatic methods. Hepatic lipids were extracted with chloroform-methanol 2:1. Total and free cholesterol were determined in liver by enzymatic methods [19]. Cholesteryl ester concentrations were calculated by subtracting free from total cholesterol. Hepatic TAG were measured according to Carr et al [20].

2.4. Lipoprotein isolation and characterization

Lipoproteins were isolated by sequential ultracentrifugation [22] in a LE-80K ultracentrifuge (Beckman Instruments, Palo Alto, CA). VLDL was isolated at $d = 1.006$ kg/L at 125,000 g at 15°C for 19 h in a Ti-50 rotor. LDL was isolated at $d = 1.019–1.09$ kg/L in quick-seal tubes at 15°C for 3 h at 200,000 g in a vertical Ti-65 rotor. [23] LDL samples were dialyzed in 0.9g/L sodium chloride-0.1g/L ethylene diamine tetra acetic acid (EDTA), pH 7.2, for 24 h and stored at 4°C for further analysis.

VLDL and LDL compositions were calculated by determining free and esterified cholesterol [19], protein by a modified Lowry method, [24] and TAG [20] and phospholipids by enzymatic kits. VLDL apo B was selectively precipitated with isopropanol [25]. The number of constituent molecules of LDL was calculated on the basis of one apo B per particle with a molecular mass of 412000 kD [26]. The used molecular weights were 885.4, 386.6, 645 and 734 for TAG, free and esterified cholesterol, and phospholipids, respectively. LDL diameters were calculated according to Van Heek and Zilversmit [27]. HDL cholesterol was also determined by selective precipitation of the apo-B containing lipoproteins [28] with a modification, which consisted of using 2 mol/L $MgCl_2$ for precipitation of apo-B containing lipoproteins [23].

2.5. In vitro determination of LDL susceptibility to oxidation

LDL isolated from individual samples was dialyzed in EDTA-free phosphate buffered saline. Copper-mediated oxidation of LDL was performed by adding 0.5 mmol/L $CuCl_2.2H_2O$ solution to 0.2g protein/L LDL. The effect of the dietary treatments on the extent of oxidation was measured by incubating samples for 3 h at 37°C. The lipid peroxide content of oxidized LDL was determined by measuring the formation of thiobarbituric acid-reactive substances (TBARS) expressed as malonaldehyde equivalents [29]. The TBARS assay was conducted by adding 2ml of TBARS reagent [26 mmol/L thiobarbituric acid (TBA), 0.92 g/L trichloroacetic acid in 0.25 mol/L HCl] to 550 μl incubation mixture at 100°C for 15 min. The phases were separated by centrifugation at 1500 x g for 15 min. The pink color was developed in the aqueous layer and extracted with n-butanol. Absorbance was read at 532 nm in a spectrophotometer.

2.6. Statistical analysis

Two-way analysis of variance (ANOVA) (GBSTAT, Silver Spring, MD) was used to evaluate the significant diet effects (saturated or polyunsaturated fat diets), group effects (male, female or ovariectomized guinea pigs) and their interaction on plasma lipids, hepatic lipids, composition of VLDL and LDL and susceptibility of LDL to oxidation. The Tukey's post hoc test was used to evaluate the differences among means in the male, female and ovariectomized groups due to intake of PUFA or SFA. Differences were considered significant at $P < 0.05$. Data are presented as the mean $\pm$ SD, $n = 8$ males, females or ovariectomized guinea pigs in each dietary group.

3. Results

Plasma total cholesterol concentrations in the PUFA groups were 7.9, 9.7 and 22.1% lower in male, female and ovariectomized guinea pigs respectively compared to those fed the SFA diet ($P < 0.05$) (Table 3). In addition, ovariectomized guinea pigs had 22 to 33% higher cholesterol concentrations than either male or females. Plasma TAG concentrations were not affected by diet or by gender/hormonal status (Table 3).

Plasma LDL-C concentrations followed a similar pattern to total cholesterol with PUFA intake resulting in 8, 21.7 and 27.7% lower LDL cholesterol concentrations in male, female and ovariectomized guinea pigs respectively, compared to the SFA groups. Ovariectomized guinea pigs also had the highest plasma cholesterol levels in both SFA and

Table 3
Plasma lipids and lipoprotein cholesterol concentrations of guinea pigs fed saturated (SFA) or polyunsaturated (PUFA) fat diets for 4 weeks. Dietary fatty acid composition is described in Table 2.

Diets	Cholesterol,[a] mmol/L	Triacylglycerol, mmol/L	VLDL-C, mmol/L	LDL-C, mmol/L	HDL-C, mmol/L
SFA					
Male	2.40 ± 0.75	1.03 ± 0.26	0.08 ± 0.04	1.95 ± 0.80	0.22 ± 0.08
Female	2.58 ± 1.28	0.97 ± 0.31	0.05 ± 0.02	2.48 ± 1.22	0.30 ± 0.14
Ovariectomized	3.62 ± 1.01	1.35 ± 0.68	0.05 ± 0.02	3.21 ± 0.99	0.39 ± 0.12
PUFA					
Male	2.21 ± 0.65	1.50 ± 0.52	0.23 ± 0.22	1.78 ± 0.57	0.19 ± 0.07
Female	2.33 ± 0.65	1.06 ± 0.39	0.20 ± 0.06	1.94 ± 0.59	0.28 ± 0.13
Ovariectomized	2.82 ± 0.58	1.56 ± 1.66	0.23 ± 0.19	2.32 ± 0.70	0.31 ± 0.16
Two-way ANOVA					
Diet effect[b]	$P < 0.05$	NS	$P < 0.01$	$P < 0.05$	NS
Group effect[c]	$P < 0.05$	NS	NS	$P < 0.05$	$P < 0.01$
Interaction	NS	NS	NS	NS	NS

[a] Data are presented as mean ± SD for n = 8 guinea, NS = non-significant, $P > 0.05$. Numbers in a column with different superscripts represent an interactive effect considered significantly different ($P < 0.01$) as determined by Tukey's post hoc test.
[b] PUFA > SFA for VLDL, SFA > PUFA for LDL.
[c] Ovariectomized > male = female for LDL-C and female = ovariectomized > male for HDL-C.

PUFA groups ($P < 0.05$) compared to male or female animals (Table 3). In contrast to effects on LDL-C, plasma VLDL-C concentrations were higher in guinea pigs fed the PUFA diet compared to the SFA group. There was no group effect on plasma VLDL-C concentrations. HDL-C were higher in female and ovariectomized guinea pigs compared to males while no diet effect was observed for this parameter (Table 3).

Free cholesterol concentrations in liver were not affected by diet or gender/hormonal status, however there was an interactive effect ($P < 0.01$). Female guinea pigs consuming the PUFA diet had the highest hepatic free cholesterol concentrations compared to the other 5 groups (Table 4). Similarly, an interactive effect was observed for cholesteryl ester concentrations in liver with female guinea pigs consuming the PUFA diet having the highest concentrations. Hepatic TAG were modified both by diet and group and there was also an interactive effect. The interactive effect consisted of the highest hepatic TAG in female guinea pigs fed the PUFA diet and the lowest values observed in the ovariectomized animals from the PUFA group ($P < 0.001$)

Lipoproteins were evaluated for composition and size and results for VLDL are presented in Table 5. The number of molecules of VLDL free cholesterol and TAG were not affected by diet or group while the number of cholesteryl ester molecules were higher in females ($P < 0.05$) and the number of phospholipid molecules were higher in guinea pigs fed the PUFA diet ($P < 0.01$). These compositional differences resulted in alterations in VLDL diameter with guinea pigs fed the SFA diets having greater VLDL diameter than the PUFA group (Table 5).

The number of molecules in LDL were not affected by diet or group while there was an interaction in the number of free cholesterol molecules with females fed the PUFA diet having the highest number of molecules of this LDL component (Table 6). The number of TAG molecules were modulated by diet with PUFA intake resulting in an LDL with a higher number of TAG molecules ($P < 0.01$). In addition, there was a group effect ($P < 0.0001$) with female guinea pigs having more TAG molecules per LDL than either male or ovariectomized guinea pigs. Also, females fed the PUFA diet had the greatest concentrations of TAG molecules (interactive effect, $P < 0.01$). LDL diameter was modified by diet and by group with female guinea pigs ($P < 0.001$) and those fed the SFA diet ($P < 0.01$) having a larger LDL diameter (Table 6). In addition, there was an interactive effect since female guinea pigs fed the SFA diet had the largest LDL particles.

Table 4
Hepatic lipids of guinea pigs fed saturated (SFA) or polyunsaturated (PUFA) fat diets for 4 weeks. Dietary fatty acid composition is described in Table 2.

Diets	FC,[a] μmol/g	CE, μmol/g	TAG, μmol/g
SFA			
Male	2.35 ± 0.81[a]	0.53 ± 0.20[a]	10.84 ± 6.43[a]
Female	2.09 ± 0.94[a]	0.44 ± 0.21[a]	8.47 ± 2.14[a]
Ovariectomized	3.11 ± 0.87[a]	0.31 ± 0.12[a]	8.02 ± 2.03[a]
PUFA			
Male	2.44 ± 0.68[a]	0.18 ± 0.15[a]	10.48 ± 6.38[a]
Female	3.12 ± 0.80[b]	0.82 ± 0.81[b]	32.30 ± 13.55[b]
Ovariectomized	2.17 ± 0.43[a]	0.22 ± 0.15[a]	4.95 ± 1.87[a]
Two way ANOVA			
Diet effect[b]	NS	NS	$P < 0.005$
Group effect[c]	NS	$P < 0.05$	$P < 0.0001$
Interaction	$P < 0.01$	$P < 0.05$	$P < 0.0001$

[a] Data are presented as mean ± SD for n = 8 guinea pigs NS = non-significant, $P > 0.05$. Numbers in a column with different superscripts are considered significantly different ($P < 0.01$) as determined by Tukey's post hoc test.
[b] PUFA > SFA for hepatic TG.
[c] Female > Male and ovariectomized for hepatic CE and TG.

Table 5
Number of molecules/VLDL apo B of free cholesterol (FC), cholesteryl ester (CE), phospholipids (PL), triacylglycerol (TAG) in VLDL and VLDL diameter of guinea pigs fed saturated (SFA) or polyunsaturated (PUFA) fat diets for 4 weeks. Dietary fatty acid composition is described in Table 2.

Diets	FC[a]	CE	PL	TAG	Diameter
	Number of molecules/VLDL apo B				Å
SFA					
Male	2197 ± 749	484 ± 594	2198 ± 908	9663 ± 2643	704.9 ± 172.2
Female	3071 ± 3023	836 ± 808	3422 ± 3953	9718 ± 5623	800.5 ± 235.5
Ovariectomized	1735 ± 874	671 ± 673	2599 ± 2305	7565 ± 3407	689.7 ± 256.8
PUFA					
Male	1217 ± 1192	235 ± 227	4305 ± 1097	5812 ± 1971	582.7 ± 115.6
Female	3584 ± 4863	1086 ± 853	4583 ± 2344	10377 ± 6637	574.7 ± 76.9
Ovariectomized	1437 ± 1867	92 ± 57	3373 ± 1446	7100 ± 6473	557.3 ± 64.0
Two way ANOVA					
Diet effect[b]	NS	NS	$P < 0.01$	NS	$P < 0.01$
Group effect[c]	NS	$P < 0.05$	NS	NS	NS
Interaction	NS	NS	NS	NS	NS

[a] Data are presented as mean ± SD for n = 8 guinea pigs NS = non-significant, $P > 0.05$. Numbers in a column with different superscripts are considered significantly different ($P < 0.01$) as determined by Tukey's post hoc test.
[b] PUFA > SFA for phospholipids and SFA > PUFA for VLDL diameter.
[c] Female > Male and ovariectomized for CE.

The resistance of LDL to oxidation was assessed by determination of TBARs formation. Both dietary fat saturation and gender/hormonal status had no effect on the susceptibility of LDL to oxidation (Table 6).

4. Discussion

In this study we have demonstrated that dietary fat saturation and gender/hormonal status interact to determine hepatic and plasma lipid concentrations and to influence composition and size of lipoproteins. These modifications in lipoproteins could be a result of specific effects of dietary fat saturation, gender and hormonal status, on hepatic cholesterol metabolism.

4.1. Effects of dietary fat saturation and gender/hormonal status on plasma lipids and lipoprotein composition

The plasma cholesterol lowering of PUFA compared to SFA diets has been documented in numerous reports in humans [4,30], and in animals [31], including guinea pigs [14]. The mechanisms of plasma LDL cholesterol lowering

Table 6
Number of molecules/LDL apo B of free cholesterol (FC), cholesteryl ester (CE), phospholipids (PL) and triacylglycerol (TAG) molecules, LDL diameter and LDL susceptibility to oxidation measured by Tbars formation of guinea pigs fed saturated (SFA) or polyunsaturated (PUFA) fat diets for 4 weeks. Dietary fatty acid composition is described in Table 2.

Diets	FC[a]	CE	PL	TAG	Diameter	TBars
	Number of Molecules				Å	nmoles MDA/LDL protein
SFA						
Male	335 ± 223[a]	860 ± 164	212 ± 73[b]	60 ± 44[a]	244 ± 31[a]	12.2 ± 4.2
Female	203 ± 64[a]	766 ± 217	142 ± 32[a]	220 ± 111[b]	378 ± 60[b]	13.0 ± 4.9
Ovariectomized	232 ± 49[a]	925 ± 107	168 ± 61[ab]	46 ± 15[a]	282 ± 62[a]	9.8 ± 3.2
PUFA						
Male	254 ± 50[a]	970 ± 380	190 ±69[ab]	159 ± 36[b]	217 ± 62[a]	8.1 ± 1.8
Female	405 ± 132[b]	1036 ± 374	219 ± 80[b]	191 ± 58[b]	273 ± 30[a]	12.4 ± 5.3
Ovariectomized	298 ± 38[a]	912 ± 223	133 ± 75[a]	147 ± 25[b]	270 ± 35[a]	8.2 ± 4.3
Two way ANOVA						
Diet effect[b]	NS	NS	NS	$P < 0.01$	$P < 0.05$	NS
Group Effect[c]	NS	NS	NS	$P < 0.0001$	$P < 0.001$	NS
Interaction	$P < 0.001$	NS	$P < 0.05$	$P < 0.01$	$P < 0.01$	NS

[a] Values are expressed as mean ± SD for the number of guinea pigs indicated in parentheses. Numbers in a column with different superscripts are considered significantly different ($P < 0.01$) as determined by Tukey's post hoc test.
[b] PUFA > SFA for TAG and SFA > PUFA for LDL diameter.
[c] Female > male = ovariectomized for TAG and VLDL diameter.

by PUFA compared to SFA diets have been reported in detail for male guinea pigs [14,32]

We have already established the ovariectomized guinea pig as a model for menopause [17]. Ovariectomized guinea pigs have more elevated plasma total and LDL-C concentrations compared to intact females, which is similar to the higher concentrations of plasma lipids observed in postmenopausal women compared to their younger counterparts [7]. In addition, ovariectomized guinea pigs and intact females present comparable levels of plasma HDL-C, which are higher than those in males, in agreement with the human situation [33]. In this study, however, although there was a trend, ovariectomized guinea pigs did not have higher plasma TAG concentrations than males or intact females as we found in our former study [18]. What is novel in this report is the response to dietary fat saturation as influenced by gender and hormonal status in the guinea pig.

In this study, a higher concentration of VLDL-C was observed in the PUFA versus the SFA group. Since lower plasma LDL-C concentrations were observed with PUFA intake, it is possible that VLDL particles are removed from plasma before they are converted into LDL. We have previously shown, that plasma LDL turnover is increased in guinea pigs fed PUFA versus SFA fat [32] and that the faster disappearance rate of LDL is correlated with an increased number of hepatic apo B/E receptors [14]. If there is up-regulation of hepatic apo B/E receptors by PUFA intake, this could be a potential route by which VLDL remnants (IDL) are removed from circulation. Although there are higher concentrations of VLDL with PUFA intake, the lower concentrations of plasma LDL-C suggest that the conversion to LDL or LDL apo B production, is decreased as shown by use of radiolabeled isotopes in guinea pigs [32].

As shown in other studies [14,32], PUFA intake resulted in lower plasma LDL-C concentrations compared to that of SFA intake. Results from the present study are consistent with reported findings on the effects of dietary fat saturation on plasma lipids in men and women [12]. The lower concentrations of LDL-C could be related to increased LDL turnover [14] and decreased conversion of VLDL to LDL [32].

4.2. Effects of dietary fat saturation and gender/hormonal status on hepatic lipids and lipoprotein composition

Previous reports in male guinea pigs have shown that dietary fat saturation has a moderate effect on hepatic cholesterol or TAG concentrations. In contrast, the present study clearly demonstrates that female guinea pigs have higher concentrations of hepatic cholesterol and TAG when fed the PUFA diet. However, these higher concentrations were not related with higher concentrations of plasma LDL-C but were associated with significant modifications in LDL composition. As calculated by ANOVA female guinea pigs had the largest calculated LDL diameters, which may be related to the higher number of TAG molecules in LDL particles of females. These findings are in agreement with the human situation where larger LDL particles have been reported in women compared to men from the Framingham study [33]. These larger LDL in women can be related to a less atherogenic particle since small dense LDL has been postulated to be more susceptible to oxidation and have a role in atherosclerosis development [34]. However, in the present study, neither gender nor diet altered the susceptibility of LDL to oxidation. In a previous study, we have shown that marginal intake of vitamin C in guinea pigs fed PUFA diets results in LDL particles more susceptible to oxidation [35]. The lack of an effect of dietary fat saturation on LDL oxidation in the current study could be related to adequate amounts of the antioxidant vitamin C provided by diet. In addition, significant differences in the susceptibility of LDL to oxidation in guinea pigs have been observed when the dietary treatment results in a cholesteryl-ester enriched LDL particle [36]. Although important modifications in LDL mediated by both diet and gender were observed in this study, there were no significant changes in the number of cholesteryl ester molecules.

In addition to effects on LDL, important compositional differences in VLDL associated to dietary fat saturation were observed. Guinea pigs fed the PUFA diet had higher concentrations of phospholipids and a smaller calculated VLDL diameter than those fed SFA diets. In other studies using guinea pigs, we have also observed that SFA intake results in larger VLDL particles, measured by electronic microscopy, compared to those fed PUFA diets [37], which were associated with faster secretion rates [38] and a faster conversion to LDL [32].

In addition to the fat saturation effects, gender affected lipoprotein composition. Female guinea pigs had the highest number of cholesteryl ester molecules in VLDL. This gender effect was more evident in the PUFA group. In human studies, it has been demonstrated that replacing SFA by PUFA results in lower plasma lanothesterol (a marker of cholesterol synthesis) in men but not in women although the plasma LDL-cholesterol lowering was similar for both genders [39]. These gender differences resulted in similar levels of lanostherol/cholesterol in men and women during the PUFA period, reducing the commonly observed greater rate of cholesterol synthesis in men compared to women [39]. We have reported that male guinea pigs have higher HMG-CoA reductase activity than females [40] when fed SFA. In this study, male guinea pigs fed the PUFA diets had lower hepatic cholesterol concentrations than females suggesting differences in hepatic cholesterol metabolism associated with gender. The higher concentrations of cholesteryl ester in liver of female guinea pigs could be related to higher ACAT activity and increased incorporation of CE into the secreted VLDL as has been reported for African green monkeys [41].

In summary, all tested groups, whether male, female or ovariectomized guinea pigs, experienced a reduction in

plasma LDL-C by taking PUFA versus SFA diets. However, there were specific differences in hepatic lipid levels and lipoprotein composition associated to gender. These different metabolic effects of dietary fat saturation between males and females in hepatic lipid pools may in part explain the formation of potentially atherogenic lipoproteins, which are gender-related.

References

[1] J. Stamler, D. Wentforth, J.D. Neaton, Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in, 356, 222 primary screens of the Multiple Risk Factor Intervention Trial (MRFlT). JAMA 256 (1986) 2823–2828.

[2] J.W. Anderson, Dietary fiber, lipids and atherosclerosis, Am. J. Cardiol. 60 (1987) 17G–22G.

[3] S.M. Grundy, M.A. Denke, Dietary influences on serum lipids and lipoproteins, J. Lipid. Res., 31 (1990) 1149–72.

[4] F.B, Hu, M.J. Stampfer, K.E. Manson, A. Ascherio, G.A. Colditz, F.E. Speizer, C.H, Henneckens, W.C. Willett, Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women, Am. J. Clin. Nutr. 70 (1999) 1001–1008.

[5] D.J. McNamara, Dietary fatty acids, lipoproteins and cardiovascular disease, Adv. Food Nutr. Res. 36 (1992) 253–361.

[6] K.A. Do, A. Green, J.R. Guthrie, E.C. Dudley, H.G. Burger, L. Dennerstein, Longitudinal study of risk factors for coronary heart disease across the menopausal transition. Am. J. Epidemiol. 151 (2000) 584–593, 2000.

[7] G. Bonithon-Kopp, P.Y. Scarabin, B, Darnel, A. Malmejac, L. Guize, Menopause-related changes in lipoproteins and some other cardiovascular risk factors, Int. J. Epidemiol. 19 (1990) 42–48.

[8] M.J. Stampfer, G.A. Colditz, Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence, Prev. Med. 20 (1991) 47–63.

[9] R.C. Wander, S. Du, S.O. Ketchum, K.E. Rowe, Effects of interaction of RRR-a-tocopheryl acetate and fish oil on low-density-Iipoprotein oxidation in postmenopausal women with or without hormone-replacement therapy, Am. J. Clin. Nutr. 63 (1996) 184–93.

[10] M. Cobb, J. Greenspan, M. Timmons, H. Teitelbaum, Gender differences in lipoprotein responses to diet, Ann. Nutr. Metab. 37 (1993) 225–236.

[11] E.E. Zanni,V.L. Zannis, C.S. Slum, P.N. Herbert, J.L. Sreslow, Effect of egg cholesterol and dietary fats on plasma lipids, lipoproteins and apoproteins of normal women consuming natural diets, J. Lipid. Res. 28 (1987) 518–527.

[12] C. Cox, J. Mann, W. Sutherland, A. Chisholm, M. Skeaff, Effects of coconut oil, butter and safflower oil on lipids and lipoproteins in persons with moderately elevated cholesterol levels, J. Lipid. Res. 36 (1995) 1787–1795.

[13] S. Vega-Lopez, R.L. Vidal-Quintanar, M.L. Fernandez, Sex and hormonal status influence plasma lipid responses to psyllium, Am J. Clin. Nutr. 74 (2001) 435–441.

[14] M.L. Fernandez, G. Abdel-Fattah, D.J. McNamara, Dietary fat saturation modifies the metabolism of LDL subfractions in guinea pigs, Arterioscler. Thromb. 14 (1993) 1418–1428.

[15] M.L. Fernandez, C. Avalos, M. Vergara-Jimenez, Differences in response between 18 carbon fatty acids and 12/14 carbon saturated fatty acids on plasma cholesterol in guinea pigs, Nutr. Res. 18 (1998) 1261–1272.

[16] M.L. Fernandez,D.J. McNamara, Dietary fat saturation and chain length modulate guinea pig hepatic cholesterol metabolism, J. Nutr. 124 (1994) 331–339.

[17] M.L. Fernandez, Guinea pigs as models for cholesterol and lipoprotein metabolism, J. Nutr. 131 (2001) 10–20.

[18] S. Roy, S. Vega-Lopez, M.L. Fernandez, Gender and hormonal status affect the hypolipidemic mechanisms of dietary soluble fiber in guinea pigs, J. Nutr. 130 (2000) 600–607.

[19] L. He, M.L. Fernandez, Dietary carbohydrate type and dietary fat saturation independently regulate hepatic cholesterol and LDL metabolism in guinea pigs, J. Nutr. Biochem. 9 (1998) 37–46.

[20] C. Allain, L. Poon, C. Chan, W. Richmond, P. Fu, Enzymatic determination of total serum cholesterol, Clin. Chem. 20 (1974) 470–475.

[21] T.P. Carr, C.J. Andressen, L.L. Rudel, Enzymatic determination of triglycerides, free cholesterol and cholesterol in tissue lipid extracts, Clin. Chem. 26 (1993) 3942.

[22] T. Redgrave, C. Roberks, C. West, Separation of plasma lipoproteins by density gradient ultracentrifugation, Anal. Biochem. 65 (1975) 42–49.

[23] M.L. Fernandez, T.A. Wilson, K. Conde, M. Vergara-Jimenez, R.J. Nicolosi, Hamsters and guinea pigs differ in their plasma lip,oprotein cholesterol distribution when fed diets varying in animal protein, soluble fiber or cholesterol content, J. Nutr. 129 (1999) 1323–1332.

[24] M.A. Markwell, S. Haas, L. Bieber, N.E. Tolbert, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples, Anal. Biochem. 87 (1978) 206–210.

[25] L. Homsquit, K. Carlson, A.L. Carlson, Comparisons between the use of isopropanol and tetramethylurea for the solubilization and quantitation of human serum very low-density lipoproteins, Anal. Biochem. 88 (1987) 457–460.

[26] J.M. Chapman, G.L. Mills, J.H. Ledford, The distribution and partial characterization of the serum apolipoproteins in the guinea pig, Biochem. J. 149 (1975) 423–436.

[27] M. Van Heek, D. Zilversmit, Mechanisms of hypertriglyceridemia in the coconut oil/cholesterol-fed rabbit. Increased secretion and decreased catabolism of VLDL, Arteriosclerosis. Thromb. 11 (1991) 918–927.

[28] G.R. Wamick, J. Benderson, J.J. Albers, Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-Iipoprotein cholesterol, Clin. Chem. 28 (1982) 1379–1388.

[29] H. Puhl, G. Waeg, H. Esterbauer, Methods to determine oxidation of lowdensity lipoproteins, Meth. Enzymol. 233 (1994) 425–441.

[30] J. Sheperd, C.J. Packard, S.M. Grundy, D. Yeshurun, A.M. Gotto, O.D. Taunto, Effects of saturated fat and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man, J. Lipid. Res. 21 (1980) 91–99.

[31] R.J. Nicolosi, A.F. Stucchi, M.C. Kowal, D.M. Hennesy, E.J. Schaefer, Effect of dietary fat saturation and cholesterol on LDL composition and catabolism: in vivo studies of receptor and non-receptor-mediated catabolism of LDL in cebus monkeys, Arteriosclerosis. 10 (1990) 119–128.

[32] M.L. Fernandez, E.C.K. Lin, D.J. McNamara, Regulation of guinea pig plasma low density lipoprotein kinetics by dietary fat saturation, J. Lipid. Res. 33 (1992) 97–109.

[33] W.P. Castelli, Cholesterol and lipids in the risk of coronary heart disease. The Framingham Heart Study, Can. J. Cardiol. 4 (1988) 5A–10A.

[34] R.M. Krauss, Dense low density lipoproteins and coronary artery disease, Am. J. Cardiol. 75 (1995) 538–578.

[35] M.L. Fernandez, S. Vega, M.T. Ayala, H. Shen, K. Conde, M. Vergara-Jimenez, A. Robbins, The interactive effects of vitamin C deficiency and dietary fat saturation on hepatic cholesterol and plasma lipoprotein metabolism in the guinea pig, J. Nutr. Biochem. 8 (1997) 414–424.

[36] M. Vergara-Jimenez, H.C. Furr, M.L. Fernandez, Pectin and psyllium decrease the susceptibility of LDL to oxidation in guinea pigs, J. Nutr. Biochem. 10 (1999) 118–124.

[37] G. Abdel Fattah, M.L. Fernandez, D.J. McNamara, Regulation of

LDLLDL apo B kinetics by dietary fat saturation and chain length in the guinea pig, Lipids. 33 (1998) 23–31.

[38] G, Abdel Fattah, M.L. Fernandez, D.J. McNamara, Regulation of hepatic VLDL secretion rates by dietary fat saturation in the guinea pig, J. Lipid. Res. 36 (1995) 1188–1198.

[39] C.M. Cox, W.H. Sutherland, M.J. Ball, J. Mann, Response of plasma lanostherol concentration to change in the quality of dietary fat in men and women, Eur. J. Clin. Nutr. 50 (1996) 358–363.

[40] M.L. Fernandez, M. Vergara, A.L. Romero, S. Erickson, D.J. McNamara, Gender differences in plasma and hepatic hypocholesterolemia induced by soluble fiber. Effects of pectin, guar gum and psyllium, J. Lipid. Res. 36 (1992) 2191–2202.

[41] T.P. Carr, S.J. Parks, L.L. Rudel, Hepatic ACA T activity in African green monkeys is highly correlated to plasma LDL cholesteryl ester enrichment and coronary artery atherosclerosis. Arterioscler. Thromb. 12 (1995) 1274–1283.