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Ovariectomized hamster: A potential model of postmenopausal hypercholesterolemia

Eugenia Sohn,* Bruce P. Daggy,† and Bahram H. Arjmandi†

**Department of Human Nutrition and Dietetics, University of Illinois at Chicago, Chicago, IL USA; and †Department of Nutritional Sciences, Oklahoma State University, Stillwater, OK USA*

A suitable and economical animal model of ovarian hormone deficiency can greatly enhance the understanding of postmenopausal-elevated risk of coronary heart disease. The male Golden Syrian hamster is a well-established small animal model of hypercholesterolemia, but the effect of ovariectomy on lipid profile in the female hamster is unclear. The objective of this study was to determine whether ovariectomized hamsters develop hypercholesterolemia and experience changes in body fat distribution consistent with changes observed in postmenopausal women. Twenty-two 90-day-old female Golden Syrian hamsters were divided into two groups and were either ovariectomized or sham-operated and given free access to a standard cholesterol-free laboratory diet for 65 days. Ovariectomized hamsters had significantly ($P < 0.05$*) elevated serum total cholesterol concentrations (16.6%) as well as abdominal fat mass (56%;* $P < 0.01$*) despite equal food intake compared with the sham-operated group. In contrast, the mean intestinal weight and in vivo rate of sterol biosynthesis were significantly (*$P < 0.002$ *and* $P = 0.01$*, respectively) lower in the ovariectomized compared with the sham-operated group. In vivo rates of hepatic sterol biosynthesis were directionally lower (*$P = 0.1$*) in the ovariectomized group. No significant differences were observed in final body weight, serum triglycerides, or liver total cholesterol and lipids between the two groups. In conclusion, ovariectomized hamsters undergo changes in serum cholesterol and fat distribution similar to those experienced by postmenopausal women, and thus may serve as an appropriate model for postmenopausal hypercholesterolemia.* (J. Nutr. Biochem. 10:660–663, 1999)

Keywords: sterol biosynthesis; intestine; hepatic; estrogen

Introduction

Elevated total and low density lipoprotein (LDL) cholesterol levels among women who have experienced natural or surgical menopause have been linked to the absence of estrogen, placing them at a higher risk for cardiovascular disease (CVD).[1] Epidemiologic data have shown that a woman's risk for CVD doubles after natural menopause and quadruples after surgical menopause.[2] A longitudinal study by Fukami et al.[3] showed increases of 14% and 19% in serum concentrations of total and LDL cholesterol concentrations, respectively, in women undergoing menopause. Bruschi et al.[4] observed that in women who had undergone bilateral ovariectomy, serum total and LDL cholesterol levels had increased by 23% and 39%, respectively, 3 months after surgery, whereas serum high density lipoprotein (HDL) levels decreased by 20%.

Estrogen replacement therapy (ERT) reduces the risk of CVD at least partially through the modulation of serum cholesterol.[5,6] However, the cardiovascular protective effects of ERT may extend beyond its influence on cholesterol and lipid metabolism and also may depend on the formulation, dose, and duration of the selected therapy.

The male golden Syrian hamster is a small animal model that is frequently used for studying cholesterol metabolism and atherogenesis.[7–11] The rate of cholesterol synthesis in male golden Syrian hamsters can be easily altered in response to changes in cholesterol intake. Low doses of dietary cholesterol inhibit synthesis of cholesterol in the hamster, allowing a considerable influx and efflux of

Address correspondence to Dr. Bahram H. Arjmandi, Department of Nutritional Sciences, 416 Human Environmental Sciences, Stillwater, OK USA 74078-6141.
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655 Avenue of the Americas, New York, NY 10010
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cholesterol and bile acids independently from cholesterol synthesis. The hamster liver reacts more sensitively than that of the rat in response to a negative sterol balance, with an increase in LDL receptor activity and a less robust alteration in hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase activity. Overall, the plasma lipoprotein profile, bile acid pool composition, and metabolic responses to dietary changes make the hamster more comparable to humans than other frequently used animal models such as the rat or rabbit.

Similar to male hamsters, female hamsters respond to high fat diets by developing hypercholesterolemia.[11] However, this diet-induced hypercholesterolemia does not model the postmenopausal-associated rise in cholesterol in humans.

The aim of this study was to determine whether hamsters rendered ovarian hormone deficient would have altered lipoprotein profiles, thus serving as a readily available small animal model of postmenopausal hypercholesterolemia. Furthermore, we examined several points of regulation of cholesterol metabolism to begin to address the mechanism underlying any observed changes. Alterations in abdominal fat also were assessed, because increases in abdominal fat have been observed in postmenopausal women.[12]

Materials and methods

Animals and diets

Twenty-two female 90-day-old Golden Syrian hamsters were given free access to a standard cereal-based, laboratory diet (Teklad diet #8640, Teklad, Madison, WI USA). Prior to surgery, hamsters were acclimated for 7 days and then were randomized by initial body weight into two treatment groups of 11 hamsters each. Hamsters were housed in an environmentally controlled laboratory with a 14-hour light:10-hour dark cycle. This study was approved by the Animal Care Committee of the University of Illinois at Chicago.

Animal surgery, necropsy, and processing of samples

Hamsters were anesthetized with an intraperitoneal injection of ketamine hydrochloride and xylazine at doses of 100 mg and 5 mg per kilogram of body weight, respectively, and ovariectomy or sham operation was performed bilaterally. After 65 days postoperatively, within 1 hour of the midpoint of the dark cycle, hamsters were slightly anesthetized (mixture of ketamine/xylazine, 100:5 ratio, respectively) and rapidly (less than 10 sec) injected with 1.85 GBq of tritiated water ([^{3}H]water; Amersham Corp., Arlington Heights, IL USA) in 0.5 mL of isotonic NaCl into the femoral vein. Exactly 60 minutes after the injection, each animal was anesthetized with ketamine/xylazine mixture and exsanguinated via the abdominal aorta for measuring serum water activity and lipid and cholesterol analyses. Serum was separated in nonheparinized tubes by centrifugation at 1,500 $\times$ g for 20 minutes at 4°C. Aliquots of serum were frozen and kept at -20°C for later analysis. The liver was immediately removed, rinsed with ice-cold 0.154 mol/L NaCl solution, and processed for determining the rates of [^{3}H]water incorporation into digitonin-precipitable sterols (DPS). Portions of the livers were kept in sealed containers and stored at -70°C for lipid and cholesterol analyses. Similarly, the entire small intestine was removed and freed from any adhering tissues and its contents flushed with isotonic-cold sodium chloride solution. The flushed intestine was blotted and weighed before measuring the rate of sterol biosynthesis.

In vivo intestinal and hepatic rates of sterol biosynthesis

The rates of sterol biosynthesis were assessed in intestinal and hepatic tissues, the key organs involved in the regulation of cholesterol metabolism, to determine whether ovarian hormone deficiency-induced hypercholesterolemia was related to higher rates of sterol synthesis. A modified method of Dietschy and Siperstein[13] was used to assess the in vivo rates of hepatic and intestinal sterol biosynthesis.[14] The specific activity of serum water used to calculate the rates of tritiated water incorporation into DPS was determined according to the method of Jeske and Dietschy.[15] The rate of [^{3}H] incorporated into DPS was calculated as nmol of [^{3}H]water per hour per gram of tissue or whole organ weight.[14]

Serum and liver lipid analyses

Serum total cholesterol and triglycerides (TG) concentrations were measured using enzymatic kits (Sigma Diagnostics, St. Louis, MO USA). Serum HDL cholesterol was determined by precipitation technique.[16] Serum non-HDL cholesterol, which represented the cholesterol carried by apolipoprotein-B-containing lipoproteins, was calculated by difference.

Portions of livers were homogenized, then extracted with a 2:1 (v/v) chloroform:methanol mixture. After the addition of 0.13 mol/L NaCl solution to the extraction and separation of phases, aliquots of the organic phase were analyzed for liver total cholesterol concentrations. Liver total cholesterol level was determined using a color reagent of glacial acetic acid-$FeSO_4$-H_2SO_4.[17] Total liver lipids were determined using the Folch gravimetric method.[18]

Organ weights and abdominal fat

All organs within the abdominal cavity, including the uterus, adrenal glands, intestines, kidneys, liver, and spleen, were removed. Prior to weighing, small incisions followed by blotting were made in the uterus to drain any excess fluid. Visceral fat and any visible fat adhering to the organs or abdominal wall were collected, blotted, and weighed to the nearest milligram.[19]

Statistical analysis

GraphPad Instat Software (version 2.0, 1993; San Diego, CA USA) was used for all statistical analyses.[20,21] Descriptive statistics are expressed as mean $\pm$ SEM. Unpaired Student's *t*-tests were performed to determine whether there were significant ($P < 0.05$) differences between groups.

Results

Food intake and body and organ weights

Mean food intakes of the hamsters were not significantly affected by ovariectomy (*Table 1*). Animals in both groups started with similar mean body weights and gained weight similarly with no differences in mean final body weights (*Table 1*). As expected, the relative mean uterine weight (g/100 g body weight) of the ovariectomized group was significantly ($P < 0.0001$) lower than the sham-operated animals, confirming the success of ovariectomy. The overiectomized group had significantly ($P < 0.01$) greater mean

Table 1 Effects of ovariectomy on food intake and body and organ weights in hamsters*

Variable	Sham	Ovariectomy
Food intake (g/day)	10.3 ± 0.3	9.9 ± 0.3
Initial body weight (g)	113 ± 3	114 ± 1
Final body weight (g)	176 ± 1	175 ± 7
Uterine weight (g/100 body wt)	0.30 ± 0.03	0.07 ± 0.01[a]
Abdominal fat (g/100 body wt)	2.5 ± 0.2	3.9 ± 0.4[a]
Liver weight (g/100 body wt)	3.9 ± 0.2	3.7 ± 0.2
Small intestine weight (g/100 body wt)	1.8 ± 0.03	1.48 ± 0.08[a]

*Values reported are mean ± SE, $n = 10–11$.
[a]Significantly ($P < 0.05$) different from sham.

abdominal fat in comparison with the sham group. No significant differences were found between relative mean liver weights, but the mean relative small intestine weight of the ovariectomized group was significantly ($P < 0.002$) lower than that of the sham-operated group.

Serum total and HDL cholesterol and TG concentrations

Serum total and HDL cholesterol levels were significantly ($P < 0.05$) higher in the ovariectomized group than in the sham-operated group (*Table 2*). There was a tendency ($P = 0.2$) for non-HDL cholesterol concentrations to be higher in the ovx group. Serum TG levels were not affected by ovariectomy (*Table 2*).

Liver lipids and liver and small intestinal rates of sterol biosynthesis

No significant differences were detected between mean liver total lipid and total cholesterol concentrations of sham-operated and ovariectomized groups (*Table 2*). The mean rate of liver sterol biosynthesis was slightly higher in the sham-operated group than in the ovariectomized group as expressed per gram of liver. These differences approached statistical significance, particularly for the whole liver ($P = 0.1$).

The intestinal rates of sterol biosynthesis were significantly ($P < 0.01$) lower in ovariectomized hamsters in comparison to the sham-operated, whether expressed on a per gram or whole organ basis.

Table 2 Effects of ovariectomy on liver total lipids and cholesterol and serum triglycerides in hamsters*

Parameters	Sham	Ovariectomy
Serum (mmol/L)		
Total cholesterol	3.31 ± 0.20	3.86 ± 0.16[a]
HDL cholesterol	1.31 ± 0.06	1.55 ± 0.08[a]
Non-HDL cholesterol[†]	2.01 ± 0.18	2.32 ± 0.15
Triglyceride	2.43 ± 0.33	2.49 ± 0.33
Liver and small intestine		
Liver total lipids (mg/g)	32 ± 0.1	32 ± 0.1
Liver total cholesterol (μmol/g)	12.2 ± 0.6	12.5 ± 0.7
nmol [^{3}H]DPS/(g liver · h)	92 ± 6	74 ± 12
nmol [^{3}H]DPS/(whole liver · h)	625 ± 52	485 ± 83
nmol [^{3}H]DPS/(g intestine · h)	179 ± 8	107 ± 11[a]
nmol [^{3}H]DPS/(whole small intestine · h)	332 ± 40	183 ± 100[a]

*Values reported are means ± SE, $n = 10–11$.
[a]Significantly ($P < 0.05$) different from sham.
[†]Non-HDL cholesterol = total cholesterol − HDL cholesterol.
HDL–high density lipoprotein. DPS–digitonin-precipitable sterol.

Discussion

The observed increase in serum total cholesterol concentrations of ovariectomized hamsters resembles those reported in postmenopausal women.[3] The tendency for non-HDL cholesterol to elevate (15%), albeit not statistically significant, is also consistent with clinical experience. These findings, coupled with the known similarities in lipid metabolism between humans and hamsters, begin to qualify the hamster as a model for studying cholesterol metabolism in ovarian hormone deficiency. However, the changes observed in HDL cholesterol concentrations with ovariectomy are not consistent with human experience.

Our observations are consistent with many previous studies in the male hamster in which hypercholesterolemic dietary modifications elevated HDL cholesterol and hypocholesterolemic agents reversed this increase.[22,23] Thus, although the hamster must be viewed primarily as a model of LDL metabolism, the increase we observed in HDL is consistent with a hypercholesterolemic effect of ovariectomy. Unfortunately, LDL cholesterol was not measured in this study, but the trend for non-HDL cholesterol to be elevated makes it likely that an LDL effect will be seen in future studies. These animals were still growing and were fed a low fat diet, which may not represent the fat intake of postmenopausal women. The effect of hamster age at the time of surgery and composition of the basal diet on the response also needs further investigation.

This study did not establish the direct cause of the hypercholesterolemia; however, the results suggest that diminished LDL clearance could be a contributing factor. Sterol synthesis rates in the liver and small intestine were depressed ($P = 0.1$ and $P < 0.01$, respectively); therefore, increased de novo cholesterol synthesis is not suggested as the mechanism. Sterol synthesis rates and LDL receptor activity in the liver tend to be positively correlated. The removal of the ovaries per se eliminates a site of LDL clearance; the hamster ovary is second only to the liver in LDL clearance capacity on a per gram tissue basis.[24] In the absence of increased LDL receptor activity, the reduced combined mass of the liver, small intestine, and uterus could be expected to further diminish LDL clearance. The liver and small intestine are responsible for approximately 80% of whole body LDL clearance in the hamster.[24] Future studies in this model should address the effects of ovariectomy on LDL receptor activity directly.

Reduced hepatic 7α-hydroxylase activity can also, in part, explain the hypercholesterolemia observed in ovariectomized hamsters. The findings of another recent hamster study by our laboratories[25] demonstrated that ovariectomy reduces hepatic 7α-hydroxylase mRNA levels and that estrogen administration significantly reverses this effect. In support of these findings, a study by Colvin et al.[26] found that oral administration of conjugated equine estrogen in

ovariectomized cynomolgus monkeys significantly elevated liver 7α-hydroxylase activity. These and perhaps other mechanisms working alone or in combination appear to contribute to the elevated cholesterol that is associated with ovarian hormone deficiency. Additional studies are needed to further illustrate the mechanisms by which ovariectomy induces hypercholesterolemia in this animal model.

Although we cannot offer an explanation for the ovariectomy-induced atrophy of the small intestine, a reasonable hypothesis is that estrogen exerts a direct effect on intestinal cells and hence in its absence the intestinal tract may become atrophic. Previously, we reported[27] that intestinal cells contain estrogen receptors and that the intestine may be a direct target for the action of estrogen.

The cause of the higher levels of abdominal fat due to ovariectomy cannot be determined by our data. Despite similar initial body weights, food intakes, and final mean body weights, the ovariectomized animals had significantly greater amounts of abdominal fat than the sham-operated animals, suggesting a shift in energy metabolism. Similar body compositional changes in postmenopausal women[28,29] and adult ovariectomized rats[30] also have been observed. A recent study[31] in monozygotic postmenopausal twins discordant for use of ERT showed that the current ERT users had similar body weight, body mass index, and total fat compared with non-users but had lower central fat.

In conclusion, our findings indicate that hamsters rendered ovarian hormone deficient have elevated serum total cholesterol levels in comparison to intact animals. Such a model is of particular relevance given the heightened cardiovascular risk experienced by women who have undergone natural or surgical menopause. These data, in addition to the observed increased levels of abdominal fat, suggest that ovariectomized hamsters may serve as a model of postmenopausal hypercholesterolemia. Further investigations as to whether ovarian hormone deficient hamsters respond similarly to estrogen or other hypocholesterolemic agents is needed. Studies examining questions of this nature are currently underway.

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