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Effects of Dietary Cholesterol and Fasting on Hamster Plasma Lipoprotein Lipids

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Summary: Hamsters are commonly utilized for comparative study of cholesterol metabolism. The present study was conducted to assess the effects of fasting on the plasma lipoprotein cholesterol concentrations of hamsters. Over a period of 3 weeks, adult male Golden Syrian hamsters (n = 32) were fed chow with or without the addition of 2 g/kg cholesterol. Half of the animals consuming each diet were fasted for 18 hours prior to blood sampling. Comparison of diets showed the following increases in those animals receiving cholesterol: total plasma cholesterol (180%) and triacylglycerols (75%), high density (75%), low density (250%), and very low density (560%) lipoprotein cholesterol. Compared with fasted animals, total plasma triacylglycerols were higher in both non-fasted diet groups. Compared with fasted hamsters that had received cholesterol, total plasma cholesterol (mean ± SE mmol/l) was greater (6.36 ± 0.18 vs 5.43 ± 0.21; $p \leq 0.05$) in the non-fasted group, due primarily to higher VLDL cholesterol (2.07 ± 0.18 vs 1.58 ± 0.18; $p \leq 0.05$). There were no differences in HDL cholesterol (2.07 ± 0.05 vs 2.17 ± 0.08) or LDL cholesterol (1.29 ± 0.08 vs 1.37 ± 0.05) between fasted and non-fasted hamsters fed cholesterol. Fasting is not necessary for the study of the plasma HDL cholesterol and LDL cholesterol of hamsters fed cholesterol.

Introduction

Hamsters have been recently used for the comparative study of cholesterol metabolism. In particular, they have been used to study in vivo low density lipoprotein cholesterol kinetics in response to excessive dietary cholesterol (1). Furthermore, hamsters readily develop atherosclerosis in response to consumption of an atherogenic diet (2). Along with moderate expense and housing requirements, these characteristics make hamsters an ideal animal model for comparative lipoprotein lipid research.

Because hamsters have been increasingly utilized to study the effects of new nutritional and pharmacological test substances on lipoprotein lipid metabolism, it was considered necessary to investigate the effects of high dietary cholesterol and fasting on plasma lipoprotein lipids prior to blood sampling from hamsters in our laboratories.

Methods

Adult male Golden Syrian hamsters (n = 32) were fed pelleted grain-based diet (certified Purina Rodent Chow, Ralston Purina, St. Louis, MO, USA) either with or without the addition of 2 g/kg cholesterol for three weeks. Half of the animals consuming each diet were fasted for eighteen hours prior to blood sampling. At the time of food withdrawal for fasting, the animals in the fasted groups were sedated by carbon dioxide gas inhalation, and the cheek pouches were manually emptied of excess food particles. The non-fasted animals were similarly sedated and a sham cheek pouch emptying procedure was performed. At the time of blood sampling, the animals were anaesthetized by carbon dioxide gas inhalation and laparotomies were performed to expose the caudal vena cavae for venipuncture. Euthanasia was effected via exsanguination. Whole blood samples were anticoagulated with potassium ethylenediamine tetraacetate and placed immediately on ice. Laboratory analyses were started immediately after centrifugal isolation of the plasma samples.

The method used for isolation of plasma lipoprotein fractions was sequential ultracentrifugal flotation (3). Very low (VLDL), low (LDL), and high density lipoprotein (HDL) fractions were isolated from KBr/NaCl-adjusted plasma density ranges of

< 1.006, 1.006–1.063, and 1.063–1.210 kg/l, respectively. Total plasma cholesterol and triacylglycerols (glycerol-blanked) concentrations were measured spectrophotometrically on each whole plasma sample in duplicate with high performance, enzymatic methods on a random-access clinical chemistry analyser (Hitachi 705, Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). Cholesterol concentrations were measured similarly on each lipoprotein fraction (VLDL cholesterol, LDL cholesterol, and HDL cholesterol). Triacylglycerol concentrations were also measured on each VLDL fraction.

Statistical analysis was performed by a computer program (RS1, BBN Software Products Corporation, Cambridge, MA) on a minicomputer (VAX 8530, Digital Equipment Corporation, Maynard, MA, USA). The *Wilk-Shapiro* test was utilized to determine group data distributions. Group comparisons were made with two-way *Student*'s t and *Wilcoxon*'s signed-rank tests for parametric and non-parametric data distributions, respectively. References to statistically significant differences were calculated at the $p < 0.05$ level of significance unless otherwise stated.

Results

The results are shown in table 1. Total plasma triacylglycerol concentrations were 9–64% higher in the non-fasted (fed) groups when compared with the fasted groups. The groups that consumed additional cholesterol had significantly higher (54–131%) total plasma triacylglycerol concentrations relative to the groups not fed additional cholesterol. This was due to an approximate 20% increase in VLDL triacylglycerol content in the animals that consumed additional cholesterol.

The animals that consumed additional cholesterol showed a significant increase (163–208%) in total plasma cholesterol when compared with animals not fed additional cholesterol. This increment in total plasma cholesterol was due to a seven-fold increase in VLDL cholesterol, a three-fold increase in LDL cholesterol, and an approximate two-fold increase in HDL cholesterol.

There was no difference in total plasma cholesterol between the fasted and non-fasted animals that consumed cholesterol-free chow only. The non-fasted animals that consumed cholesterol-free chow showed a significant decrease in HDL cholesterol and an increase in VLDL cholesterol when compared with the fasted animals.

The total plasma cholesterol was significantly higher in the non-fasted hamsters that consumed additional cholesterol when compared with those that fasted. This difference was due primarily to higher VLDL cholesterol levels in the non-fasted animals. There was no difference in HDL cholesterol and LDL cholesterol between fasted and non-fasted hamsters fed cholesterol.

Discussion

Plasma cholesterol concentrations in hamsters readily increase with consumption of a diet containing additional cholesterol. Consumption of a chow-based diet containing 2 g/kg cholesterol for three weeks resulted in total plasma cholesterol levels of 5.17–6.46 mmol/l. Similar levels of hypercholesterolaemia have been induced in hamsters by feeding chow containing 5 g/kg cholesterol for 14 days (4). This moderate level of diet-induced hypercholesterolaemia in hamsters is similar to the level of hypercholesterolaemia in human beings that would initiate further analysis of individual plasma lipoprotein lipids for coronary heart disease risk assessment. The increase in total plasma

Tab. 1. Mean ± SE hamster plasma lipid concentrations (mmol/l)

	Chow/Fasted	Chow/Fed	Cholesterol/Fasted	Cholesterol/Fed
Total plasma triacylglycerols	1.24 ± 0.14[b,c,d]	2.03 ± 0.09[a,c,d]	2.87 ± 0.38[a,b]	3.13 ± 0.49[a,b]
Total plasma cholesterol	2.07 ± 0.10[c,d]	2.07 ± 0.08[c,d]	5.43 ± 0.21[a,b,d]	6.36 ± 0.18[a,b,c]
HDL cholesterol	1.29 ± 0.05[b,c,d] (67%)[e]	1.11 ± 0.05[a,c,d] (62%)	2.07 ± 0.05[a,b] (42%)	2.17 ± 0.08[a,b] (39%)
LDL cholesterol	0.41 ± 0.03[c,d] (21%)	0.36 ± 0.03[c,d] (21%)	1.29 ± 0.08[a,b] (26%)	1.37 ± 0.05[a,b] (24%)
VLDL cholesterol	0.23 ± 0.03[b,c,d] (12%)	0.31 ± 0.03[a,c,d] (17%)	1.58 ± 0.18[a,b] (32%)	2.07 ± 0.18[a,b] (37%)
VLDL triacylglycerol	0.68 ± 0.11[b,c,d] (55%)	1.35 ± 0.08[a,c,d] (67%)	2.28 ± 0.37[a,b] (80%)	2.57 ± 0.43[a,b] (82%)

[a] $p < 0.05$ vs Chow/Fasted
[b] $p < 0.05$ vs Chow/Fed
[c] $p < 0.05$ vs Cholesterol/Fasted
[d] $p < 0.05$ vs Cholesterol/Fed
[e] % of total plasma cholesterol or total plasma triacylglycerols, respectively

cholesterol was due primarily to increases of VLDL cholesterol and LDL cholesterol. This finding has also been observed in rabbits (5), pigs (6), and monkeys (7). Human beings fed excessive cholesterol in the form of eggs develop hypercholesterolaemia due primarily to an increase in LDL cholesterol (8). The large increment of plasma VLDL cholesterol in hamsters may be the result of increased circulation of β-VLDL particles caused by consumption of excessive dietary cholesterol (9). The large dietary cholesterol-induced increase in VLDL was accompanied by higher total plasma triacylglycerol values. Increased plasma cholesteryl ester transfer activity has been reported in hamsters fed an atherogenic diet (10), and this would enhance the VLDL cholesterol content. The cholesterol-fed hamsters also showed increased plasma HDL cholesterol when compared with animals fed cholesterol-free chow. Previous work in this laboratory has shown that the HDL particle induced in hamsters by cholesterol consumption is enriched in apolipoprotein A (*B. P. Daggy* unpublished data). This HDL particle does not appear to be similar to the apolipoprotein E-enriched HDL commonly induced by consumption of excessive dietary cholesterol (9). These dietary cholesterol-induced increments in HDL cholesterol and total plasma triacylglycerols differ from the decrease or lack of an effect observed in human beings and other laboratory animals and warrant further investigation.

The need for fasting of hamsters prior to blood sampling for lipoprotein lipid analyses was uncertain. Fasting of hamsters involves more than simply removing the food source, as they often hide food in their bedding and store food in their cheek pouches. Although a period of fasting is routinely recommended for patients before blood sampling for lipoprotein lipid analyses, no significant differences have been observed in the total plasma cholesterol and HDL cholesterol of non-fasted patients and patients fasted for 12 h (11).

There was no difference in total plasma cholesterol between fasted and non-fasted hamsters that consumed cholesterol-free chow. In contrast, total plasma cholesterol values were higher in non-fasting hamsters when compared with fasting animals that had consumed additional cholesterol. No differences in LDL cholesterol were observed between fasting and non-fasting hamsters fed either diet in this study. Compared with the fasting animals, VLDL cholesterol was higher in non-fasting animals irrespective of diet. Non-fasting animals that consumed cholesterol-free chow had lower HDL cholesterol than their fasting counterparts. In contrast, plasma HDL cholesterol values were slightly higher in the non-fasted group than in the fasted animals that had consumed additional cholesterol. The fasting—non-fasting differences in lipoprotein lipids of the hamsters fed cholesterol-free chow resemble the changes observed in human beings after consumption of a fatty meal (12).

Cholesterol-fed hamsters are being increasingly utilized for the comparative study of LDL cholesterol kinetics (1) and the development of atherosclerosis (2). The present study shows that, as in humans, the plasma LDL cholesterol of hamsters increases in response to additional dietary cholesterol. Fasting is not required for the study of plasma HDL cholesterol or LDL cholesterol from hamsters fed cholesterol.

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