Effects of Dietary Cholesterol and its Oxidation Products on Pathological Lesions and Cholesterol and Lipid Oxidation in the Rabbit Liver

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Summary and Implications

The effects of dietary cholesterol (CHO) and cholesterol oxidation productions (COPs) on the induction of pathological lesions in rabbit liver tissues were determined. Rabbits were fed with a chow containing no additives or added with 1 g CHO, 2 g CHO, 0.9 g CHO + 0.1 COPs, 0.8 g CHO + 0.2 g COPs, 0.5 g CHO + 0.5 g COPs, 1.6 g CHO + 0.4 g COPs, or 1.2 g CHO + 0.8 g COPs per kg diet. Liver lesions were induced only when the levels of CHO and COPs in the diet were very high. The amount of CHO in the liver increased when dietary CHO was increased; by comparison, dietary COPs affected liver CHO amounts to a lesser extent. The TBARS (thiobarbituric acid reactive substances) value of the liver samples also increased when dietary CHO and COP levels were elevated, and the TBARS value was more strongly affected by the amount of COPs in the diet than by the amount of CHO. At 6 and 12 weeks, COP levels were highest in the group that received 1.2 g CHO + 0.8 g COPs, followed by the 0.5 g CHO + 0.5 g COPs and 1.6 g CHO + 0.4 g COPs groups; the control (0 g) group showed the lowest COP levels among all groups. This indicated that not only dietary CHO but also COPs were involved in hypercholesterolemia-induced liver lesions when the amount of CHO and COPs was high.

Introduction

Cholesterol (CHO) is a crucial component of the human body, but a high level of CHO is considered a major risk factor for the development of atherosclerosis and coronary heart disease (CHD). Moreover, the intake of cholesterol oxidation products (COPs) also typically leads to the development of atherosclerosis and CHD. CHO is widely distributed in living organisms and is localized mainly in cell membranes in its non-esterified form and in the blood as a component of lipoproteins mainly in its esterified form, and CHO plays a role in numerous physiological and pathological processes. CHO may be derived either from the diet or by means of endogenous synthesis, which occurs primarily in the liver. The liver is also the major site where CHO is removed by being directly secreted into the bile or by being broken down to bile acids; moreover, the liver plays a key role in maintaining whole body CHO homeostasis by controlling the uptake of extracellular CHO, CHO synthesis, and CHO storage. An elevated level of plasma CHO is typically a risk factor for atherosclerosis, and several COPs are cytotoxic, atherogenic, mutagenic, or carcinogenic; furthermore, COPs injure endothelial cells, leading to atherosclerosis. Alterations in hepatic CHO homeostasis caused by dietary or drug interventions potently influence CHO balance and plasma low density lipoprotein (LDL) CHO levels in the body. Thus, dietary CHO and COPs critically affect health because LDL CHO levels are positively correlated with the risk of cardiovascular disease. Metabolic changes in liver tissues serve as a barometer of the potential risk of atherosclerosis and CHD, but the relationship between atherosclerosis and metabolic changes in liver tissues, CHO type, and changes in liver lipids are not well understood.

The objective of this study was to determine the relationship between dietary CHO and COPs and the induction of lesions in liver tissues and the composition of CHO, COPs, TBARS, and fatty acids in the liver tissues of rabbits.

Materials and Methods

- A total of 64 young male New Zealand White rabbits (average weight 3 kg) were used.
- Diets were formulated to have eight CHO and COPS combinations (0 g CHO + 0 g COPs) or chow containing, per kg diet, 1 g CHO, 2 g CHO, 0.9 g CHO + 0.1 g COPs, 0.8 g CHO + 0.2 g COPs, 0.5 g CHO + 0.5 g COPs, 1.6 g CHO + 0.4 g COPs, or 1.2 g CHO + 0.8 g COPs and fed for 12 weeks.
- Four rabbits per treatment were sacrificed by means of pentobarbital overdose (200 mg/kg bodyweight) after 45 and 90 days of feeding.
- Liver tissues were collected and analyzed for liver pathological lesions, cholesterol and COPS content, and TBARS.

Results and Discussion

- The rabbits that were fed the diets containing (per kg diet) 1 g CHO, 0.9 g CHO + 0.1 g COPs, 0.8 g CHO + 0.2 g COPs, and 0.5 g CHO + 0.5 g COPs showed the least vacuolation of hepatocytes in the periacinar region of the hepatic lobule at 6 and 12 weeks.
- At both 6 and 12 weeks, high levels of CHO in the diet significantly increased ($P < 0.05$) CHO content in the liver compared with low levels of CHO in the diet.
- The TBARS values in the liver increased significantly ($P < 0.05$) with an increase in dietary intake of CHO and COPs.
- At 6 weeks, TBARS levels in the liver were significantly higher ($P < 0.05$) in the 1.2 g CHO + 0.8 g COPs diet group than in the other diet groups. At 12

weeks, the 1.2 g CHO + 0.4 g COPs diet group showed the highest levels of TBARS ($P < 0.05$), followed by the 1.6 g CHO + 0.4 g COPs, 0.5 g CHO + 0.5 g COPs, 2 g CHO, and 0.8 g CHO + 0.2 g COPs diet groups, and then the remaining diet groups.

- The COP content in the liver increased significantly ($P < 0.05$) with an increase in feeding periods in all diet groups.
- Both at 6 and 12 weeks, COP levels were highest ($P < 0.05$) in the 1.2 g CHO + 0.8 g COPs group, followed by the 0.5 g CHO + 0.5 g COPs group or 1.6 g CHO + 0.4 g COPs group; the 0-g group showed the lowest COP levels ($P < 0.05$) among all diet groups.
- The rabbits that received 1.6 g CHO + 0.4 g COPs and 1.2 g CHO + 0.8 g COPs exhibited the most severe vacuolation of hepatocytes in the periacinar region of the hepatic lobule, followed by the 2-g-CHO group; however, the 0-g group showed no abnormalities at 6 or 12 weeks.
- After 12 weeks, rabbits fed with 1.6 g CHO + 0.4 g COPs and 1.2 g CHO + 0.8 g COPs developed diffuse severe vacuolation of hepatocytes throughout all areas of the hepatic lobule and exhibited marked hepatocyte swelling.

Conclusion

CHO levels in the liver were significantly increased when the amounts of dietary CHO were increased; by comparison, increasing dietary COPs affected liver CHO levels to a lesser extent. TBARS and COP levels in the liver were elevated with increases in dietary CHO and COPs, and the amount of COPs in diet exerted a larger effect than CHO did on increasing the contents of TBARS and COPs in the liver. The percentages of palmitoleic acid and linolenic acid increased, whereas that of stearic acid decreased with increasing dietary CHO and COPs. In this study, we determined that not only dietary CHO but also COPs were involved in hypercholesterolemia-induced liver lesions when the amount of CHO and COPs was high. However, dietary COPs exhibited a greater influence than did CHO on liver cell damage.

Table 1. Effect of dietary cholesterol and cholesterol oxidation products on cholesterol content in liver

	Cholesterol in liver (mg/g)		
Dietary CHO[1)] + COPs g/kg diet	6 week	12 week	SEM
0 g	1.123^{by}	3.120^{az}	0.156
1g chol	3.231^{bx}	16.727^{aw}	0.405
2g chol	17.795^{bv}	27.735^{av}	0.940
0.9chol + 0.1COPs	2.787^{bx}	10.737^{ax}	0.813
0.8chol + 0.2COPs	2.271^{bxy}	9.004^{axy}	0.714
0.5chol + 0.5COPs	2.580^{bx}	6.890^{ay}	0.948
1.6chol + 0.4COPs	11.072^{bw}	16.218^{aw}	0.894
1.2chol + 0.8COPs	10.558^{w}	11.665^{x}	0.555
SEM	0.446	0.929	-

[a and b] Distinct letters within a row indicate significant differences ($P < 0.05$). [v,w,x,y and z] Distinct letters within a column indicate significant differences ($P < 0.05$). [1)] CHO: natural cholesterol, COPs: cholesterol oxidation products.

Table 2. Effect of dietary cholesterol and cholesterol oxidation products on TBARS in liver

	TBARS in liver (MA[2)] mg/kg)		
Dietary CHO[1)] + COPs g/kg diet	6 week	12 week	SEM
0 g	0.564^{y}	0.598^{y}	0.024
1g chol	0.691^{x}	0.712^{x}	0.016
2g chol	0.736^{bx}	0.832^{aw}	0.021
0.9chol + 0.1COPs	0.745^{x}	0.740^{x}	0.034
0.8chol + 0.2COPs	0.695^{bx}	0.833^{aw}	0.032
0.5chol + 0.5COPs	0.753^{bx}	0.858^{aw}	0.032
1.6chol + 0.4COPs	0.864^{w}	0.874^{w}	0.021
1.2chol + 0.8COPs	0.968^{v}	1.029^{v}	0.020
SEM	0.032	0.016	-

[a and b] Distinct letters within a row indicate significant differences ($P < 0.05$). [v,w,x and y] Distinct letters within a column indicate significant differences ($P < 0.05$). [1)] CHO: natural cholesterol, COPs: cholesterol oxidation products. [2)] MA: malonaldehyde.

Table 3. Effect of dietary cholesterol and cholesterol oxidation products on cholesterol oxidation products in liver

COPs in liver (μg/g)			
Dietary CHO[1)] + COPs g/kg diet	6 week	12 week	SEM
0 g	trace	0.048^{z}	0.003
1g chol	0.030^{by}	0.068^{ay}	0.005
2g chol	0.053^{bwx}	0.109^{awx}	0.006
0.9chol + 0.1COPs	0.042^{bx}	0.074^{ay}	0.004
0.8chol + 0.2COPs	0.055^{bw}	0.094^{ax}	0.005
0.5chol + 0.5COPs	0.055^{bw}	0.096^{awx}	0.004
1.6chol + 0.4COPs	0.054^{bwx}	0.112^{aw}	0.006
1.2chol + 0.8COPs	0.082^{bv}	0.146^{av}	0.006
SEM	0.004	0.006	-

[a and b] Distinct letters within a row indicate significant differences ($P < 0.05$). [v,w,x,y and z] Distinct letters within a column indicate significant differences ($P < 0.05$). [1)] CHO: natural cholesterol, COPs: cholesterol oxidation products.

Table 4. Effect of dietary cholesterol and cholesterol oxidation products on liver lesions: Pathological lesions in liver

Dietary CHO[1)] + COPs g/kg diet	6 week	12 week
0 g	0	0
	0	0
	0	0
	0	0
1g chol	+	+
	+	+
	+	+
	+	+
2g chol	+	++
	++	++
	++	++
	++++	+++
0.9chol + 0.1COPs	+	+
	+	+
	+	+
	+	+
0.8chol + 0.2COPs	+	+
	+	+
	+	+
	+	+
0.5chol + 0.5COPs	+	+
	+	+
	+	+
	+	+
1.6chol + 0.4COPs	+	++
	++	+++
	+++	+++
	++++	++++
1.2chol + 0.8COPs	+	++
	+++	+++
	+++	++++
	++++	++++

0, no abnormalities detected; +, mild vacuolation of hepatocytes in the periacinar region of the hepatic lobule; ++, mild vacuolation of hepatocytes in the periacinar and midzonal areas of the hepatic lobule; +++, moderate vacuolation of cells in the periacinar and midzonal areas of the hepatic lobule, with affected cells showing diffuse vacuolation of the cytoplasm; ++++, diffuse and severe vacuolation of hepatocytes in all areas of the hepatic lobule, with marked swelling of the hepatocytes

[1)] CHO: natural cholesterol, COPs: cholesterol oxidation products.