

Effect of High-Cholesterol Diet on Serum Leptin and Blood Lipid in Rabbits

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Abstract: The purpose of this study was to investigate the effect of high-cholesterol diet on serum leptin levels and blood lipid in rabbits. About 20 rabbits were randomly divided into two groups: high-cholesterol group (n = 10) which was maintained on high-cholesterol diet for 12 weeks and control group (n = 10) which was fed with normal diet for 12 weeks. Blood lipid and leptin analyses were performed at 0, 6 and 12 weeks after the high-cholesterol diet. Compared with the control group, rabbits fed with high-cholesterol diets showed higher levels of serum total cholesterol, low-density lipoprotein cholesterol and leptin.

Key words: Leptin, blood lipid, high-cholesterol diet, hypercholesterolemia, serum, China

INTRODUCTION

Leptin, a product of *ob* gene is synthesized and released mainly by adipose tissue and is involved in the control of body weight through the effects on food intake and energy expenditure (Pellemounter *et al.*, 1995; Halaas *et al.*, 1995). Leptin also has pleiotropic effects including involvement in the development of atherosclerosis (Peelman *et al.*, 2004). Atherosclerosis is formed through the accumulation of Low Density Lipoprotein (LDL-C) that has a direct action and may be oxidized in the intima of the arteries in contact with free radicals generated on this site. It causes the plasma monocytes migrate into the arterial wall and becomes into macrophages to phagocytose the Low Density Lipoproteins (LDL-C) and to form the foam cells. Atherosclerosis is a multifactorial disease determined by the individual's genetic and exacerbated by external factors such as dietary foods high in cholesterol (Cooper *et al.*, 2000). A growing body of evidence shows that leptin is an independent risk factor for cardiovascular diseases (Leyva *et al.*, 1998; Soderberg *et al.*, 1999) even after adjustment for traditional risk factors (Wallace *et al.*, 2001). Furthermore, the leptin Receptor (*ob-R*) was detected in human atherosclerotic lesions (Kang *et al.*, 2000) and the leptin-deficient (*ob/ob*) mice had a reduced inflammatory response and a reduced ability to accumulate cholesterol in macrophage, suggesting a role for leptin in foam cell formation and atherothrombosis development (Kjerrulf *et al.*, 2006; Bodary *et al.*, 2002). Leptin may therefore, be a potential target for treatment of atherosclerosis. Thus, the major purpose of the present study was to investigate the effects of high-cholesterol

diet on lipid profile and serum leptin. The significance of this study has been mainly explained by potential application of its findings to preventive medicine.

MATERIALS AND METHODS

Animals and treatment: All animal experiments were conducted according to the guidelines of Guangxi animal ethical committee for animal experimentation in China. Aout 20 female New Zealand rabbits were housed individually. They were fed with normal diet for 7 days before the study began. Then the animals were randomly assigned to be fed with normal diet (control group, n = 10) and high-cholesterol diet (1% cholesterol, 7.5% protein, 8% fat, n = 10). Peripheral blood sample was collected from ear middle artery. Blood lipid and leptin analyses were performed at 0, 6 and 12 weeks after the high-cholesterol diet. Plasma Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C) and Triglyceride (TG) concentrations were measured by Enzymatic Method (bioMerieux, Lyon, France) using an automated analyzer (Type 7170A, Hitachi).

Leptin protein measurement: Leptin concentrations were measured in serum using ELISA (Market Inc., USA) with a sensitivity of 1 ng mL⁻¹ and no cross-reactivity against other cytokines according to the manufacturer's recommendations. Each sample was assayed in duplicate. Intra-assay and interassay precision variability was <8%.

Statistical analysis: Data were analyzed with SPSS (Ver. 17.0) and are presented as mean±SD unless otherwise

indicated. Comparisons between the intra and intergroup means were analyzed by t-test or one-way ANOVA. Coefficients of correlation (r) were calculated by Pearson correlation analysis. Differences were considered significant at a value of $p < 0.05$ for all tests.

RESULTS AND DISCUSSION

Effects of high-cholesterol diet on serum lipids and body weight parameters: There were no significant differences in serum lipid levels and body weight between the two groups at baseline. After 6 and 12 weeks of high-cholesterol diet, serum concentrations of TC and LDL-C were significantly increased ($p < 0.001$) and serum concentrations of TG and HDL-C were slightly increased (Table 1). There was no significant difference in body weight between the two groups throughout the experiment.

Effect of high-cholesterol diet on serum concentration level of leptin: The baseline concentrations of circulating leptin were not significantly different between the two groups. High-cholesterol diet for 6 and 12 weeks induced significantly increased serum leptin levels ($p < 0.01$) while no change was observed in the control group (Table 2).

Leptin, a circulating hormone secreted mainly by adipose tissues is involved in the regulation of food intake and body weight. The proatherosclerotic effect of leptin has recently received a great deal of attention. Leptin stimulates vascular smooth muscle proliferation (Oda *et al.*, 2001), accelerates vascular calcification (Parhami *et al.*, 2001) and induces oxidative stress in endothelial cells (Yamagishi *et al.*, 2001) which may levels that positively correlated with the development of the contribute to atherogenesis.

Table 1: Blood lipid and lipoprotein levels of all rabbits in the experiment

Groups	n	0 week	6 weeks	12 weeks
TC (mmol L⁻¹)				
Control	10	3.58±0.38	3.720±0.43	3.67±0.63
High-cholesterol	10	3.66±0.79	25.31±1.42*	27.55±2.03*
LDL-C (mmol L⁻¹)				
Control	10	1.33±0.32	1.260±0.34	1.18±0.36
High-cholesterol	10	1.67±0.65	22.47±1.62*	24.28±2.03*
TG (mmol L⁻¹)				
Control	10	0.69±0.18	0.760±0.05	0.88±0.08
High-cholesterol	10	0.73±0.14	1.850±1.17	1.91±0.20
HDL-C (mmol L⁻¹)				
Control	10	1.25±0.06	1.430±0.11	1.28±0.23
High-cholesterol	10	1.39±0.16	1.660±0.32	1.36±0.06

Data are presented as mean±SD; * $p < 0.001$: compared to baseline levels

Table 2: Serum leptin levels of all rabbits in the experiment

Groups	n	Serum leptin (ng mL ⁻¹)		
		0 week	6 weeks	12 weeks
Control	10	3.11±1.03	3.23±0.71	3.21±1.08
High-cholesterol	10	3.25±0.89	8.15±0.65*	8.36±1.12*

Data are presented as mean±SD; * $p < 0.01$: Compared to baseline levels

Interestingly, increased leptin cardiovascular disease were observed in obesity (Leyva *et al.*, 1998; Soderberg *et al.*, 1999; Wallace *et al.*, 2001). Schafer *et al.* (2004) reported that compared with mice maintained on normal chow, there were significantly increased (9 fold) leptin levels observed in wild-type mice placed on an atherogenic and high-fat diet. In the present study, researcher found that high-cholesterol diet induced hypercholesterolemia and elevated serum leptin levels. The changes of leptin were nearly identical to the changes of serum cholesterol concentrations throughout the study while body weights were not significantly different among the groups. These data indicate that hypercholesterolemia itself might induce increased serum leptin levels.

CONCLUSION

High-cholesterol diet induce hypercholesterolemia and elevated serum leptin levels in rabbits. This study indicate that hypercholesterolemia itself might induce increased serum leptin levels.

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