Effects of dietary minerals on cholesterol metabolism in rabbits fed cholesterol-free atherogenic diets

S. Sammam, E.M. Kurowska, N. Guthrie, L. Eckel, and K.K. Carroll

Department of Biochemistry, University of Western Ontario, London, Ontario, Canada

To investigate the effect of dietary minerals on cholesterol metabolism, four groups of rabbits were fed semipurified diets with modified mineral mixes for 7 weeks. Compared with the control diet that contained minerals at the 4% (wt/wt) level (diet A), LDL-cholesterol was significantly higher ($P < 0.05$) in those fed a diet with a lower amount of minerals (diet B). The inclusion of a supplement to diet B to restore the more abundant minerals to the level found in diet A (diet C) had no effect on LDL-cholesterol, however, the inclusion of a trace element supplement (diet D) resulted in a lowering of LDL-cholesterol to the level observed in the control diet. Hepatic, fecal, and total plasma cholesterol concentrations were not statistically different in the four groups. Fecal bile acids were also unaffected by the dietary variations. The data suggest that a reduction in dietary minerals increases the concentration of LDL cholesterol by a mechanism unrelated to the rates of cholesterol and bile acid excretion, and the trace elements are more likely to be involved than the abundant minerals.

Keywords: minerals; cholesterol; bile acids; lipoproteins; casein; rabbits

Introduction

Dietary animal proteins, such as casein, have been shown to raise plasma cholesterol concentrations in a variety of animal species when compared with proteins of plant origin.[1,2] In rabbits, the hypercholesterolemia can be modulated by the type and amount of other dietary components such as carbohydrates, fat, and fiber.[3–5] More recently, the role of dietary minerals in this effect has been highlighted, particularly as the reduction in the amount of mineral mix in the diet exacerbates the hypercholesterolemia.[6,7]

The rise in the plasma cholesterol concentration of rabbits fed casein-containing diets relative to those fed soy protein occurs rapidly[8] and in the absence of dietary cholesterol,[6,8] that is, the hypercholesterolemia is of endogenous origin. The enterohepatic circulation is thought to play an important role in this effect,[9,10] particularly as lower amounts of sterols and bile acids are excreted in animals fed casein relative to controls fed soy protein.[9] Because dietary minerals, such as calcium, can interfere with the enterohepatic circulation,[11] it is possible that a reduction in dietary minerals will reduce the excretion of bile acids by allowing them to remain soluble and available for reabsorption. This could be responsible for the observed increase in the concentration of plasma lipoproteins.

The aims of this study were first to test the hypothesis that the reduction in dietary minerals reduces the excretion of cholesterol and bile acids, and second to determine which group of minerals, those found in larger amounts or the trace elements, is associated with the increase in plasma cholesterol.

Materials and methods

Young male New Zealand White rabbits (approximately 1.5 kg) were obtained from Reimen's Fur Ranches (Guelph, Ontario, Canada) and housed under conditions described previously.[12] The animals were randomly divided into four groups of four animals each and maintained on commercial

Supported by the Heart and Stroke Foundation of Ontario and by Protein Technologies International.
S. Samman is a Postdoctoral Fellow of the Canadian Heart Foundation and recipient of a Travel Grant from the National Heart Foundation of Australia.
K. K. Carroll is a Career Investigator of the Medical Research Council of Canada.
Address reprint requests to Dr. Samir Samman at the Human Nutrition Unit, Department of Biochemistry, University of Sydney, N.S.W., 2006, Australia.
Received November 25, 1991; accepted February 27, 1992.

pelleted Purina Rabbit Chow (Ralston Purina Co., St. Louis, MO, USA) for 1 week.

All animals were adapted to semipurified diets by feeding them a mixture of powdered chow and their respective semipurified diets, for 1 week. Following the week of adaptation, rabbits were fed the diets for an additional 6 weeks. The diets were provided ad libitum and the animals had access to deionized water. The composition of the diets is described in *Table 1.*

The mineral mix was varied among the diets. Diet A, the control diet, contained the Philips-Hart mineral mix at the 4% level. Diet B contained the same mineral mix as diet A but at a lower level (2.5%), with the balance being made up with dextrose. Diet C contained the smaller amount of minerals in addition to a supplement of the more abundant minerals to equal their level in diet A. This supplement contained potassium, calcium, sodium, magnesium, and iron. Diet D consisted of the lower level of minerals with a supplement of the trace elements to equal their level in diet A. This supplement included manganese, iodine, copper, zinc, and cobalt. Thus, the diets were designed to narrow the group of minerals associated with the hypercholesterolemia induced by a reduction in dietary minerals by selectively replenishing the mineral mix with either the abundant minerals or the trace elements. These modifications of the mineral mix are shown in *Table 2.*

Table 1 Composition of the semipurified diets fed to rabbits

Component	Composition
Casein[a]	270
Dextrose[b]	598
Cellufloura[a]	50
Mineral mix[c]	40
Molasses[b] (50% (vol/vol)	30
Corn oil[b]	10
Vitamins[d]	2

[a]Obtained from ICN Life Science Group, Nutritional Biochemicals Division, Cleveland, OH, USA.
[b]Obtained from a local feed mill.
[c]Mineral mix A, B, C, or D. Composition described in more detail in *Table 2.*
[d]Composition of the vitamin mix has been described previously.[13]

At weekly intervals, blood samples were collected from the marginal ear vein of fasted animals and plasma cholesterol concentrations were measured. After 7 weeks on the diet, the concentration of plasma cholesterol and its distribution among the lipoproteins was determined. VLDL ($d < 1.006$ g/mL), IDL (1.006–1.019), LDL (1.019–1.063), and HDL (1.063–1.21) were isolated from plasma by discontinuous ultracentrifugation using an SW 40 rotor.[14] Cholesterol concentrations in the plasma and lipoprotein fractions were determined using an enzymatic method (CHOD-PAP kit; Boehringer Mannheim, Montreal, Quebec, Canada).

Feces were collected over a 24-hour period after the animals had been on the diet for 6 weeks. Feces were freeze dried, homogenized, and a representative sample taken for the analysis of cholesterol and total bile acids. Total bile acids were measured using an enzymatic method.[15] Fecal cholesterol was measured enzymatically (CHOD-PAP kit) after saponification of the feces in ethanolic KOH and extraction with petroleum ether[16] as modified by Huff and Carroll.[9]

The animals were subsequently killed by anesthetic overdose (Euthanyl; Canada Packers, Cambridge, Ontario, Canada) and the livers were excised. Lipids were extracted using the Folch method[17] and total cholesterol concentrations were measured enzymatically (CHOD-PAP kit).

Statistical analysis was carried out using one-way analysis of variance and the Kruskal-Wallis test was used to confirm the results. Subsequently Tukey's test was used to assess differences between the groups. Differences were considered significant at $P < 0.05$.

Results

The plasma cholesterol concentration of all rabbits increased from initial values regardless of the diet fed such that a marked hypercholesterolemia was evident within 2–3 weeks. Compared with group A, plasma cholesterol levels were elevated in groups B and C, while those in group D had a similar plasma cholesterol

Table 2 Composition of the mineral mix[a] included in the semipurified diets (g/100g diet)

	A[b]	B[c]	C[d]	D[e]
Minerals				
Potassium phosphate dibasic	1.288	0.805	1.288	0.805
Calcium carbonate	1.20	0.75	1.20	0.75
Sodium chloride	0.668	0.4175	0.668	0.4175
Magnesium sulphate	0.408	0.255	0.408	0.255
Calcium phosphate monobasic	0.3	0.1875	0.3	0.1875
Ferric citrate (16–17% Fe)	0.11	0.06875	0.11	0.06875
Trace elements				
Manganese sulphate-H_2O	0.0204	0.01275	0.01275	0.0204
Potassium iodide	0.0032	0.002	0.002	0.0032
Copper sulphate-$5H_2O$	0.0012	0.00075	0.00075	0.0012
Zinc chloride	0.001	0.000625	0.000625	0.001
Cobalt chloride-$6H_2O$	0.0002	0.000125	0.000125	0.0002
Dextrose	0	1.5	0.0008	1.49025

[a]Phillips-Hart mineral mix IV, plus cobalt. Obtained from ICN Biochemicals, Cleveland, OH, USA.
[b]This was provided in the diet at 4g/100g.
[c]Provided in the diet at 2.5g/100g with dextrose added (1.5g/100g).
[d]Mix B with abundant minerals restored to the level found in A.
[e]Mix B with trace elements restored to the level found in A.

concentration. However, differences between these values did not reach statistical significance. The hypercholesterolemia was associated with a significant rise in LDL cholesterol in groups B (7.23 ± 0.98 mmol/L, mean ± SE) and C (7.09 ± 0.68) compared with group A (4.48 ± 0.50) ($P < 0.05$) *(Figure 1)*. VLDL, IDL, and HDL were similar in the four groups *(Figure 1)*.

All animals gained weight over the experimental period, with animals fed diet A exhibiting the highest weight gain followed by diets C, D, and B, respectively (mean values shown in legend to *Figure 2*). However, the rate of body-weight gain was variable and poorly correlated with plasma LDL cholesterol concentrations (*Figure 2*).

Despite the differences in LDL cholesterol, hepatic cholesterol was not statistically different among the four groups (*Table 3*), but there was a tendency ($P < 0.09$) for animals in groups B and C to have higher concentrations of hepatic cholesterol. When liver weights were taken into account, animals in group D had a lower hepatic cholesterol content relative to group A, reflecting the smaller liver weight of group D (*Table 3*).

The rate of cholesterol excretion tends to be higher as the amount of dietary minerals is reduced, that is, in diets B, C, and D (*Table 4*). The excretion of bile acids in groups B and C was 28–33% higher than group A, and excretion in group D was 61% lower. Differences in the excretion of bile acids and cholesterol did not reach statistical significance, possibly due to the small number of animals in each group (*Table 4*).

Discussion

These data confirm our previous findings, which showed a rise in LDL cholesterol when the level of dietary

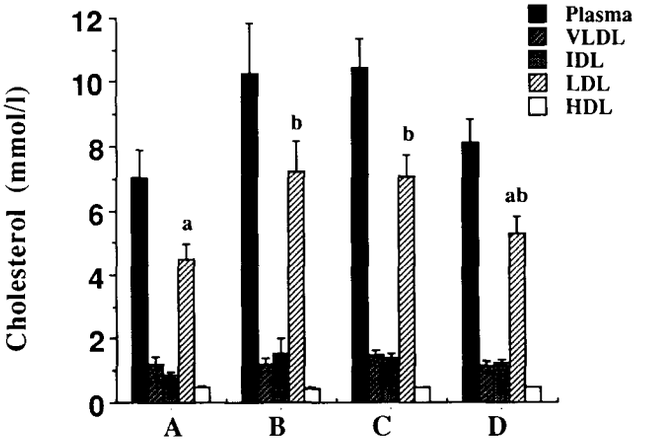

Figure 1 Distribution of cholesterol in plasma and lipoprotein fractions[a] of rabbits fed semipurified diets[b] with varying amounts of minerals.
[a]LDL values not sharing a common superscript are significantly different ($P < 0.05$). Differences between all other means are not statistically significant. Values expressed as mean ± SE.
[b]Diets are defined in *Tables 1 and 2*.

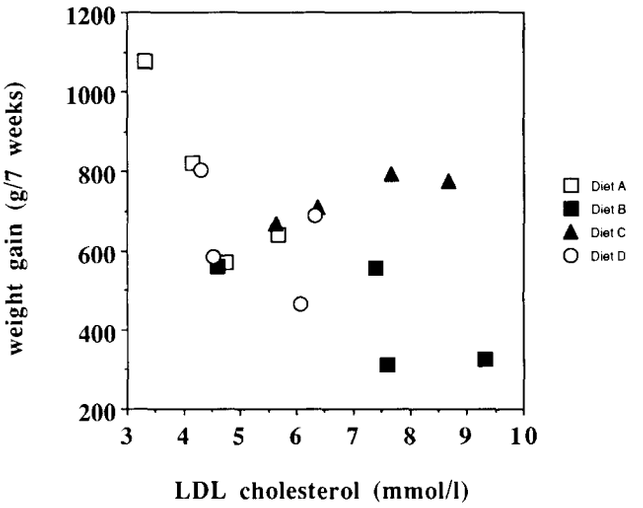

Figure 2 Relationship[a] between weight gain[b] (g/7 weeks) and LDL cholesterol (mmol/L) in rabbits fed semipurified diets[c] with varying amounts of minerals.
[a]The relationship is defined as $y = 976.6 - 1.67x$, $r^2 = 0.21$.
[b]Mean weight gain (g/7 weeks) ± SE are: group A: 777^{a} ± 113, group B: 439^{ab} ± 69, group C: 738^{b} ± 29, group D: 636 ± 73. Values sharing a common superscript are significantly different from each other ($P < 0.02$) using ANOVA and Tukey's test.
[c]Diets are defined in *Tables 1 and 2*.

Table 3 Liver weights and cholesterol concentrations

	Weight	Cholesterol	
	(g, wet weight)	mg/g	mg/total liver weight
A	67.9 ± 6.9	1.48 ± 0.29	102.4 ± 40.4
B	49.6 ± 4.7	1.71 ± 0.22	84.6 ± 17.7
C	59.9 ± 3.2	1.64 ± 0.25	97.1 ± 4.3
D	53.8 ± 5.4	1.29 ± 0.44	67.4 ± 18.2

Values expressed as mean ± SE.

Table 4 Fecal cholesterol and bile acid excretion

	Total bile acids (μmol/day)	Cholesterol (mg/day)
A	30.9 ± 7.8	3.66 ± 1.09
B	43.1 ± 32.3	7.18 ± 5.77
C	46.2 ± 22.5	6.90 ± 4.64
D	12.2 ± 8.1	6.61 ± 8.35

Values expressed as mean ± SE.

minerals was reduced.[6,7] In addition, the data suggest that trace elements are more likely to be involved in this effect than are the more abundant minerals.

Consistent with previous reports,[6–8,10,12,13] regardless of the mineral mix, the plasma cholesterol concentration of all rabbits increased from initial values such that a marked hypercholesterolemia was evident among the four groups (*Figure 1*). However, there were some group differences in LDL cholesterol as the mineral

mix was manipulated. Compared to the control diet (A), LDL cholesterol concentrations were significantly higher for groups B and C. That is, the reduction in minerals induced a rise in LDL cholesterol (group B) and the supplementation of the diet with the more abundant minerals (group C) had no apparent effect on plasma cholesterol or its distribution among the lipoproteins. The replenishment of the low mineral diet with a trace element supplement (group D) resulted in this difference being negated.

Casein-fed animals excrete less fecal sterols than controls fed soy protein,[9] possibly due to a lower content of minerals associated with the diet, particularly the protein source.[6] It was hypothesized that a reduction in the dietary minerals may further decrease the excretion of sterols, causing an increase in the hepatic pool, which in turn stimulates the secretion of cholesterol-rich lipoproteins.[7] However, despite the differential in LDL cholesterol, the excretion of cholesterol and bile acids was not statistically different, possibly due to large variations in the response.

The lack of effect of mineral supplements on plasma cholesterol concentrations (diet C) is in contrast to previous findings. Van der Meer et al.[11] showed that calcium supplements reduce plasma cholesterol, an effect similar to that shown in humans.[18] However, an important difference in this study is the use of a mixed supplement (*Table 2*), not just calcium, which results in greater alterations in amount and ratios of minerals in the diet.

Although the mechanism by which trace elements affect lipoprotein metabolism remains unknown, the effect is likely to represent a balance of the influences of copper, zinc, manganese, iodine, and cobalt (*Table 2*). A review of numerous studies in rats[19] and a case report in a human[20] demonstrated the hypercholesterolemic effect of copper deficiency. The effect appears to be due to an increase in the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase.[21] However, the role of copper is not entirely clear, because in the Watanabe Heritable Hyperlipidemic (WHHL) rabbit[22] (a model for familial hypercholesterolemia) and in hyperlipidemic humans,[23] plasma copper levels are elevated. In addition, the effect is partly dependent on the ratio of zinc:copper.[19]

A limited number of well-controlled studies of the effect of zinc deficiency on cholesterol metabolism have been carried out. They suggest that the deficiency is associated with depressed cholesterol levels,[24] but paradoxically, the activity of HMG-CoA reductase is elevated.[25] From a recent review of the literature, it was concluded that the association between zinc and cholesterol metabolism may be non-specific.[24]

In rats fed high dietary iodine, plasma triacylglycerol and cholesterol levels were lower than controls, an effect thought to be mediated by altered thyroid function.[26] The effect of a decrease in dietary manganese on cholesterol levels in rats is strain-dependent and requires further study. In one report, plasma and HDL cholesterol were reduced in Sprague-Dawley rats,[27] whereas no effect was reported when Wistar or genetically hypercholesterolemic RICO rats were used.[28]

In conclusion, elucidating the mechanism by which minerals affect cholesterol homeostasis is confounded by the complex dietary interactions displayed by minerals. Because their bioavailability and absorption is dependent on the amounts and ratios present in the diet, altering a single constituent of a mineral mix may result in non-direct effects caused by another. To control for non-direct effects, and to help clarify the role of specific minerals, future studies should include measurements of biochemical markers of mineral status.

References

1 Carroll, K.K. (1982). Hypercholesterolemia and atherosclerosis: Effect of dietary protein. *Fed. Proc.* **41,** 2792–2796

2 Terpstra, A.H.M., Hermus, R.J.J., and West, C.E. (1983). The role of dietary protein in cholesterol metabolism. *World Rev. Nutr. Diet.* **42,** 1–55

3 Hamilton, R.M.G. and Carroll, K.K. (1976). Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets. Effects of dietary proteins, carbohydrates and fibre from different sources. *Atherosclerosis* **24,** 47–62

4 Beynen, A.C. and West, C.E. (1981). The distribution of cholesterol between lipoprotein fractions of serum from rabbits fed semipurified diets containing casein and either coconut oil or corn oil. *Z. Tierphysiol. Tierernahrg. U. Futtermittelkde.* **46,** 233–239

5 Bauer, J.E. (1987). Serum lipoproteins of rabbits fed semipurified diets varying in protein and carbohydrate source. *J. Am. Oil Chem. Soc.* **64,** 1183–1192

6 Allotta, E.C., Samman, S., and Roberts, D.C.K. (1985). The importance of the non-protein components of the diet in the plasma cholesterol response of rabbits to casein. *Br. J. Nutr.* **54,** 87–94

7 Samman, S., Khosla, P., and Carroll, K.K. (1990). Influence of dietary minerals on apolipoprotein B metabolism in rabbits fed semipurified diets containing casein. *Atherosclerosis* **82,** 69–74

8 Khosla, P., Samman, S., and Carroll, K.K. (1991). Decreased LDL-receptor activity in casein-fed rabbits precedes the increase in plasma cholesterol levels. *J. Nutr. Biochem.* **2,** 203–209

9 Huff, M.W. and Carroll, K.K. (1981). Effects of dietary protein on turnover, oxidation, and absorption of cholesterol, and on steroid excretion in rabbits. *J. Lipid Res.* **21,** 546–558

10 Beynen, A.C., Van der Meer, R., West, C.E., Sugano, M., and Kritchevsky, D. (1986). Possible mechanisms underlying the differential cholesterolemic effects of dietary casein and soy protein. In *Nutritional Effects on Cholesterol Metabolism,* (A.C. Beynen, ed.), p. 29–45, Transmondial, Voorthuizen, The Netherlands

11 Van der Meer, R., De Vries, H., West, C.E., and De Waard, H. (1985). Casein-induced hypercholesterolemia in rabbits is calcium-dependent. *Atherosclerosis* **56,** 139–147

12 Hrabek-Smith, J.M. and Carroll, K.K. (1987). A comparative study of serum lipoproteins in rabbits fed a natural ingredient diet or low-fat, cholesterol-free semipurified diet containing casein or isolated soy protein. *Biochem. Cell Biol.* **65,** 610–616

13 Roberts, D.C.K., Stalmach, M.E., Khalil, M.W., Hutchinson, J.C., and Carroll, K.K. (1981). Effect of dietary protein on composition and turnover of apoproteins in plasma lipoproteins of rabbits. *Can. J. Biochem.* **59,** 642–647

14 Redgrave, T.G., Roberts, D.C.K., and West, C.E. (1975). Separation of plasma lipoproteins by density-gradient ultracentrifugation. *Anal. Biochem.* **63,** 42–49

15 Van Der Meer, R., De Vries, H., and Glatz, J.F.C. (1985). t-Butanol extraction of feces: a rapid procedure for enzymatic determination of fecal bile acids. In *Cholesterol Metabolism in Health and Disease: Studies in the Netherlands,* (A.C. Beynen,

M.J.H. Geelen, M.B. Katan, and J.A. Schouten, eds.), p. 113–119, Ponsen & Looijen, Wageningen, The Netherlands

16 Miettinen, T.A., Ahrens, E.H., and Grundy, S.M. (1965). Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* **6,** 411–424

17 Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226,** 497–509

18 Bierenbaum, M.L., Fleischman, A.I., and Raichelson, R.I. (1972). Long-term human studies on lipid effects of oral calcium. *Lipids* **7,** 202–206

19 Samman, S. and Roberts, D.C.K. (1985). Dietary copper and cholesterol metabolism. *Nutr. Res.* **5,** 1021–1034

20 Klevay, L.M., Inman, L., Johnson, L.K., Lawler, M.R., Mahalko, J.R., Milne, D.B., Lukaski, H.C., Bolonchuk, W., and Sandstead, H.H. (1984). Increased cholesterol in plasma in a young man during experimental copper depletion. *Metabolism* **33,** 1112–1118

21 Yount, N.Y., McNamara, D.J., Al-Othman, A.A., and Lei, K.Y. (1990). The effect of copper deficiency on rat hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. *J. Nutr. Biochem.* **1,** 21–27

22 Allain, P., Krari, N., Chaleil, D., Balanant, Y., Bled, F., and Girault, M. (1989). The distribution of elements in the tissues of Watanabe Heritable Hyperlipidemic rabbits. *Biol. Trace Elem. Res.* **19,** 153–160

23 Uza, G., Gabor, S., Kovats, A., Vlaicu, R., and Cucuianu, M. (1985). Serum zinc and copper in hyperlipoproteinemia. *Biol. Trace Elem. Res.* **8,** 167–172

24 Samman, S. and Roberts, D.C.K. (1988). Zinc and cholesterol metabolism. *Nutr. Res.* **8,** 559–570

25 Gebhard, R.L., Karouani, R., Prigge, W.F., and McLain, C.J. (1983). The effect of severe zinc deficiency on activity of intestinal disaccharides and 3-hydroxy-3-methylglutaryl coenzyme A reductase in the rat. *J. Nutr.* **113,** 855–859

26 Katamine, S., Hoshino, N., Totsuka, K., and Suzuki, M. (1985). Effects of the long-term (17–19 months) feeding of high-iodine eggs on lipid metabolism and thyroid function in rats. *J. Nutr. Sci. Vitaminol.* **31,** 339–353

27 Davis, C.D., Ney, D.M., and Greger, J.L. (1990). Manganese, iron and lipid interactions in rats. *J. Nutr.* **120,** 507–513

28 Klimis-Tavantzis, D.J., Leach, R.M., Kris-Etherton, P.M. (1983). The effect of dietary manganese deficiency on cholesterol and lipid metabolism in the Wistar rat and in the genetically hypercholesterolemic RICO rats. *J. Nutr.* **113,** 328–336