

## Diets deficient in selenium and vitamin E affect plasma lipoprotein and apolipoprotein concentrations in the rat

BY A. MAZUR, F. NASSIR\*, E. GUEUX, C. MOUNDRAS, J. BELLANGER,  
P. GROLIER, E. ROCK AND Y. RAYSSIGUIER

*Centre de Recherche en Nutrition Humaine de Clermont-Ferrand, Unité Maladies Métaboliques et Micronutriments, INRA, Theix, 63122 Saint-Genès-Champanelle, France*

(Received 20 November 1995 – Revised 25 March 1996 – Accepted 17 April 1996)

The present study examined the effects of Se, vitamin E and combined Se and vitamin E deficiencies in rats on plasma lipid, lipoprotein and apolipoprotein (apo) concentrations. Deficiencies were induced by feeding rats the respective diets for 6 weeks. The study shows that Se deficiency results in increased concentrations of plasma cholesterol and apo E. Both could be explained by an increase in the HDL1 fraction. Vitamin E deficiency alone had no significant effect on plasma lipid, lipoprotein and apo concentrations. Se deficiency in combination with vitamin E deficiency leads to an increase in plasma LDL and apo B concentrations. These results point to the need for further investigations on the mechanism by which Se deficiency affects lipoprotein metabolism.

Selenium: Vitamin E: Cholesterol: Apolipoproteins: Lipoproteins

Animal studies, ecological comparisons, and epidemiological and clinical studies provide several indications that Se may be related to heart diseases, including cardiovascular diseases (for review, see Oster & Prellwitz, 1990). Se, as an essential component of glutathione peroxidase (EC 1.11.1.9; GSH-Px), is implicated in the protection of cells from oxygen-radical-initiated injury (Rotruck *et al.* 1973). A metabolic interrelationship between Se and vitamin E is well known, and the metabolism of one of these is influenced by the other (Fisher & Whanger, 1977). It has been demonstrated that the oxidative damage in many tissues is more severe during double Se and vitamin E deficiency than with deficiency of either antioxidant alone (Arthur *et al.* 1988; You-Zhen *et al.* 1993; Lee & Csallany, 1994; Kuo *et al.* 1995). Additionally, Se and vitamin E may have important roles in biological processes of importance for the cardiovascular system that do not necessarily relate to their antioxidant function (Arthur *et al.* 1993; Dutta-Roy *et al.* 1994).

Hypercholesterolaemia in human subjects is frequently related to an increased risk of atherosclerosis. Even if human studies on relationships between Se and plasma lipids and lipoproteins are inconclusive, animal studies indicate that Se deficiency results in a significant increase in plasma cholesterol (Stone *et al.* 1986). This hypercholesterolaemic effect of Se deficiency appears to be potentiated by the addition of cholesterol to the diet (Stone, 1988; Stone *et al.* 1994). On the other hand, the addition of Se and vitamin E to the diet of cholesterol-fed rabbits has been shown to have a beneficial effect on lipid concentrations and atherosclerotic lesions (Wojcicki *et al.* 1991). However, studies on the influences of Se and vitamin E on lipid and lipoprotein metabolism are scarce. Therefore, the present investigation was undertaken to assess further the influence of Se and vitamin

\* Present address: Department of Medicine, University of Chicago, Chicago, IL 60637, USA.

E deficiencies on plasma lipoproteins and particularly on apolipoproteins (apo) in view of their essential role in the structural and functional specificity of lipoproteins.

## MATERIALS AND METHODS

### *Animals and diets*

Male Wistar weanling rats (IFFA-CREDO, L'Arlesle, France) weighting about 60 (SE 2) g were used for the present study. They were randomly divided into four groups of eight and fed on either the control diet, the Se-deficient diet, the vitamin E-deficient diet or the Se and vitamin E-deficient diet. The basal doubly-deficient diet (containing (g/kg): Torula yeast 300, sucrose 590, tocopherol-stripped lard 50, salt and vitamin mixture without Se and vitamin E 60) was purchased from ICN Nutritional Biochemicals (Orsay, France). Se (0.16 mg/kg) as sodium selenite was added to make the vitamin E-deficient diet, vitamin E (50 mg/kg) as DL- $\alpha$ -tocopheryl acetate (Sigma, l'Isle d'Abeau, France) was added to make the Se-deficient diet, and the control diet was made by adding both nutrients. Rats were housed in pairs in wire-bottomed cages in a temperature-controlled room (22°) with a dark period from 20.00 to 08.00 hours. Diet and distilled water were provided *ad libitum*. Body weight and feed intake were recorded at 1-week intervals. After 6 weeks of the experimental period, on the day of sampling, feed was withdrawn at 08.00 hours. Animals were killed between 09.00 and 11.00 hours by exsanguination from the abdominal aorta after being anaesthetized with sodium pentobarbital (40 mg/kg body weight) given intraperitoneally. Blood was collected into chilled tubes containing EDTA (1 g/l) and plasma was obtained by low-speed centrifugation (2000 g). The liver was excised, blotted free of residual blood on the filter paper, weighed and portions from the right lobe were immediately plunged into liquid N<sub>2</sub> and then stored at -80° for further analyses.

All procedures were in accord with the Institute's guide for the care and use of laboratory animals.

### *Biochemical analyses*

Liver Se was determined spectrofluorimetrically, as previously described (Bellanger *et al.* 1992). Liver vitamin E content was assayed by reverse-phase HPLC apparatus (Kontron serie 400; Kontron, St-Quentin-en-Yvelines, France) using a hexane extract and  $\alpha$ -tocopheryl acetate (Sigma) as an internal standard (Gueux *et al.* 1995). Erythrocyte GSH-Px activity was measured according to the method of Paglia & Valentine (1967) using cumene hydroperoxide as a substrate. Plasma triacylglycerol (Biotrol, Paris, France), cholesterol and phospholipid concentrations (Biomérieux, Charbonnières-les-Bains, France) were determined by enzymic procedures using commercial kits. Liver samples were homogenized and lipids were extracted with chloroform-methanol (2/1, v/v; Folch *et al.* 1957). Triacylglycerol and cholesterol concentrations were measured in the lipid extracts (Mazur *et al.* 1990). To analyse cholesterol distribution among lipoproteins, plasma was subjected to density-gradient ultracentrifugation, as described previously (Moundras *et al.* 1995). Cholesterol concentrations in isolated lipoprotein fractions were measured enzymically (Biomérieux).

### *Plasma apolipoprotein analyses*

Plasma apo concentrations were determined by radial immunodiffusion using sheep anti-rat apo antisera obtained in our laboratory (Felgines *et al.* 1994). Plasma samples were diluted with Nonidiet P40 (5 ml/l final concentration) for apo B and apo E assays and with urea (7 M final concentration) for apo A-I and A-IV assays. Serially-diluted purified rat LDL and HDL fractions were used as assay standards.

### Statistics

Results are expressed as means with their standard errors. Comparison between groups was made by two-way ANOVA using the main effects of Se and vitamin E status. When a significant *F* value was obtained, differences between group means were assessed by Student–Newman–Keuls multiple-range test. *P* < 0.05 was considered statistically significant.

### RESULTS

Body weights and feed intake of rats in the four groups at the end of the experimental feeding are shown in Table 1. The doubly-deficient diet resulted in low body weight and feed intake compared to the other groups. One rat from the doubly-deficient group died after 5 weeks on the experimental diet. Se status was assessed by GSH-Px activity in erythrocytes and by Se content in the liver. In both groups of rats fed on diets without Se, low levels of these measures were consistent with severe Se deficiency (Table 1). Vitamin E status was assessed by the measurement of its hepatic concentration. Both groups of animals fed on diets without vitamin E had low vitamin E levels in the liver (Table 1).

Plasma cholesterol concentrations were significantly greater in Se-deficient, vitamin E-supplemented rats than in other groups (Table 2). The density-gradient-ultracentrifugation study indicated marked alteration in the distribution of lipoproteins in this group compared with other groups (Fig. 1). Hypercholesterolaemia associated with Se deficiency was related to an increased concentration of HDL1. These modifications in lipoprotein distribution in Se-deficient rats were associated with an increase in plasma apo E concentration (Table 2). Doubly-deficient rats had increased apo B and decreased apo A-IV concentrations in the plasma compared with the other experimental groups (Table 2). This increase in apo B concentration was concomitant with an increase in the LDL fraction (Fig. 1). Compared with the control group, vitamin E deficiency alone had no significant effect on plasma lipid, lipoprotein and apo concentrations (Table 2; Fig. 1). Lipid concentrations in the liver were not significantly different among experimental groups (Table 3).

### DISCUSSION

The present results, in agreement with those of Stone and coworkers (Stone 1988; Stone *et al.* 1986, 1994), show that Se-deficiency results in increased plasma cholesterol concentration. Additionally, we have demonstrated that Se-deficient rats displayed an increase in apo E concentration. Both increased plasma cholesterol and apo E concentrations in these rats are related to an increase in the HDL1 fraction, which is particularly rich in apo E (Lusk *et al.* 1979). In rats, most of the plasma cholesterol is associated with HDL which could be separated into at least two major subclasses: the particles with a density ranging between 1.040 and 1.080 kg/l have been referred to as HDL1. Because of its richness in apo E, HDL1 plays an important role in the reverse transport of cholesterol in the rat. It has been found in our previous studies that this lipoprotein fraction in the rat is particularly responsive to dietary conditions (Mazur *et al.* 1990; Nassir *et al.* 1994; Sérougne *et al.* 1995).

The specific increase in HDL1 fraction in Se-deficient rats differs from the earlier study of Stone *et al.* (1986) demonstrating an increase in the LDL fraction. This difference could be explained by the method used for the separation of plasma lipoproteins. In fact, Stone *et al.* (1986) separated lipoproteins by the heparin–Mn(II) precipitation method. Since, in contrast to human studies, there are no data on the specificity of the precipitation methods in the rat, we preferred to isolate lipoproteins by gradient-density ultracentrifugation. Even if some overlap may occur between LDL and HDL1 particles in the rat plasma lipoprotein

Table 1. Final weight, feed intake, selenium and vitamin E status of rats fed for 6 weeks on the control, selenium-deficient, vitamin E-deficient or Se- and vitamin E-deficient diets\*

(Mean values with their standard errors for eight rats per group, except for the group fed on Se- and vitamin E-deficient diet (n 7))

Dietary group ...	Control		Se deficient		Vitamin E deficient		Se and vitamin E deficient		Statistical significance† of effect of:		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Se	Vitamin E	Se x vitamin E
Body wt (g)	213 <sup>a</sup>	9	208 <sup>a</sup>	7	226 <sup>a</sup>	8	160 <sup>b</sup>	11	P < 0.001	NS	P = 0.002
Feed intake‡ (g/d)	17.3 <sup>a</sup>	1.1	15.6 <sup>a</sup>	1.0	18.9 <sup>a</sup>	1.2	12.4 <sup>b</sup>	0.8	P < 0.001	P < 0.001	P < 0.001
Relative liver wt (g/100 g body wt)	4.62 <sup>a</sup>	0.09	4.54 <sup>a</sup>	0.11	4.44 <sup>a</sup>	0.12	5.17 <sup>b</sup>	0.22	P = 0.026	NS	P = 0.006
Liver Se (µg/g dry wt)	1.79 <sup>a</sup>	0.06	0.08 <sup>b</sup>	0.01	1.21 <sup>c</sup>	0.21	0.12 <sup>b</sup>	0.01	P < 0.001	P = 0.028	P = 0.013
Erythrocyte GSH-Px	303 <sup>a</sup>	22	77 <sup>b</sup>	5	311 <sup>a</sup>	13	78 <sup>b</sup>	11	P < 0.001	NS	NS
Liver vitamin E (µg/g wet wt)	10.6 <sup>a</sup>	1.9	8.6 <sup>a</sup>	1.9	1.9 <sup>b</sup>	1.2	2.4 <sup>b</sup>	1.1	NS	P < 0.001	NS

<sup>a, b, c</sup> Group means with different superscript letters in the same row were significantly different (P < 0.05).

GSH-Px, Glutathione peroxidase (EC 1.11.1.9 activity; µmol/min per g haemoglobin).

\* For details of diets and procedures, see pp. 900-901.

† Two-way ANOVA and Student-Newmann-Keuls multiple-range test (NS P > 0.05).

‡ Measured during the last week of the experimental period.

Table 2. Plasma lipids (mmol/l) and apolipoproteins (apo; g/l) in rats fed for 6 weeks on the control, selenium-deficient, vitamin E-deficient or Se- and vitamin E-deficient diets\*

(Mean values with their standard errors for eight rats per group, except for the group fed on Se- and vitamin E-deficient diet (n 7))

Dietary group ...	Control		Se deficient		Vitamin E deficient		Se and vitamin E deficient		Statistical significance† of effect of:		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Vitamin E	Se x vitamin E	
Triacylglycerols	1.19 <sup>a</sup>	0.26	0.65 <sup>a</sup>	0.05	0.97 <sup>a</sup>	0.10	0.83 <sup>a</sup>	0.14	P = 0.040	NS	NS
Total cholesterol	1.63 <sup>a</sup>	0.06	2.01 <sup>b</sup>	0.09	1.80 <sup>ab</sup>	0.09	1.66 <sup>a</sup>	0.09	NS	NS	P = 0.005
Phospholipids	1.70 <sup>ab</sup>	0.02	1.82 <sup>a</sup>	0.04	1.80 <sup>a</sup>	0.03	1.61 <sup>b</sup>	0.08	NS	NS	P = 0.006
Apo B	0.15 <sup>a</sup>	0.01	0.17 <sup>a</sup>	0.01	0.14 <sup>a</sup>	0.01	0.28 <sup>b</sup>	0.06	NS	NS	P = 0.027
Apo E	0.14 <sup>a</sup>	0.01	0.21 <sup>b</sup>	0.01	0.17 <sup>a</sup>	0.01	0.17 <sup>a</sup>	0.01	P = 0.002	NS	P = 0.002
Apo A-I	0.79	0.01	0.84	0.02	0.85	0.03	0.79	0.03	NS	NS	NS
Apo A-IV	0.35 <sup>a</sup>	0.01	0.40 <sup>a</sup>	0.01	0.38 <sup>a</sup>	0.02	0.30 <sup>b</sup>	0.01	NS	P = 0.039	P < 0.001

<sup>a, b</sup> Group means with different superscript letters in the same row were significantly different (P < 0.05).

\* For details of diets and procedures, see pp. 900-901.

† Two-way ANOVA and Student-Newmann-Keuls multiple-range test (NS, P > 0.05).

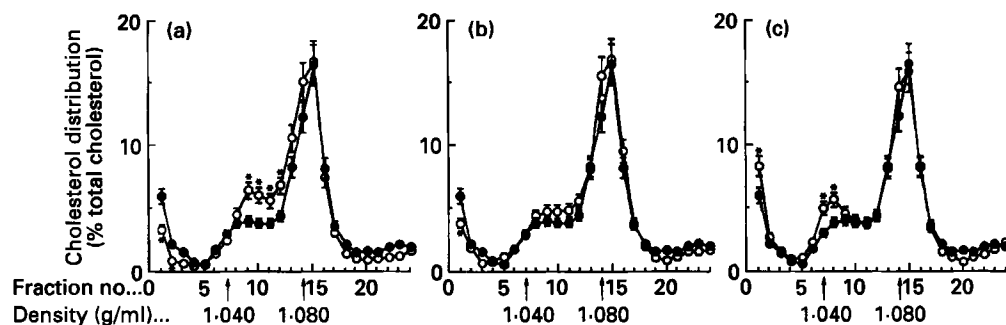


Fig. 1. Cholesterol distribution of lipoprotein fractions separated by density-gradient ultracentrifugation of plasma from control (●) and (a) selenium-deficient (○), (b) vitamin E-deficient (○), and (c) Se- and vitamin E-deficient (○) rats. For details of diets and procedures, see pp. 900–901. Results are expressed as means with their standard errors. Mean values were significantly different from those of the corresponding fractions isolated from control rats: \*  $P < 0.05$ .

profile, an increase in apo E concentration, without an increase of apo B, in Se-deficient rats provides an unequivocal demonstration of the specific increase in HDL1 apo E-rich fraction in these animals.

An increase in HDL1 in Se-deficient rats observed in our study may result from its enhanced formation and/or decreased removal. Increased formation of HDL1 could result from an increased supply of the surface components of triacylglycerol-rich lipoproteins (Eisenberg, 1980). In rat, a species devoid of cholesteryl ester transfer protein (Oschry & Eisenberg, 1982), HDL1 is generated in plasma from more-dense HDL particles that are progressively loaded with cholesteryl esters and enriched with apo E. The surface material released from VLDL lipolysis represents a major source of HDL1-apo E (Oschry & Eisenberg, 1982; Gavish *et al.* 1987). Hepatic synthesis of VLDL in Se-deficient spontaneously-hypertensive rats (SHR) fed on a cholesterol-enriched diet (10 g/kg) was evaluated by using the isolated liver perfusion system (Scott *et al.* 1991). It was found in this work that Se deficiency resulted in increased hepatic production of the lipid moiety of VLDL. The liver perfusate from these Se-deficient rats showed decreased fatty acid oxidation. Thus, it has been suggested that the decreased fatty acid oxidation in the liver of Se-deficient rats provides a stimulus for the secretion of newly-synthesized VLDL. Since Se is a part of the enzyme that catalyses the conversion of thyroxine to the more metabolically active 3,5,3'-triiodothyronine (Arthur *et al.* 1993), a defect in thyroid hormone metabolism has been proposed as a possible mechanism for such a modification in lipid metabolism (Stone *et al.* 1994). An enhanced secretion of VLDL by the liver of Se-deficient rats might support the hypothesis of increased formation of HDL1 by the mechanism described previously. However, the latter study was carried out on cholesterol-enriched (10 g/kg) diet-fed rats and it is well known that cholesterol feeding of animals leads to profound modifications in lipoprotein and apo metabolism (Mahley & Holcombe, 1977; Sérougne *et al.* 1995).

Vitamin E deficiency for 6 weeks, in agreement with the findings of Stone *et al.* (1986), had no discernable effect on plasma lipid and lipoprotein concentrations. In the present work and as shown by several authors (Hafeman *et al.* 1974; Kuo *et al.* 1995), it appears that even if Se and vitamin E deficiency alone have limited effect on animal growth and health, the combination of both leads to growth retardation, reduced feed intake and premature deaths. We showed that doubly-deficient rats had a different pattern of plasma lipoproteins and apo from Se-deficient animals. An increased concentration of plasma LDL which corresponded to increased apo B levels occurred in doubly-deficient rats. This observation concurs with previous work showing that Fischer-344 rats fed on a cholesterol-

Table 3. Liver lipids ( $\mu\text{mol/g}$  wet weight) in rats fed for 6 weeks on the control, selenium-deficient, vitamin E-deficient or Se- and vitamin E-deficient diets\*  
 (Mean values with their standard errors for eight rats per group, except for the group fed on Se- and vitamin E-deficient diet ( $n=7$ ))

Dietary group...	Control		Se deficient		Vitamin E deficient		Se and vitamin E deficient		Statistical significance† of effect of:		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Se	Vitamin E	Se $\times$ vitamin E
Triacylglycerols	5.36	0.91	4.30	0.63	6.10	0.84	5.35	0.59	NS	NS	NS
Total cholesterol	5.35	0.16	5.12	0.13	5.25	0.18	6.28	0.62	NS	NS	NS
Phospholipids	23.0	2.3	23.3	1.5	24.3	1.0	21.6	2.0	NS	NS	NS

\* For details of diets and procedures, see pp. 900-901.

† Two-way ANOVA (NS  $P > 0.05$ ).



free diet deficient in Se and vitamin E developed elevated levels of LDL-cholesterol (Stone *et al.* 1986). The observed decrease in plasma apo A-IV concentrations on doubly-deficient rats indicates that the intestinal absorption of dietary lipids may be affected in these animals since this apo is closely linked to the chylomicron secretion and metabolism (Mahley *et al.* 1984). Previous work has shown that poor growth may lead to an increase in plasma cholesterol in rats (Lefevre & Schneeman, 1983) and that dietary restriction affected LDL metabolism in rabbits (Lacombe *et al.* 1988). Therefore, it may be hypothesized that reduced feed intake and growth retardation in doubly-deficient rats contribute to the decrease in apo A-IV and increase in apo B plasma concentrations. Further work comparing pair-fed Se- and vitamin E-adequate animals and doubly-deficient animals is necessary to determine whether these modifications are due to differences in feed intake *per se* or to the deficiencies.

In conclusion, Se deficiency alone or in combination with vitamin E deficiency affects plasma lipoprotein profiles and apo concentrations differently. Mechanisms related to the increased synthesis and/or impaired catabolism of HDL1 should be evaluated to explain the origin of hypercholesterolaemia in Se-deficient rats. Particular attention should be focused on oxidative modifications of lipoproteins that may occur during Se deficiency and may be of importance for their metabolic fate.

We thank D. Bayle, A. Bellanger, and C. Lab for their excellent technical assistance. This project was supported by grant from ARCOL.

#### REFERENCES

- Arthur, J. R., McPhail, D. B. & Goodman, B. A. (1988). Spin trapping of free radicals in homogenates of heart from selenium and vitamin E deficient rats. *Free Radical Research Communications* **4**, 311–315.
- Arthur, J. R., Nicol, F. & Beckett, G. J. (1993). Selenium deficiency, thyroid hormone metabolism, and thyroid hormone deionidases. *American Journal of Clinical Nutrition* **57**, Suppl., 236S–239S.
- Bellanger, J., Bouvier, J. C. & Lab, C. (1992). Dosage du sélénium dans le vin par spectrofluorimétrie. Application à quelques vins français (Determination of selenium in wine by spectrofluorimetry. Application to some French wines). *Journal International des Sciences de la Vigne et du Vin* **26**, 287–293.
- Dutta-Roy, A. K., Gordon, M. J., Campbell, F. M., Duthie, G. G. & James, W. P. T. (1994). Vitamin E requirements, transport, and metabolism: Role of  $\alpha$ -tocopherol-binding proteins. *Journal of Nutritional Biochemistry* **5**, 562–570.
- Eisenberg, S. (1980). Plasma lipoprotein conversion: the origin of low density and high-density lipoproteins. *Annals of the New York Academy of Sciences* **348**, 30–47.
- Felgines, C., Sérougne, C., Mathé, D., Mazur, A. & Lutton, C. (1994). Effect of simvastatin treatment on plasma apolipoproteins and hepatic apolipoprotein mRNA levels in the genetically hypercholesterolemic rats (RICO). *Life Sciences* **54**, 361–367.
- Fisher, W. C. & Whanger, P. D. (1977). Effects of selenium deficiency on vitamin E metabolism in rats. *Journal of Nutritional Sciences and Vitaminology* **23**, 273–280.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–506.
- Gavish, D., Oschry, Y. & Eisenberg, S. (1987). In vivo conversion of human HDL3 to HDL2 and apo E-rich HDL1 in the rat: effects of lipid transfer protein. *Journal of Lipid Research* **28**, 257–267.
- Gueux, E., Azaïs-Braesco, V., Bussière, L., Grolier, P., Mazur, A. & Rayssiguier, Y. (1995). Effect of magnesium deficiency on triacylglycerol-rich lipoprotein and tissue susceptibility to peroxidation in relation to vitamin E content. *British Journal of Nutrition* **74**, 849–856.
- Hafeman, D. G., Sunde, R. A. & Hoekstra, W. G. (1974). Effect of dietary selenium and liver glutathione peroxidase in the rat. *Journal of Nutrition* **104**, 580–587.
- Kuo, C.-F., Cheng, S. & Burgess, J. R. (1995). Deficiency of vitamin E and selenium enhances calcium-independent phospholipase A2 activity in rat lung and liver. *Journal of Nutrition* **125**, 1419–1429.
- Lacombe, C., Corraze, G. & Nibbelink, M. (1988). Low-calorie diets amplify the response to dietary cholesterol. *Medical Science Research* **16**, 367–371.
- Lee, H.-S. & Csallany, A. S. (1994). The influence of vitamin E and selenium on lipid peroxidation and aldehyde dehydrogenase activity in rat liver and tissue. *Lipids* **29**, 345–350.
- Lefevre, M. & Schneeman, B. O. (1983). Relationship between plasma cholesterol and protein quality in weanling rats. *Nutrition Reports International* **28**, 1369–1374.



- Lusk, L. T., Walker, L. F., Dubien, L. H. & Getz, G. S. (1979). Isolation and characterization of high-density lipoprotein HDL1 from rat plasma by gradient centrifugation. *Biochemical Journal* **183**, 83–90.
- Mahley, R. W. & Holcombe, K. S. (1977). Alterations of the plasma lipoproteins and apolipoproteins following cholesterol feeding in the rat. *Journal of Lipid Research* **18**, 314–324.
- Mahley, R. W., Innerarity, T. L., Rall, S. C. Jr & Weisgraber, K. H. (1984). Plasma lipoproteins: apolipoprotein structure and function. *Journal of Lipid Research* **25**, 1277–1294.
- Mazur, A., Rémésy, C., Gueux, E., Levrat, M. A. & Demigné, C. (1990). Effects of diets rich in fermentable carbohydrates on plasma lipoprotein levels and on lipoprotein catabolism in rats. *Journal of Nutrition* **120**, 1037–1045.
- Moundras, C., Demigné, C., Mazur, A. & Rémésy, C. (1995). The cholesterol lowering effect of steroid sequestrans is modulated by large intestine fermentations. *Journal of Nutritional Biochemistry* **6**, 158–162.
- Nassir, F., Mazur, A., Gueux, E., Sérougne, C. & Rayssiguier, Y. (1994). Apolipoprotein A-I, A-IV and E synthesis in the liver of copper-deficient rats. *Lipids* **29**, 727–729.
- Oschry, Y. & Eisenberg, S. (1982). Rat plasma lipoproteins: reevaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity. *Journal of Lipid Research* **23**, 499–507.
- Oster, O. & Prellwitz, W. (1990). Selenium and cardiovascular disease. *Biological Trace Element Research* **24**, 91–103.
- Paglia, D. E. & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine* **70**, 158–169.
- Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G. & Hoekstra, W. G. (1973). Selenium: biochemical role as a component of glutathione peroxidase. *Science* **179**, 588–590.
- Scott, R. L., Kheshti, A., Heimberg, M., Wilcox, H. G. & Stone, W. L. (1991). The role of selenium in the secretion of very-low-density lipoprotein in the isolated perfused liver. *Biochemical Journal* **279**, 741–745.
- Sérougne, C., Felgines, C., Férézou, J., Hajri, T., Bertin, C. & Mazur, A. (1995). Hypercholesterolemia induced by cholesterol- or cystine-enriched diets is characterized by different plasma lipoprotein and apolipoprotein concentrations in rats. *Journal of Nutrition* **125**, 35–41.
- Stone, W. L. (1988). The effects of cholesterol supplementation on plasma lipoprotein cholesterol levels in rats fed diets deficient in vitamin E and/or selenium. *Nutrition Research* **8**, 1061–1071.
- Stone, W. L., Scott, R. L., Stewart, E. M. & Kheshti, A. (1994). Lipoprotein alterations in the spontaneously hypertensive rat fed diets deficient in selenium and vitamin E. *Proceedings of the Society for Experimental Biology and Medicine* **206**, 130–137.
- Stone, W. L., Stewart, M. E., Nicholas, C. & Pavuluri, S. (1986). Effects of dietary selenium and vitamin E on plasma lipoprotein cholesterol levels in male rats. *Annals of Nutrition and Metabolism* **30**, 94–103.
- Wojcicki, J., Ruzewicka, L., Barcew-Wiszniowska, B., Samochowiec, L., Juzwiak, S., Kadlubowska, D., Tustanowski, S. & Juzyszyn, Z. (1991). Effect of selenium and vitamin E on the development of experimental atherosclerosis in rabbits. *Atherosclerosis* **87**, 9–16.
- You-Zhen, W., Xi-An, J., Jun-Yong, Z. & Guang-Lu, X. (1993). Effect of selenium deficiency on Ca transport function of sarcoplasmic reticulum and lipid peroxidation in rat myocardium. *Biological Trace Element Research* **36**, 159–166.