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The role of selenium in the secretion of very-low-density lipoprotein in the isolated perfused rat liver

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A recirculating liver perfusion system was used to study the effects of dietary selenium (Se) on the hepatic secretion of very-low-density lipoprotein (VLDL). The perfusate from livers of rats fed on a Se-deficient diet incorporated about 50% more [1-^{14}C]oleic acid into triacylglycerol (TG) and cholesteryl esters (ChoEs) than did the perfusate from livers of rats fed on a Se-supplemented diet. Similarly, livers from rats fed the Se-deficient diet secreted more VLDL and incorporated about 60% more [1-^{14}C]oleic acid into VLDL TG and ChoEs than did livers from rats fed the Se-supplemented diet. The liver perfusate from rats in the Se-deficient group also showed significantly decreased fatty acid oxidation. We conclude that Se is a potent modulator of lipoprotein metabolism. A primary action of Se deficiency appears to be a decrease in fatty acid oxidation and a stimulation of fatty acid esterification, leading to increased VLDL TG and ChoEs formation and secretion.

INTRODUCTION

The essential trace element selenium (Se) and vitamin E are micronutrients that play a major role in preventing lipid peroxidation *in vivo*. Se is an essential component of glutathione peroxidase [1], a selenoenzyme that can reduce lipid hydroperoxide (or H_2O_2) to the corresponding alcohols (or water in the case of H_2O_2) [2]. Increasing evidence suggests that lipid peroxidation may be an important factor in the aetiology of atherosclerosis [3,4]. In past experiments, we have observed that Fischer-344 rats fed on a cholesterol-free diet deficient in Se developed elevated levels of plasma total cholesterol [cholesteryl ester (ChoEs) plus cholesterol (Cho); TCho] and low-density lipoprotein (LDL)-TCho compared with age-matched rats fed on an identical diet but supplemented with Se [5]. Our previous data also indicated that this effect could be: (*a*) reversed by dietary Se repletion [5], and (*b*) amplified by the addition of 1% dietary cholesterol [6]. Moreover, the Fischer-344 rats fed the 1% cholesterol diet deficient in both vitamin E and Se [the basal (B)+Cho diet] developed elevated levels of very-low-density lipoprotein (VLDL)-TCho compared with values obtained from rats fed identical diets supplemented with vitamin E alone (the B+E+Cho diet), with Se alone (the B+Se+Cho diet) or with both micronutrients (the B+E+Se+Cho diet) [6].

Lipoprotein-TCho levels were previously measured in spontaneously hypertensive (SHR) rats and in normotensive WKY rats fed on the various diets [7]. SHR rats fed the diet deficient in Se alone (B+E+Cho diet) had increased ($P < 0.05$) levels of VLDL-TCho and LDL-TCho compared with corresponding values in age-matched SHR rats fed the B+E+Se+Cho diet. WKY rats fed the B+E+Cho diet also showed increased ($P < 0.05$) VLDL-TCho, but not LDL-TCho, levels when compared with values for WKY rats fed the B+E+Se+Cho diet. For these reasons, we have selected the SHR rat model to further define the role of Se in modulating lipoprotein metabolism. No differences in systolic blood pressure were observed between SHR rats fed the four experimental diets [7].

In the experiments reported here, we explored the hypothesis that Se deficiency increases the hepatic synthesis and secretion of VLDL, the precursor of LDL. In particular, we measured the net hepatic synthesis of VLDL using the isolated rat liver perfusion system. SHR rats were fed 1% cholesterol diets either supplemented with or deficient in Se. Livers from these animals were perfused *in vitro* and the incorporation of [1-^{14}C]oleic acid into perfusate lipids, perfusate VLDL lipids and ketone bodies was measured. A preliminary account of this work has appeared [8].

EXPERIMENTAL

Chemicals

All chemicals used were reagent grade. Oleic acid (99% purity) was obtained from Nu-Chek-Prep, Elysian, MN, U.S.A. BSA (fraction V; powder; Sigma, St. Louis, MO, U.S.A.) was delipidated and purified as previously described [9]. Silica gel G plates (250 mm thick) were obtained from Analtech, Newark, DE, U.S.A. [1-^{14}C]Oleic acid was purchased from New England Nuclear, Boston, MA, U.S.A.

Animals and diets

Male inbred SHR rats (4 weeks old; 40–50 g) were obtained from Charles River Breeding Laboratories, Wilmington, MA, U.S.A., and randomly divided into two dietary groups (six rats per group). The B+E+Cho group was fed on a Se-deficient diet (less than 0.03 p.p.m. of Se) containing 1.0% cholesterol and 50 mg of all-*rac*-α-tocopherol (1.00 units/mg)/kg of diet. This torula-yeast-based basal diet contained all other necessary nutrients as proposed by the National Research Council [10]. The B+E+Se+Cho group was fed a diet identical to that of the B+E+Cho group, but supplemented with 0.4 p.p.m. of Se in the form of sodium selenite. The diets were prepared in 2 kg batches every 3 weeks (or less) by slowly mixing the constituents to avoid heating, and were stored at 4 °C in airtight plastic bags from which all possible air was excluded during closure. Glass and stainless steel animal feeders (Hazelton Systems, Aberdeen, MD,

Abbreviations used: VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; Cho, cholesterol; ChoEs, cholesteryl ester; TG, triacylglycerol; PL, phospholipid; TCho, total cholesterol (Cho plus ChoEs); SHR, spontaneously hypertensive; NEFA, non-esterified fatty acids.

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Table 1. Composition of basal diet with 1% cholesterol (B + Cho diet)

The vitamin mixture provided (in mg/100 g of diet): ascorbic acid, 99; inositol, 11; choline chloride, 16.5; *p*-aminobenzoic acid, 11; niacin, 9.9; riboflavin, 2.2; pyridoxine hydrochloride, 2.2; thiamin hydrochloride, 2.2; calcium pantothenate, 6.6; biotin, 0.05; folic acid, 0.2; vitamin B_{12}, 0.003. In addition, the vitamin mixture contains (in units/100 g of diet): vitamin A acetate, 1980; calciferol (vitamin D_3), 220. The mineral mix provided (in mg/100 g of diet): $CaCO_3$, 654; $CuSO_4,5H_2O$, 0.72; $Ca_3(PO_4)_2$, 1422; ferric citrate,-$3H_2O$, 64; $MnSO_4,H_2O$, 5.5; potassium citrate, H_2O, 946; KI, 0.16; K_2HPO_4, 309; NaCl, 432; $ZnCO_3$, 1.8; $MgCO_3$, 164.

Ingredients	Content (g/100 g)
Torula yeast	36.00
Sucrose	42.05
Corn oil, tocopherol-stripped	14.50
Vitamin mix	2.20
Mineral mix (Draper)	4.00
L-Methionine	0.25
Cholesterol	1.0

U.S.A.) were filled every 2 days and any uneaten food was discarded to minimize rancidity. The drinking water was deionized water with 3 p.p.m. of chromium added. Both the experimental diets and water were provided *ad libitum*; food intake and body weight were recorded at 2 week intervals. The rats were maintained on a 12 h-light/12 h-dark cycle (light 07:00–19:00 h) and were housed in suspended wire-bottomed cages. The detailed composition of the B + Cho diet is provided in Table 1. All dietary supplies were purchased from United States Biochemical Co., Cleveland, OH, U.S.A. The purified Cho used was analytical grade.

Perfusion studies

The rats were maintained on the experimental diets for 22 weeks. For experiments, rats were anaesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg), and livers were surgically removed from fed rats between 08:00 and 10:10 h and perfused *in vitro* using a recycling perfusion apparatus as described previously [11,12]. Immediately before cannulation of the portal vein, 5 ml of blood was obtained from the abdominal aorta, and the plasma and erythrocytes were separated by centrifugation (15600 *g* for 10 min).

The initial perfusion medium (80 ml) consisted of 25% (v/v) bovine erythrocytes, 40 mg of BSA/ml and 1 mg of glucose/ml in Krebs–Henseleit bicarbonate buffer, pH 7.4 [12]. After a 20 min equilibration period, a 2.5 ml sample of an albumin–oleate complex (containing 6 g of BSA, 1419 μmol of oleic acid and 10 μCi of [1-^{14}C]oleic acid per 100 ml of Ca^{2+}- and Mg^{2+}-free Krebs–Henseleit buffer) was added to the reservoir. About 3 min later (0 h of perfusion), 10 ml of perfusate was removed and analysed as detailed below. The complex was then pumped into the perfusion system at a constant rate of 11.7 ml/h. In this manner the perfusate concentration of non-esterified fatty acids (NEFA) remained constant (0.7 μmol/ml), as did the specific radioactivity of the [1-^{14}C]oleic acid. Samples of perfusate (10 ml) were also collected after 2 h and 4 h of perfusion. The haematocrit of each perfusate was immediately determined and a 2 ml aliquot of perfusate was then added to a tube containing 2 ml of 15% $HClO_4$. The tube was centrifuged at 1000 *g* for 15 min and a 70 μl aliquot of the resulting supernatant was used to estimate the conversion of [1-^{14}C]oleic acid into $HClO_4$-soluble radioactivity, representing the ketone bodies (acetoacetate plus β-hydroxybutyrate) and small amounts of citric acid cycle intermediates and acetylcarnitine [13,14].

The remaining 8 ml of perfusate was centrifuged at 1000 *g* for 15 min and the resulting cell-free supernatant was used to determine the incorporation of [1-^{14}C]oleic acid into newly synthesized lipids, which were extracted and isolated as detailed below. In addition, the 4 h cell-free perfusate was directly analysed for ChoEs [15] and triacylglycerol (TG) mass (Sigma Diagnostics Triglycerides no. 320-A). ChoEs was calculated as the difference between TCho (with cholesterol esterase in the assay) and free Cho (without cholesterol esterase in the assay). After 4 h the remainder of the perfusate was taken and the erythrocytes were removed by low-speed centrifugation. The VLDL secreted by the perfused liver, i.e. the perfusate VLDL, was isolated from the cell-free perfusate by ultracentrifugation as previously described [16,17]. At the end of the perfusion, the liver was flushed with 50 ml of ice-cold 0.9% NaCl, cleansed of any adherent extrahepatic tissue, blotted and weighed. A 0.5 g portion of liver was homogenized in 100 ml of ice-cold 1.15% KCl for measurement of glutathione peroxidase activity. A 1.0 g liver sample was also homogenized in 6.5 ml of methanol (with 1 mg of butylated hydroxytoluene/ml), and the lipids were extracted, separated and analysed as detailed below.

Lipid extraction and analyses

Lipids were extracted from samples of cell-free perfusate, perfusate VLDL and liver homogenate with chloroform/methanol (2:1, v/v) by the Folch method [18]. The extractions of the perfusate VLDL and the liver homogenate were done in duplicate in order to measure both the mass of each individual lipid component and the amount (μmol) of [1-^{14}C]oleic acid incorporation into each lipid fraction. The washed and filtered chloroform lipid extracts were quantitatively applied to silica gel G plates (250 μm pore size) in order to separate the lipid classes [TG, Cho, NEFA, ChoE and phospholipid (PL)]. The plates were developed in a light petroleum (b.p. 35–65 °C)/diethyl ether/acetic acid (84:15:1, by vol.) and the lipid classes were visualized with 0.01% Rhodamine 6G under u.v. light. The radioactivity in each lipid class (from one plate) was determined by liquid-scintillation spectroscopy using a Scintiverse II Scintanalyzed scintillation cocktail. The masses of NEFA [19], Cho, ChoEs [20], PL [21] and TG were determined using the second duplicate plate. For each perfusion the specific radioactivity of perfusate NEFA (d.p.m. of ^{14}C/μmol) was calculated (at each time point). The specific radioactivity was used to calculate the amount (μmol) of [1-^{14}C]oleic acid incorporated into a lipid class. The measured haematocrit at each time point was used to relate the radioactivity in a given volume of the cell-free perfusate to the total volume of perfusate. The protein content of VLDL was determined by a modification of the Lowry procedure [22].

Glutathione peroxidase activity

Glutathione peroxidase is a selenoenzyme and its activity in the liver is a good measure of Se status [23]. Glutathione peroxidase activity was measured by the coupled method of Paglia & Valentine [24] using cumene hydroperoxide, a water-soluble organic hydroperoxide, as a substrate.

Statistics

Values are reported as means ± S.E.M. The statistical significance of the data was determined using a two-tailed Student's *t* test.

Table 2. Glutathione peroxidase activities, liver weights and body weights for SHR rats fed either the Se-deficient (B+E+Cho) or the Se-supplemented diet (B+E+Se+Cho) containing 1% cholesterol for 22 weeks

Data are means ± S.E.M. ($n = 6$). Glutathione peroxidase activity is given as nmol of NADPH oxidized/min per mg of protein. *$P < 0.01$ compared with B+E+Cho.

Dietary group...	B+E+Cho	B+E+Se+Cho
Glutathione peroxidase (nmol/min per mg)	118±8	1015±80*
Liver weight (g)	15.2±0.5	15.3±0.5
Body weight (g)	340.6±8.1	321.3±7.3

Table 3. Incorporation of [1-^{14}C]oleic acid into perfusate TG, ChoEs and acid-soluble radioactivity

Livers from SHR rats fed on the B+E+Cho diet or the B+E+Se+Cho diet were perfused with [1-^{14}C]oleic acid for 4 h. The incorporation of [1-^{14}C]oleic acid (per g of liver) into perfusate TG, ChoEs and acid-soluble radioactivity is shown as a function of time. Data are means ± S.E.M. *$P < 0.05$ compared with B+E+Cho.

		Incorporation of [1-^{14}C]oleic acid (μmol/g of liver)	
	Dietary group	2 h	4 h
TG	B+E+Cho	0.952±0.120	2.52±0.21
	B+E+Se+Cho	0.592±0.087*	1.49±0.09*
ChoEs	B+E+Cho	0.162±0.017	0.302±0.032
	B+E+Se+Cho	0.106±0.008*	0.196±0.011*
Acid-soluble radioactivity	B+E+Cho	5.69±0.62	11.44±1.10
	B+E+Se+Cho	7.64±0.41*	16.13±1.05*

RESULTS

Glutathione peroxidase activities, liver weights and body weights

The data in Table 2 show that SHR rats fed the Se-deficient diet had a lower hepatic glutathione peroxidase activity than rats fed the Se-supplemented diet. The hepatic glutathione peroxidase activity obtained for the Se-supplemented rats was similar to that previously observed in rats fed a standard laboratory diet (Rodent Laboratory Chow 5001; Ralston Purina Co., St. Louis, MO, U.S.A.) [23]. Hepatic levels of glutathione peroxidase activity correlate very well with hepatic levels of Se, as determined by neutron activation analyses [23]. The data in Table 2 demonstrate that the experimental diets produced the anticipated state of Se deficiency in rats fed the B+E+Cho diet.

The body and liver weights for rats in the B+E+Cho group were similar to the values observed for the B+E+Se+Cho group (Table 2). Food consumption of rats in the B+E+Cho (59.5±1.2 g/day per kg body wt.) and B+E+Se+Cho (55.3±1.9 g/day per kg) groups was similar at week 22.

Incorporation of [1-^{14}C]oleic acid into perfusate lipids and VLDL lipids

Table 3 shows the incorporation of [1-^{14}C]oleic acid into the TG and ChoEs synthesized by the perfused livers and secreted into the perfusion medium. After both 2 h and 4 h of perfusion, livers from rats fed the Se-deficient diet showed about a 50% increase ($P < 0.05$) in the amount of [1-^{14}C]oleic acid (μmol) incorporated into the perfusate TG and ChoEs compared with the corresponding values obtained for the livers of Se-supplemented rats. More than 90% of the amount of [1-^{14}C]oleic acid incorporated into the perfusate lipids was found in TG plus ChoEs. Incorporation of [1-^{14}C]oleic acid after 4 h into perfusate PL was significantly less ($P < 0.05$) in livers from the B+E+Cho-fed rats than in those from the B+E+Se+Cho-fed rats (0.114±0.011 versus 0.158±0.012 μmol/g respectively). The specific radioactivities [(3.28±0.14)×10^4 and (3.44±0.14)×12^4 d.p.m./μmol] and the amounts of NEFA (64.2±5.8 and 62.0±10.6 μmol) present in the perfusates at the end of the experiment were very similar for the B+E+Se+Cho and B+E+Cho groups respectively. The net secretion of newly synthesized TG (2.4±0.3 μmol/g of liver) and ChoEs (1.08±0.08 μmol/g of liver) isolated from the liver perfusates of B+E+Cho-fed rats at 4 h were also significantly higher ($P < 0.05$) than corresponding values (1.5±0.1 μmol/g for TG and 0.81±0.06 μmol/g for ChoEs) for the B+E+Se+Cho group.

We also measured the amounts of [1-^{14}C]oleic acid incorporated (μmol/g of liver) into TG and ChoEs of VLDL produced by livers from rats fed the Se-supplemented or -deficient diets. Rats fed the Se-deficient diet showed about a 60% increase ($P < 0.05$) in the incorporation of [1-^{14}C]oleic acid into both TG (2.64±0.28 μmol/g of liver) and ChoEs (0.30±0.02 μmol/g of liver) compared with corresponding values (1.68±0.15 μmol/g of liver for TG and 0.177±0.016 μmol/g of liver for ChoEs) obtained for the B+E+Se+Cho group. About 96% of the total [1-^{14}C]oleic acid label recovered in the perfusate VLDL was found in TG plus ChoEs. The incorporation of [1-^{14}C]oleic acid into perfusate VLDL PL, Cho and NEFA was not significantly different between the Se-supplemented and Se-deficient groups.

Table 4 shows the lipid analyses of perfusate VLDL secreted by livers from rats fed on the experimental diets for 22 weeks. The total output of VLDL lipid (total μmol of lipid/g of liver) produced, as well as the output of TG plus ChoEs, was greater ($P < 0.05$) in the B+E+Cho group than in the B+E+Se+Cho group. Perfusate VLDL TG output was about 52% higher in the B+E+Cho group (2.95±0.42 μmol/g of liver) than in the B+E+Se+Cho group (1.94±0.12 μmol/g). The 52% increase in perfusate VLDL TG output for the B+E+Cho group was similar to the 60% increase in incorporation of [1-^{14}C]oleic acid observed for this dietary group.

The molar relationships between the VLDL lipids for the B+E+Se+Cho and B+E+Cho groups were calculated from the data in Table 4. Lower molar ratios of surface lipids (Cho or PL) to the core lipids (TG plus ChoEs) would be expected for larger VLDL particles. The Cho/TG+ChoEs ratio was 5.7 for the Se-deficient groups and 8.9 for the Se-supplemented group. Similarly, the PL/TG+ChoEs ratio was 13.0 for the Se-deficient group and 18.1 for the Se-supplemented group. The lower molar ratio of surface lipids to core lipids found for the Se-deficient group suggests the presence of larger VLDL particles. The compositions (w/w) of perfusate VLDLs from the B+E+Cho and the B+E+Se+Cho rat livers are also shown in Table 4. VLDL isolated from the B+E+Cho group perfusate was somewhat lower in PL, Cho and protein, but higher in TG content than VLDL isolated from the B+E+Se+Cho group perfusate.

Liver composition

Analyses of the liver lipids at the end of perfusions are provided in Table 5. There were no significant differences in the hepatic lipid levels expressed as μmol/g of liver for rats maintained on either the B+E+Cho or the B+E+Se+Cho

Table 4. Effects of dietary Se on hepatic VLDL lipid mass output and composition

VLDL produced by the livers of rats fed on the B+E+Cho diet or the B+E+Se+Cho diet (for 22 weeks) was isolated after 4 h of perfusion. The data in (*a*) indicate the net secretion of VLDL lipids, and the data in (*b*) indicate the percentage composition. Data are means ± S.E.M. *$P < 0.05$ compared with B+E+Cho.

	(*a*) Output of VLDL lipids (μmol/g of liver) or (*b*) composition (%, w/w)	
Lipid class	B+E+Cho group	B+E+Se+Cho group
(*a*)		
PL	0.46±0.04	0.43±0.03
Cho	0.20±0.01	0.21±0.02
NEFA	0.11±0.04	0.06±0.01
TG	2.95±0.42	1.94±0.12*
ChoEs	0.58±0.03	0.43±0.02*
Total lipid mass	4.31±0.53	3.06±0.18*
(*b*)		
PL	9.8±0.5	12.4±0.5*
Cho	2.3±0.2	3.0±0.1*
NEFA	0.8±0.2	0.7±0.07
TG	69.9±1.7	64.3±1.0*
ChoEs	10.6±0.5	10.5±0.3
Protein	6.6±0.6	9.2±0.3*

Table 5. Hepatic lipid composition and hepatic incorporation of [1-^{14}C]oleic acid

Livers from rats fed on the B+E+Cho diet or the B+E+Se+Cho diet (for 22 weeks) were perfused for 4 h. At the end of the perfusion the hepatic lipid composition (μmol/g of liver) and the incorporation of [1-^{14}C]oleic acid into hepatic lipids (μmol incorporated/g of liver) were determined. Data are means ± S.E.M. *$P < 0.05$ compared with B+E+Cho.

	(*a*) Lipid mass or (*b*) [^{14}C]oleic acid incorporation (μmol/g)	
Lipid class	B+E+Cho	B+E+Se+Cho
(*a*)		
PL	26.6±0.9	25.1±1.3
Cho	3.9±0.2	4.4±0.8
NEFA	0.8±0.5	1.2±0.6
TG	15.0±0.7	13.8±1.0
ChoEs	39.4±3.6	49.9±5.8
Total	85.8±3.0	94.5±6.6
(*b*)		
PL	3.09±0.23	3.31±0.19
Cho	0.10±0.02	0.19±0.02*
NEFA	0.06±0.04	0.14±0.05
TG	8.02±0.59	10.29±0.56*
ChoEs	1.90±0.08	2.58±0.22*
Total	13.17±0.72	16.52±0.90*

diet for 22 weeks. The hepatic incorporation of [1-^{14}C]oleic acid at the end of the perfusion experiment is also shown in Table 5. The amount of [1-^{14}C]oleic acid incorporated (μmol/g of liver) into Cho, TG and ChoE was found to be significantly greater in the perfused livers of the Se-supplemented animals. Moreover, the total amount of [1-^{14}C]oleic acid incorporated into hepatic lipids was greater for the Se-supplemented group.

Effects of Se on fatty acid oxidation

Table 3 shows the amount of [1-^{14}C]oleic acid (μmol) converted into $HClO_4$-soluble radioactivity in the perfusate as a function of time. For both the Se-deficient and the Se-supplemented groups the formation of acid-soluble radioactivity in the liver perfusates was linear with time. Livers from rats fed on the Se-deficient diet showed lowered ($P < 0.05$) levels of acid-soluble radioactivity after both 2 and 4 h of perfusion compared with values obtained for rats fed on the Se-supplemented diet. There was a 29% decrease in the rate of appearance of acid-soluble radioactivity in the perfusate of livers from rats fed the Se-deficient diet compared with the Se-supplemented diet. At the zero time point essentially no acid-soluble radioactivity was found, indicating that the [1-^{14}C]oleic acid label was precipitated with the protein fraction. Similarly, long-chain acyl-CoA and long-chain acylcarnitine were also precipitated with protein in the presence of $HClO_4$ [25].

DISCUSSION

The primary findings of the studies reported here are consistent with the hypothesis that increased levels of plasma TCho, VLDL-TCho and LDL-TCho observed in Se-deficient rats (compared with Se-supplemented rats) result from increased hepatic production of VLDL, which is the metabolic precursor of LDL. In the experiments reported here, plasma levels of Cho and ChoEs were higher ($P < 0.05$) in the Se-deficient rats (38.5±3.6 and 106.6±5.3 μmol/dl of plasma respectively) compared with the values found in the Se-supplemented rats (25.1±1.2 and 87.2±5.0 μmol/dl of plasma respectively). The increased level of plasma non-esterified Cho cannot, however, be directly attributed to an increased secretion of VLDL non-esterified Cho, since this was not observed.

The mechanism whereby Se deficiency causes an increased hepatic production of VLDL is not known, but the data reported here suggest some possibilities for further study. The amount of acid-soluble radioactivity in the perfusate provides a good estimate of ketone body formation [13,14]. As shown in Table 3, the acid-soluble radioactivity measured in the liver perfusates of rats fed the Se-deficient diet was decreased. A decreased ability of Se-deficient livers to oxidize fatty acids could shift the metabolic fate of incoming fatty acids more towards esterification and increased hepatic secretion of VLDL. This notion was not, however, supported by the data shown in Tables 3 and 4, which show that the total amount of [1-^{14}C]oleic acid incorporated into perfusate plus hepatic lipids (i.e. TG plus ChoEs) was not greater in Se-deficient livers (15.9 μmol/g of liver) than in Se-supplemented livers (18.2 μmol/g of liver). Evidently, the decreased oxidation of [1-^{14}C]oleic acid in the Se-deficient livers was not sufficient to cause a measurable shift towards esterification. We cannot, however, rule out the possibility that the decreased fatty acid oxidation in Se-deficient livers provided a stimulus for the secretion of newly synthesized VLDL. Increased VLDL secretion by Se-deficient livers should decrease the level of newly formed hepatic lipid, particularly TG, which is the probable primary stimulus for VLDL formation and secretion. This expectation was validated by the data in Table 4, which show a significantly lower level of [1-^{14}C]oleic acid incorporation into hepatic TG, ChoEs and total hepatic lipids in the Se-deficient group compared with the Se-supplemented group.

The potential relationship between Se deficiency and decreased β-oxidation of fatty acids is not clear. The best characterized role for Se in vertebrates is as an essential cofactor for the antioxidant enzyme glutathione peroxidase. This enzyme is found in the mitochondrial matrix where it helps protect the mitochondrial inner membrane from oxidative damage [26]. Table 2 shows that

Se deficiency resulted in a dramatic decrease in hepatic glutathione peroxidase activity, using a water-soluble organic hydroperoxide as a substrate. It is possible that lipid peroxidation *in vivo* caused by decreased glutathione peroxidase activity has damaged some mitochondrial components necessary for β-oxidation. Carnitine palmitoyltransferase II is also located on the inner face of the mitochondrial inner membrane [27], and oxidative damage to this enzyme could inhibit β-oxidation of fatty acids.

Our experiments suggest that Se is a potent nutritional modulator of lipoprotein metabolism: the only difference between the B+E+Cho diet and the B+E+Se+Cho diet was 400 μg of Se/kg of diet. The relationship between dietary Se status and lipoprotein metabolism may conceivably be of importance in hyperlipoproteinaemia and atherogenesis. Although controversial, some epidemiological studies suggest that Se deficiency in humans is related to an increased incidence of coronary atherosclerosis [28] and ischaemic heart disease [29].

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