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Cholesterol and copper in the liver of rabbit inbred strains with differences in dietary cholesterol response

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Abstract

In order to investigate whether cholesterol intake influences the hepatic copper content of rabbits, we compared the hepatic copper content of two rabbit inbred strains after feeding the animals a control or a cholesterol-rich diet. One strain was not reactive to dietary cholesterol (IIIVO/JU), whereas the other strain was reactive to dietary cholesterol (AX/JU). The coefficient of inbreeding (F) >0.95 for both strains. Dietary cholesterol-reactive rabbits when compared with their non-reactive counterparts had a higher hepatic copper content. The consumption of a hypercholesterolemic diet decreased liver copper concentration (expressed in μg/g dry weight) in both strains of rabbits, which was (in part) due to dietary-induced hepatomegaly. A decrease in the absolute hepatic copper content was found only in the dietary cholesterol-reactive inbred strain. It is discussed that differences in glucocorticoid levels may be responsible for the strain difference in liver copper content. The cholesterol effect on the hepatic copper content in the reactive strain might be caused by an increased bilirubin secretion.

Keywords: Cholesterol; Copper; Inbred strain; Liver; Rabbit

1. Introduction

Copper is an essential trace element that is necessary for adequate functioning of various fundamental biochemical processes [1]. Copper deficiency may have serious, and even lethal, consequences as is shown in Menkes' Disease [2]. Too much copper may also be harmful as is evident from the liver and brain damage seen in the copper storage disorder Wilson's Disease [2]. Maintaining copper homeostasis via a well-functioning copper metabolism is thus a very critical process. Copper metabolism, however, is affected by numerous internal and external factors, among which the pH in the gastrointestinal system, the hepatic and biliary function and the composition of the diet [3,4]. One of the nutrients associated with copper metabolism is cholesterol. For the rabbit, a dramatic decrease in liver copper concentration has been found after feeding a cholesterol-rich diet [5]. In rats, a decrease in liver copper concentration has also been described after feeding a cholesterol-rich diet, while a reversed relationship has also been observed [6,7]. In previous research, we confirmed the decrease in liver copper concentration after feeding rats a hypercholesterolemic diet[1]. However, dietary cholesterol did not reduce the absolute nor the relative copper store of the rats. We concluded that the decrease in liver copper concentration in rats under influence of a hypercholesterolemic diet was not due to a decrease in the amount of hepatic copper, but due to dietary-induced hepatomegaly[1].

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1 De Wolf, I.D. et al. (submitted) Liver copper content of rats hypo- or hyper-responsive to dietary cholesterol.

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These previous findings and the apparent relationship between liver copper concentration and dietary cholesterol in rabbits prompted us to compare the hepatic copper content of rabbit inbred strains that were non-reactive to dietary cholesterol and rabbit inbred strains that were reactive to dietary cholesterol on a diet without added cholesterol and to test whether reactive rabbits show a more pronounced decrease in hepatic copper content than non-reactive rabbits when fed a diet with cholesterol.

2. Materials and methods

The research project was approved by the Animal Experimentation Committee of the Utrecht Faculty of Veterinary Medicine.

2.1. Animals, housing, diets and preparation of samples

At the Department of Laboratory Animal Science (Utrecht, The Netherlands) two rabbit (*Oryctolagus cuniculus*) inbred strains are available: AX/JU, which is a strain reactive after dietary cholesterol strain and IIIVO/JU, which is a strain that is non-reactive after dietary cholesterol[2] [8]. The strains originated from the Jackson Laboratory colony, Bar Harbor, ME, USA [9]. In 1983, the Jackson Laboratory stopped research on rabbits. Breeding pairs of IIIVO/J ($F > 0.99$) and AX/J ($F > 0.80$) were taken over by Van Zutphen, who continued inbreeding at the Department of Laboratory Animal Science, Utrecht University. Since their arrival in Utrecht, the two strains are indicated as IIIVO/JU and AX/JU, and have been propagated by strictly brother x sister mating. From 1983 to 2001, AX/JU and IIIVO/JU were inbred for more than 16 generations ($F > 0.95$ for both strains). The two inbred strains are maintained by brother-sister mating. The rabbits were housed and studied in the Central Laboratory Animal Institute from the Utrecht University.

From weaning (i.e. at the age of 10 weeks) until the start of the experiment, the rabbits were fed a commercial, pelleted, natural-ingredient diet (LKK-20®, Hope Farms BV, Woerden, The Netherlands), containing 25.04 mg Cu/kg diet. According to the manufacturer, the commercial diet (energy density 12.9 MJ/kg) consisted of the following (g/kg diet): total protein, 180; fat, 32; fiber, 152; other carbohydrates, 517. A detailed chemical composition of this commercial rabbit diet has been described previously [10]. The rabbits were housed individually in stainless steel cages with wire mesh floors (Ruco BV, Waalre, The Netherlands) as previously described [11]. The cages were located in rooms with controlled lighting (light from 07:00 to 19:00 hr), temperature (16-19°C) and relative humidity (55-65%).

In the experiment we used adult AX/JU and IIIVO/JU rabbits from both sexes. The animals were fed daily the commercial pelleted diet with or without added cholesterol (0.3 g/100 g diet) for 42 days. Per strain the experimental groups had similar distributions of age (mean age: AX/JU, 79 weeks; IIIVO/JU 99 weeks).

To maintain palatability, the high-cholesterol diet was the commercial chow to which 0.3% (w/w) of cholesterol was added. The cholesterol (USP; Solvay Pharmaceuticals BV, Weesp, The Netherlands) was mixed into the test diets by the manufacturer (Hope Farms BV). During the experiment restricted amounts of diet were given each day at 10:00 a.m. The daily amount of pellets was 100 g for each rabbit. Acidified tap water was provided *ad libitum.* The rabbits were allowed to practice caecotrophy. The cholesterol-rich diet was stored at 4°C until feeding. Body weight was measured at the beginning (day 0) and at the end (day 42) of the experimental period. Food intake was recorded once a week throughout the entire test period. All animals consumed all of the administered food during the test period.

Blood samples were taken on days 0, 7, 14, 21, 28, 35 and 42 in random order between 08.00 and 10.00 a.m. after a 16 hr fasting period. Samples of blood were taken from the lateral ear vein without anesthesia (days 0, 7, 14, 21, 28 and 35) or via heart punction with anesthesia (day 42). Blood was collected in tubes without anticoagulant. To collect serum, the blood in the tubes was allowed to clot and serum was prepared by low-speed centrifugation. The serum samples were stored at - 70°C in the freezer until use.

At the end of the test period, the fasted rabbits were anesthetized in a random order by an intravenous injection of Hypnorm®(Janssen Pharmaceutica BV, Beerse, Belgium) sufficient to reach the surgical phase (approximately 0.3 ml/rabbit). Subsequently, the animals were killed by cardiac exsanguination and the liver was removed. The autopsy wet weight of the livers (without the gallbladder) was determined. From each animal aliquots of the liver (two pieces of 0.5 g from the quadrate liver lobe) were frozen immediately.

2.2. Chemical analyses

Lipids were extracted from liver homogenates according to a modification of the method of Abell *et al.* [12]. The liver samples were homogenized on ice in ten volumes 12.5% (v/v) ethanol with a 180 s burst of an UltraTurrax tissue homogenizer (Janke and Kunkel, Staufen, Germany) at 20000 rev./min. The homogenates were frozen at -20°C, thawed and firmly stirred. From each homogenate 200 μl was taken and 2.0 ml of an ethanol-solution containing KOH (ethanolic alkali: 6 ml of 50%-KOH in a final volume of 100 ml absolute-ethanol) was added. The saponification was carried out in closed tubes overnight at 50°C. After this reaction the tubes were adjusted to room temperature and 2.0 ml distilled water plus 4.0 ml warm petroleum ether (40°C-60°C) was added. The tubes were closed and shaken

2 Meijer, G.W. (1991). Cholesterol metabolism in inbred rabbits with low or high cholesterolemic response to dietary cholesterol. Characterization of an animal model. *Ph.D.-thesis,* Utrecht University.

for 10 min with a frequency of 500 movements/min. The liquids were allowed to separate for 10 min. Three mL of the petroleum-ether fraction was evaporated under nitrogen at 70°C. The residue was dissolved in 0.5 ml of absolute-ethanol and the cholesterol concentration was determined in duplo.

Total cholesterol in the liver lipid extracts and the serum was measured enzymatically according to Siedel *et al.* [13], using a kit (Monotest®) supplied by Boehringer Mannheim GmbH (Mannheim, Germany). Cholesterol analyses were performed on a Cobas-BIO automatic micro-centrifugal analyser (Roche Diagnostics Systems, Hoffmann-La Roche, Basel, Switzerland). The precision of the Cobas-BIO for cholesterol determinations was 2.4% (expressed as coefficient of variation) and its accuracy was 0.6% (expressed as % error). In both experiments for each individual animal the area under the curve (AUC) for the total experimental period was derived from the measured concentrations by the trapezoidal rule. The AUC is frequently used as a summary of the magnitude of the serum cholesterol response.

Copper in the liver was determined by drying liver samples overnight at 105°C, after which the dry weights were determined. Subsequently, the samples were ashed at 200°C for one hour, 300°C for two hours, 400°C for three hours and 500°C for ten hours. The remaining ash was dissolved in 1.0 ml concentrated $HClO_4$which was then evaporated at 225°C. This step was repeated until the ash was completely white. The ash was then dissolved in 1.0 ml 6 M HCl. Copper was measured by using flame atomic absorption spectrophotometry on a Varian-AA275 (Varian, Springville, Australia).

2.3. Statistical analyses

Since the rabbits were housed individually, each animal formed an experimental unit in itself. The Kolmogorov-Smirnov one-sample test was used to check normality of the data. All results within groups were normally distributed. The significance of the differences between groups was calculated by a three-way analysis of variance (ANOVA). Homogeneity of the variances was tested using Bartlett's test. When necessary, the variances were equalized by ranking-transformation [14] of the data. After transformation the variances were similar and the transformed within-group data were still normally distributed. Thus, application of an analysis of variance on the (transformed) data is then straightforward. If the analyses of variance showed significant effects the group means were further compared with the unpaired Student's *t* test. These tests were performed with pooled (for equal variances) or separate (for unequal variances) variance estimates. The equality of variances was then tested using a F-test. To take into account the greater probability of a type I error due to multiple comparisons, the level of significance for the unpaired Student's *t* tests was pre-set at $P < 0.05$/times a group is used for a comparison (i.e. $P < 0.05/3 = 0.0167$) instead of $P < 0.05$, according to Bonferroni's adaptation. In all other cases, the probability of a type I error < 0.05 was taken as the criterion of significance. Between selected parameters, Spearman's coefficient of rank correlation (R) was calculated; significance was assessed by a two-tailed test. Two-side probabilities were estimated throughout. All statistical analyses were carried out according to Petrie and Watson [15] using a SPSS PC+ computer program [16].

3. Results

3.1. Growth performance

At the beginning of the test period IIIVO/JU rabbits were significantly heavier than AX/JU rabbits (results not shown) (two-way ANOVA, n = 38: strain effect, $p = 0.001$; gender effect, $p < 0.001$; interaction effect, $p = 0.231$). Compared to females rabbits, male rabbits had a slightly lower initial (results not shown) and final body weight (Table 1). Since the experiment was carried out with adult rabbits, this strain difference could not be explained by the difference in age (IIIVO/JU rabbits were on average 10 weeks older than AX/JU rabbits; see Materials and Methods). Furthermore, it is known for a long time that in the rabbit the female, when mature, is usually the larger one of the two sexes, which differs from most other mammals [17]. During the course of the experiment, body weights of the rabbits on the high-cholesterol diet did not change, whereas body weights of the rabbits on the control diet were slightly, but significantly diminished. As a consequence, in the analysis of variance, a diet effect was detected (Table 1).

3.2. Serum cholesterol

Baseline serum cholesterol levels of the IIIVO/JU rabbits were significantly higher than those of AX/JU rabbits. Female rabbits when compared with male rabbits have higher initial serum cholesterol levels (IIIVO/JU: ♂ 28±32mg/dl n = 10, ♀ 43±8mg/dl n = 9; AX/JU: ♂ 16±3mg/dl n = 10, ♀ 29±3mg/dl n = 9; two-way ANOVA, n = 38: strain effect, $p < 0.001$; gender effect, $p < 0.001$; interaction effect, $p = 0.704$). This is in line with earlier observations [18]. Baseline serum total cholesterol values of the IIIVO/JU strain are in agreement with early work on the IIIVO/J strain by Laird *et al.* [19] and Van Zutphen and Fox [8]. Values for the AX/J strain were higher in the paper of Laird *et al.* [19], but the AX/J strain was not inbred then. The cholesterol-rich diet dramatically increased the AUC in the two strains, the increment being significantly greater in the AX/JU rabbits. On the control diet female rabbits also have a higher AUC when compared with their male counterparts. In contrast, on the cholesterol-rich diet the AUCs for male and female rabbits were similar (Table 1).

Table 1
Body weight serum total cholesterol content and liver weight of non-reactive and reactive rabbits fed diets with or without added cholesterol[1]

Measure	Gender	Diet without added cholesterol HIVO/JU (n=5M,5F)	AX/JU (n=5M,5F)	Diet with added cholesterol IIIVO/JU (n=5M,4F)	AX/JU (n=5M,4F)	Sign.[2]
Final body weight	*Males:*	2718 ±71	2650 ± 82	2782 ± 131	2692 ± 121[a]	D,G
(*g*)	*Females:*	2768 ± 52	2691 ± 129	2838 ± 170	2903 ± 44[a]	
Serum total cholesterol level (*AUC, day 0 to day 42*)						
(*mg.day/dL*)	*Males:*	131[c] ± 114[eg]	692 ± 90[bfg]	7595 ± 2008[ah]	28556 ± 4817[bh]	S,D,G,SxD,
	Females:	1858 ± 187[cei]	1280 ± 85[dfi]	7514 ± 1749[cj]	28312 ± 3173[dj]	SxG,SxD, SxDxG[3]
Liver wet weight						
Absolute	*Males:*	64.78 ± 3.50[ac]	54.10 ± 1.97[ad]	107.78 ± 8.21[bc]	82.52 ± 9.91[bd]	S,D,SxG[3]
(*g*)	*Females:*	63.62 ± 2.19	56.50 ± 7.46[e]	87.45 ± 16.05	83.90 ± 8.12[e]	
Relative	*Males:*	23.82 ± 0.79[ac]	20.43 ± 0.92[ad]	38.74 ± 2.08[bcf]	30.62 ± 3.15[bd]	S,D,SxG,
(*g/kg body wt*)	*Females:*	22.98 ± 0.86	20.98 ± 2.35[e]	30.65 ± 4.06[f]	28.90 ± 2.73[e]	DxG[3]
Liver dry weight						
Absolute	*Males:*	19.16 ± 1.22[ac]	15.93 ± 0.46[ad]	32.71 ± 2.67[bd]	24.58 ± 3.10[bd]	S,D,SxG[3]
(*g*)	*Females:*	18.45 ± 0.59	16.68 ± 1.94[c]	26.09 ± 5.29	25.83 ± 3.11[e]	
Relative	*Males:*	7.04 ± 0.30[ac]	6.01 ± 0.13[ad]	11.75 ± 0.60[bcf]	9.12 ± 0.98[bd]	S,D,G,SxG,
(*g/kg body wt.*)	*Females:*	6.67 ± 0.22	6.20 ± 0.60[e]	9.14 ± 1.38[f]	8.90 ± 1.05[e]	DxG[3]

[1] Values are means ± SD; n is the number of male (M) and female (F) animals per group.

[2] Significance ($P<0.05$) based on three-way ANOVA with main factors *strain, diet* and *gender*. S, effect of strain; D, effect of diet; G, effect of gender, SxG, interaction; DxG, interaction.

[3] ANOVA after ranking of the data.

[4]Contrast significance (Student's *t* test; $P<0.0167$). Within two rows (i.e. the males plus females row), values bearing the same superscript letter are significantly different.

3.3. Liver weight

Irrespective of the diet, IIIVO/JU male rabbits have statistically significantly higher absolute and relative liver (wet and dry) weights than AX/JU male rabbits (Table 1). In female rabbits this difference did not reach the level of statistical significance in the multiple comparison procedure. The consumption of cholesterol raised in both strains absolute and relative liver (wet and dry) weights, albeit in the female IIIVO/JU this cholesterol effect was not statistically significant in the multiple comparison procedure.

3.4. Liver cholesterol

The consumption of cholesterol raised liver cholesterol concentration in both strains and both sexes, although for female IIIVO/JU rabbits this increase was not statistically significant in the multiple comparison procedure (Table 2). The magnitude of the increase in the male IIIVO/JU and AX/JU is more than 5.3 and 9.5 times, respectively. In the female IIIVO/JU and AX/JU the increase is more than 2.4 and 8.2 times, respectively. This diet effect was also found for liver cholesterol content. On the diet without added cholesterol, liver cholesterol content (absolute and relative) of the two strains and two genders was similar. The consumption of cholesterol drastically raised liver cholesterol content in both strains and both genders. However, the effect is most pronounced in the AX/JU strain and in males. The magnitude of the increase in liver cholesterol content in the male IIIVO/JU and AX/JU rabbit is about 9.0 and 14.5 times, respectively. In the female IIIVO/JU and AX/JU rabbit this increase is about 3.5 and 11.8 times, respectively. The increase in liver weight of test animals when compared with control animals (wet weight: 23.83 to 43.00 g; dry weight: 7.64 to 13.55 g) can only partly be attributed to the increase in hepatic cholesterol amount (mg/whole liver); the latter represented about 0.48 to 2.33 g.

3.5. Liver copper

Irrespective of the dietary composition, there was a statistically significant strain effect on the concentration and store of hepatic copper; the AX/JU rabbits having a much higher copper concentration and total store than the IIIVO/JU animals (Table 2). The diet with added cholesterol when compared with the control diet produced lower liver copper concentrations and contents in AX/JU, but not in the IIIVO/JU rabbits.

Liver copper concentration, but not liver copper store, was weakly correlated with serum cholesterol response.

Table 2
Liver total cholesterol and copper content of non-reactive and reactive rabbits fed diets with or without added cholesterol[1]

Measure	Gender	Diet without added cholesterol		Diet with added cholesterol				Sign.[2]
		IIIVO/JU (n=5M,5F)	AX/JU (n=5M,5F)	IIIVO/JU (n=5M,4F)		AX/JU (n=5M,4F)		
Liver total cholesterol concentration								
(*mg/g wet weight*)	*Males:*	2.71 ± 0.12^{ac}	3.19 ± 0.23^{ad}	14.96 ± 4.57^{bc}	(5.521)[5]	30.70 ± 3.74^{bd}	(9.62)	S,D,SxG,
	Females:	2.90 ± 0.11	2.96 ± 0.21^{e}	7.21 ± 3.61^{f}	(2.486)	25.45 ± 2.92^{ef}	(8.591)	SxDxG[3]
(*mg/g wet weight*)	*Males:*	9.18 ± 0.44^{ac}	10.83 ± 0.73^{ad}	49.23 ± 14.73^{bc}	(5.363)	103.17 ± 13.25^{bd}	(9.526)	S,D,SxG,
	Females:	9.99 ± 0.43	10.01 ± 0.60^{e}	24.02 ± 11.46^{f}	(2.404)	82.86 ± 10.23^{ef}	(8.278)	DxG[3]
Liver cholesterol content								
(*mg/whole liver*)	*Males:*	175 ± 6^{a}	172 ± 7^{b}	1618 ± 508^{a}	(9.246)	2504 ± 78^{bc}	(14.558)	D,SxD,
	Females:	184 ± 9	166 ± 14^{d}	666 ± 440^{e}	(3.620)	2120 ± 154^{cde}	(12.771)	DxG[3]
(*mg/100 g body wt.*)	*Males:*	6.46 ± 0.17^{a}	6.51 ± 0.41^{c}	58.21 ± 18.55^{ab}	(9.01)	93.10 ± 2.62^{bcd}	(14.301)	D,G,SxD,
	Females:	6.66 ± 0.35	6.18 ± 0.32^{e}	22.92 ± 14.11^{f}	(3.441)	73.09 ± 5.71^{def}	(11.827)	SxG[3]
Liver copper concentration								
(μ*g/g wet weight*)	*Males:*	5.33 ± 0.65^{a}	74.23 ± 17.84^{ac}	4.19 ± 0.86^{b}	(0.786)	18.06 ± 5.66^{bc}	(0.243)	S,D,SxG[3]
	Females:	4.61 ± 0.38^{d}	47.63 ± 3.65^{d}	4.09 ± 0.45	(0.887)	19.10 ± 19.32	0.401)	
(μg/g dry weight)	*Males:*	18.06 ± 2.41^{a}	252.93 ± 64.03^{ac}	13.81 ± 2.76^{b}	(0.765	60.69 ± 19.30^{bc}	(0.240)	S,D,G[3]
	Females:	15.88 ± 1.24^{d}	161.29 ± 15.06^{d}	13.76 ± 1.55	(0.866)	61.35 ± 60.56	(0.380)	
Liver copper store								
(μg whole liver)	*Males:*	344 ± 25^{ad}	4020 ± 968^{ac}	449 ± 77^{b}	(1.305)	1472 ± 438^{bc}	(0.366)	S,D,G[3]
	Females:	293 ± 27de	2702 ± 494^{e}	356 ± 72	(1.215)	1714 ± 1917	(0.634)	
(μ*g/100 g body wt.*)	*Males:*	12.67 ± 1.22^{ad}	152.50 ± 40.37^{ac}	16.19 ± 3.11^{b}	(1.278)	54.52 ± 15.54^{bc}	(0.358)	S,G,SxD[3]
	Females:	10.58 ± 0.85^{de}	100.47 ± 17.52^{e}	12.51 ± 1.94	(1.182)	58.79 ± 65.65	(0.585)	

[1] Values are means ± SD; n is the number of male (M) and female (F) animals per group.
[2] Significance ($P<0.05$) based on three-way ANOVA with main factors *strain, diet* and *gender*, S, effect of strain; D, effect of diet; G, effect of gender, SxD, interaction; SxG, interaction; DxG, interaction.
[3] ANOVA after ranking of the data.
[4] Contrast significance (Student's *t* test; $P<0.0167$). Within two rows (i.e. males plus females row), values bearing the same superscript letter are significantly different.
[5] The "fold increase" is given in parenthesis.

Both for μg Cu/g liver dry weight and μg Cu/g liver wet weight the R was -0.3739 (n = 38, p = 0.021). In rabbits, none of the parameters for liver copper store were significantly associated with the parameters for liver cholesterol content.

4. Discussion

In the literature substantial evidence for a relationship between cholesterol and copper has been described [5-7]. The aim of the present work was to determine whether cholesterol in the diet influences liver copper content in rabbits. For this purpose we used two inbred rabbit strains which differ markedly in their cholesterolemic response to dietary cholesterol[2][8].

The mean liver copper concentration of male IIIVO/JU rabbits fed a diet without added cholesterol was 18.06 μg/g dry weight; for female IIIVO/JU animals the value for this parameter was 15.88 (Table 2). The liver copper concentrations of male and female AX/JU rabbits on the control diet were remarkably higher than those of the IIIVO/JU, being 252.93 and 161.29 μg/g dry weight, respectively (Table 2). Klevay [5] reported that liver copper concentrations in New Zealand White rabbits fed a control diet ranged from 9.1-87.0 μg/g dry weight. Allain et al. [20] reported similar copper levels in the livers of Watanabe Heritable Hyperlipidemic (WHHL) rabbits and New Zealand White rabbits. Thus on a control diet, IIIVO/JU rabbits, which like WHHL rabbits originate from a New Zealand White stock [9], have liver copper concentrations (range 14.5-21.7 μg/g dry weight) that fall in the range described in the literature. In contrast, control AX/JU rabbits with liver copper concentrations that vary between 144.4 and 343.2 μg/g dry weight have hepatic copper concentrations much higher than those reported thus far [5,20].

The strain difference in hepatic copper content for these rabbits fed a normal diet might be explained by differences

[2] Meijer, G.W. (1991). Cholesterol metabolism in inbred rabbits with low or high cholesterolemic response to dietary cholesterol. Characterization of an animal model. *Ph.D.-thesis,* Utrecht University.

in the serum glucocorticoid levels. Glucocorticoids are able to stimulate the synthesis of metallothionein and ceruloplasmin in the liver [21]. Dietary copper is absorbed from the diet through the intestine and is transported to and taken up by the liver. There, copper is partly incorporated into newly synthesized apoceruloplasmin, which is then excreted into the plasma. Besides incorporation of copper in copper-containing liver proteins, a part of the copper is stored as metallothionein, while the remaining copper is excreted into the bile. Thus, synthesis of metallothionein and ceruloplasmin may result in an increase in plasma and liver copper concentration. Recently we have reported that AX/JU when compared with IIIVO/JU rabbits have higher levels of circulating corticosterone [22]. A difference in copper intake between the two strains could be excluded. During the experiment, the rabbits were fed restricted amounts of diet and the animals consumed all the administered food.

The cholesterol-rich diet resulted in a lower liver copper store in AX/JU rabbit, whereas no significant change was found in IIIVO/JU rabbits (Table 2). It is well-known that in rabbits a cholesterol-rich diet leads to an increased biliary excretion of cholesterol and its metabolites, (conjugated) bile acids. It is generally agreed that the bile is the main excretory route for copper and that copper is excreted as complexes of amino acids and/or (conjugated) bile acids. During biliary flow, the copper also becomes complexed with bilirubins; these complexes are unavailable for reabsorption. Keeping this in mind, Klevay [5] hypothesized that dietary cholesterol and (conjugated) bile acids produce increased biliary loss of copper, which results in a decreased liver copper content. However, bile acid excretion has been reported to be consistently higher in the IIIVO/JU strain both on a control diet and on a cholesterol-rich diet [11].

Meijer *et al.* [23] showed that adding cholesterol to the diet caused significantly higher bilirubin concentrations in the serum of AX/JU rabbits, but not in IIIVO/JU animals. Dietary cholesterol induced hypercholesterolemia associated with hemolytic anemia has also been reported [24]. Abnormally shaped erythrocytes are formed as a result of an increase in erythrocyte membrane cholesterol and these red cells are removed from the blood stream by the reticuloendothelial system. Within the phagocytic cells, hemoglobin and other heme proteins are catabolized to bilirubin. After its formation within phagocytic cells, bilirubin is released into the circulation. In this form the bilirubin is unconjugated and is cleared from the blood by the liver. In the liver bilirubin is conjugated with glucuronic acid and excreted via the bile into the duodenum. Thus an increase in the serum concentration of bilirubin will be associated with an enhanced biliary secretion of bilirubins. As pointed out above, during biliary flow, copper becomes complexed with bilirubins, which cannot be absorbed. Therefore, it could be anticipated that on a cholesterol-rich diet the biliary excretion of bilirubin and thus of copper is enhanced in AX/JU when compared with IIIVO/JU rabbits.

In conclusion, the AX/JU and IIIVO/JU rabbit inbred strains show differences in liver copper content. These strain effects perhaps could be explained by differences in circulating glucocorticoid levels. In reactive when compared with non-reactive rabbits, cholesterol loading produced a marked decrease in liver copper store. We hypothesized that this cholesterol effect in rabbits is due to an enhanced hepatobiliary transport of copper via increased bilirubin production.

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