Cholesterol-Lowering Nature of Unsaturated Fat in Rats May Be Due to Its Inability to Increase Hepatic Iron

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The present investigation was conducted to determine whether the cholesterol-raising properties of saturated fat and cholesterol-lowering properties of unsaturated fat are associated with levels of hepatic iron. The magnitude of hepatic iron retention was manipulated by feeding rats diets that were either copper-deficient or -adequate, iron-adequate or -supplemented, and contained either beef tallow or corn oil. Weanling male Sprague-Dawley rats were randomly divided into eight dietary groups according to the type of dietary fat (beef tallow or corn oil) and level of dietary copper (0.74 or 6.9 μg Cu/g diet) or iron (44.4 or 86.7 μg Fe/g diet). Beef tallow and copper deficiency alone increased hepatic iron levels, which in turn were associated with increased plasma cholesterol. When the three dietary factors were combined, ie, iron, beef tallow, and copper deficiency, they induced the highest magnitude of hepatic iron retention, which in turn was associated with the highest concentration of plasma cholesterol. In contrast, when hepatic iron retention was not increased, such as by feeding a diet containing corn oil or by consumption of a copper-adequate diet, plasma cholesterol was not elevated. Based on these data, it is suggested that nutrients that have the ability to increase hepatic iron have the potential to increase plasma cholesterol.

COPPER DEFICIENCY is associated with the spontaneous retention of hepatic iron.[1,2] We have recently reported that hypercholesterolemia and hypertriglyceridemia were associated with elevated hepatic iron concentrations in copper-deficient rats.[3] There was a direct relationship between the magnitude of blood lipids and the concentration of hepatic iron.[3] Based on these data, we hypothesized that iron was responsible for the development of lipemia of copper deficiency. In another study, we have shown that the type of dietary fat, saturated versus unsaturated, played an important part in modifying the pathogenesis of copper deficiency.[4] In that study, rats were fed a diet containing either beef tallow, a rich source of saturated fat known for its hypercholesterolemic properties, or corn oil. However, saturated fat is also responsible for elevating hepatic iron.[5,6] As expected, the consumption of beef tallow caused hypercholesterolemia in all rats compared with rats fed corn oil.[4] The greatest degree of lipemia was associated with the highest concentration of liver iron.[4] In contrast, rats that were fed corn oil did not exhibit elevated levels of hepatic iron, and their cholesterol and triglycerides were not elevated.[4] In those two studies,[3,4] all rats were fed the recommended levels of iron for optimal growth. If the inability of corn oil to increase blood lipids is due to insufficient hepatic iron retention, then higher levels of dietary iron may be needed to increase liver iron. On the other hand, the hypocholesterolemic properties of unsaturated fat may result from its inability to increase liver iron.

This study was conducted to test the hypothesis that dietary fats (saturated *v* unsaturated) exert effects on blood cholesterol through their ability or inability to increase the hepatic iron concentration.

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MATERIALS AND METHODS

Forty-eight weanling male Sprague-Dawley rats weighing approximately 40 to 45 g each were randomly divided into eight dietary groups depending on the type of dietary fat and level of copper and iron: group 1, corn oil, copper-adequate, iron-adequate; group 2, corn oil, copper-adequate, high-iron; group 3, beef tallow, copper-adequate, iron-adequate; group 4, beef tallow, copper-adequate, high-iron; group 5, corn oil, copper-deficient, iron-adequate; group 6, corn oil, copper-deficient, high-iron; group 7, beef tallow, copper-deficient, iron-adequate; and group 8, beef tallow, copper-deficient, high-iron.

All diets contained 627 g/kg carbohydrate as fructose, 35 g/kg salt mix[7] prepared in our laboratory without copper and iron, 10 g/kg vitamin mix,[8] 0.002 g/kg biotin, 2.7 g/kg choline bitartrate, 30 g/kg fiber, 200 g/kg egg white, and 95 g/kg fat (corn-oil or beef tallow). The concentrations of copper and iron in the diets were measured by atomic absorption spectophotometry. The copper-deficient diet contained 0.74 ± 0.09 mg Cu/kg, and the copper-adequate diets contained 6.9 ± 0.6 mg Cu/kg diet and were prepared by adding cupric carbonate to the copper-deficient diets. Ferric citrate was also added to the diets. Iron-adequate diets contained 44.4 ± 3.4 mg Fe/kg diet, and the high-iron diet contained 86.7 ± 3.6 mg Fe/kg. Both levels of dietary iron are within the normal range recommended for the optimal growth of rodents.

Rats were fed their respective diets for 4 weeks. They were allowed free access to food and distilled water, and were weighed weekly. At the end of the fourth week, rats were fasted overnight and then killed. The liver, pancreas, and heart were removed and weighed, and portions of the liver were taken for determination of copper and iron concentrations. Blood was collected for hematocrit determination. Cholesterol and triglyceride levels were measured in plasma by an automated procedure (Centrifichem; Union Carbide, Rye, NY) and using conventional methods.

This study was designed to answer the question of whether any of the dietary nutrients that can affect iron status, such as the type of fat and levels of iron and copper, are able to determine the magnitude of hypercholesterolemia and hypertriglyceridemia. Therefore, data were analyzed by a 2 × 2 × 2 ANOVA (two types of fat, two levels of copper, and two levels of iron). The main effects of fat, copper, and iron were tested, and the interaction among them at a P level less than .05 was considered significant.

RESULTS

The body mass, relative organ size, and hematocrit are presented in Table 1. Copper deficiency was responsible for a reduced body mass. The consumption of beef tallow compared

Table 1. Body Mass, Relative Organ Size, and Hematocrit

	Copper-Adequate				Copper-Deficient			
	Corn Oil		Beef Tallow		Corn Oil		Beef Tallow	
Parameter	Adequate Fe	High Fe	Adequate Fe	High Fe	Adequate Fe	High Fe	Adequate Fe	High Fe
Body mass (g)	183 ± 8	194 ± 3	220 ± 3	220 ± 3	148 ± 7	143 ± 5	167 ± 5	174 ± 16
Relative organ size (g/100 g)								
Liver	4.0 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.09	5.0 ± 0.09	5.3 ± 0.2	4.5 ± 0.1	4.7 ± 0.1
Heart	0.42 ± 0 07	0.40 ± 0.01	0.47 ± 0.1	0.47 ± 0.01	0 67 ± 0.05	0.76 ± 0.03	0.81 ± 0.04	0.72 ± 0.4
Hematocrit (%)	41.0 ± 1.1	40.2 ± 1.62	39.5 ± 1.1	40.8 ± 1.6	23.6 ± 1.8	18 8 ± 2.6	21.1 ± 0.8	16.5 ± 1.8

ANOVA	Cu	Fat	Cu × Fat	Fe	Cu × Fe	Fat × Fe	Cu × Fat × Fe
Body mass	S	S	NS	NS	NS	NS	NS
Liver	S	S	S	S	NS	NS	NS
Heart	S	S	NS	NS	NS	NS	S
Hematocrit	S	S	NS	S	S	NS	NS

NOTE. Results are the mean ± SEM of 5 observations per group in the corn oil diet and the mean ± SEM of 7 observations per group in the beef tallow diet.

Abbreviations: S, significant; NS, nonsignificant.

with corn oil was responsible for a greater body mass. Copper deficiency resulted in enlarged liver size. However, copper deficiency in combination with beef tallow consumption caused a reduction in liver size. Iron supplementation increased liver size. All copper-deficient rats exhibited heart enlargement. The greatest magnitude of heart enlargement was noted in rats fed the copper-deficient diet containing beef tallow and adequate iron. Regardless of iron intake, all copper-deficient rats were anemic. The combination of copper deficiency and high iron intake resulted in the greatest magnitude of anemia.

Liver copper and iron and plasma cholesterol and triglycerides are presented in Table 2. As expected, all rats that consumed a copper-deficient diet exhibited reduced hepatic copper compared with copper-adequate controls. The consumption of corn oil elevated hepatic copper levels compared with consumption of beef tallow. The combination of copper deficiency, beef tallow, and high iron resulted in the lowest concentration of hepatic copper. All copper-deficient rats exhibited hepatic iron overload. The consumption of beef tallow by all rats caused a greater magnitude of hepatic iron retention than the consumption of corn oil. Both cholesterol and triglycerides were elevated by copper deficiency. The consumption of beef tallow increased plasma cholesterol compared with the consumption of corn oil. The highest levels of plasma cholesterol were found in copper-deficient rats fed beef tallow and high iron.

DISCUSSION

This study was conducted to test the hypothesis that the ability of dietary fat to increase blood cholesterol is associated with an elevated hepatic iron concentration. Data from the present study support this hypothesis and show that regardless of the mechanisms responsible for elevating hepatic iron, high levels of liver iron were associated with hypercholesterolemia. The data also show that when liver iron was not elevated, plasma cholesterol remained unchanged.

In the present investigation, three different dietary means were used to increase liver iron retention: copper deficiency,

Table 2. Hepatic Copper and Iron and Plasma Cholesterol and Triglycerides

	Copper-Adequate				Copper-Deficient			
	Corn Oil		Beef Tallow		Corn Oil		Beef Tallow	
Parameter	Adequate Fe	High Fe	Adequate Fe	High Fe	Adequate Fe	High Fe	Adequate Fe	High Fe
Liver Cu (μg/g wet weight)	4.94 ± 0.39	7.16 ± 0.56	4.49 ± 0.14	3.78 ± 0.25	0.88 ± 0.11	1.24 ± 0.19	0.86 ± 0.10	0.71 ± 0.04
Liver Fe (μg/g wet weight)	103 ± 108	96 ± 9	201 ± 4	192 ± 10	187 ± 24	191 ± 22	252 ± 17	296 ± 9
Cholesterol (mg/dL)	106 ± 3	114 ± 6	134 ± 5	140 ± 3	161 ± 7	155 ± 8	181 ± 5	222 ± 5
Triglycerides (mg/dL)	27 4 ± 2.8	34.1 ± 7.0	28.9 ± 3.5	32.9 ± 5.3	49.2 ± 1 7	47.2 ± 6.9	45.5 ± 2.8	52.8 ± 1.9

ANOVA	Cu	Fat	Cu × Fat	Fe	Cu × Fe	Fat × Fe	Cu × Fat × Fe
Copper	S	S	S	S	NS	S	S
Iron	S	S	NS	NS	NS	NS	NS
Cholesterol	S	S	S	S	NS	S	S
Triglycerides	S	NS	NS	NS	NS	NS	NS

NOTE. Refer to Table 1 for details.

high dietary iron, and saturated fat (beef tallow). Copper deficiency by itself is responsible for the spontaneous increase of hepatic iron, due to the antagonistic relationship that exists between copper and iron.[1,2] In addition, the consumption of saturated fat such as beef tallow or coconut oil also has the ability to increase liver iron by increasing iron absorption and hepatic retention.[5,6,9] As expected, copper deficiency and beef tallow increased hepatic iron. The combination of copper deficiency with beef tallow produced higher levels of hepatic iron than were produced by copper deficiency or beef tallow alone. Elevating the level of dietary iron alone did not increase hepatic iron retention. Higher levels of iron than those used in the present study may be needed to achieve such an effect. However, when the three dietary factors, ie, high level of dietary iron, inadequate level of copper, and beef tallow, were combined, they produced the highest level of hepatic iron, which in turn caused the greatest magnitude of hypercholesterolemia.

Unlike rats fed beef tallow, no further increase in hepatic iron could be found in rats fed copper-deficient diets that contained corn oil and double the concentration of dietary iron. Since hepatic iron could not be further elevated by the combination of supplemental iron and corn oil, blood cholesterol was not elevated. It was surprising and difficult to accept that a fourfold increase in dietary iron would not elevate hepatic iron concentrations in copper-deficient rats.[10] However, examination of the diets used in that study revealed that they contained corn oil.[10] The inability of corn oil to increase hepatic iron may explain the lack of change in the magnitude of lipemia.[10] It is interesting that regardless of the type of dietary fat, consumption of a copper-adequate diet also did not produce higher levels of hepatic iron even when the diet was supplemented with iron. The lack of an effect on liver iron resulted in a lack of change in plasma cholesterol. These findings further support our hypothesis that a direct relationship exists between hepatic iron and blood cholesterol.

The mechanism responsible for hypercholesterolemia when hepatic iron is elevated is not fully understood. None of the enzymes participating in cholesterol biosynthesis require iron. There is no information in the literature that would link iron to blood cholesterol, but there are studies that attribute iron deficiency anemia to lipogenesis.[11-14] Iron deficiency in the rat has been shown to reduce the activity of stearoyl coenzyme A desaturase and to alter essential fatty acid metabolism.[15-17] Although both copper and iron deficiencies result in anemia, unlike iron deficiency, the anemia of copper deficiency is not due to a lack of iron, but rather to increased hepatic iron stores and impaired mobilization of iron from storage to the bone marrow for heme synthesis, leading to impaired erythropoiesis.

The influence of the amount and type of dietary fat on the utilization of minerals such as copper and iron has hardly been studied. The association between copper deficiency and the degree of fat saturation,[4] between copper deficiency and levels of dietary fat,[18,19] between dietary fat and iron deficiency,[11-14] between iron supplementation and lipemia,[14] and between fat saturation and iron[5,6,9,20] has been reported. However, in all of these studies,[5,6,9-14,18-20] the association between hepatic iron overload and blood lipids was not determined.

It has been suggested that hyperlipidemia is induced by a dietary deficiency of antioxidant nutrients such as vitamin C,[21] vitamin E,[22] selenium,[23] and copper.[24,25] All rats that were fed a copper-deficient diet were similarly copper-depleted, but not all exhibited the same levels of blood cholesterol. The magnitude of lipemia was positively associated with levels of hepatic iron but inversely associated with hepatic copper. All correlations between liver copper and plasma cholesterol and between liver iron and plasma cholesterol were highly significant ($P < .001$), with coefficients of determination (R^2) from .573 for liver copper and cholesterol to .627 for liver iron and cholesterol. The inverse correlation between liver copper and cholesterol in plasma has been previously reported.[25,26] In copper deficiency, elevated liver iron appeared to be associated with high plasma cholesterol.[25]

It is well established that an association exists between the degree of fat saturation and blood lipids. Saturated fat possesses cholesterol-raising properties. In contrast, unsaturated fat decreases blood cholesterol.[27] The data presented here show that the potential dietary fat has to increase blood cholesterol is dependent on its ability to elevate liver iron. The data also show that the protection provided by unsaturated fat, such as corn oil, against an elevation of blood lipids is achieved by preventing an increase in liver iron. Unfortunately, there are limited data to support this hypothesis, because the association between hepatic iron and lipemia is rarely studied and reported. Amine et al[11] and Cunnane and McAdoo[14] have shown that iron supplementation caused a significant increase in blood cholesterol; however, levels of hepatic iron were not measured. Other researchers used a mixture of saturated and unsaturated fat.[18,19] In those studies, cholesterol was measured but the hepatic iron concentration was not reported.[18] or when hepatic iron was determined, plasma cholesterol was not assessed.[19] Lynch and Strain[28] reported that saturated fat was responsible for elevating hepatic iron and plasma triglycerides but not cholesterol in copper-deficient rats. It is imperative to assess iron status in iron-feeding studies. It should not be assumed that the consumption of high levels of dietary iron guarantees the spontaneous elevation of liver iron. Consumption of supplemental iron by copper-adequate rats and by rats that were fed corn oil did not elevate hepatic iron.

In addition to hyperlipidemia, pathologies associated with copper deficiency were magnified by the consumption of beef tallow and high iron. This diet also produced the highest degree of hepatic iron. Reducing the level of hepatic iron either by chelation or by decreasing the intake of dietary iron resulted in amelioration and prevention of the pathologies induced by copper deficiency.[29,30]

In summary, under the experimental conditions presented here, the data show that liver iron could play an important role in determining hypercholesterolemia. It should be pointed out that caution is warranted when interpreting these data. Sherman[12] observed no elevations in serum cholesterol by copper and iron deficiencies in female rats. The animals in that study did not exhibit the typical signs of copper deficiency, although they were fed their respective diets for 18 weeks. The sex of the rat,[31] age, type of diet, and levels of iron and copper are important factors that contribute to the pathogenesis and development of copper deficiency.

The enhancement of hepatic iron retention in copper deficiency and by the consumption of saturated fat is of great concern. Diets consumed by individuals living in industrialized societies are low in copper.[32] In addition, high-fat and high–saturated fat diets are prevalent in many socioeconomic groups.[33] Furthermore, a large segment of our population uses supplements with iron and multivitamins containing iron, and there widespread use of iron fortification.[34] Higher levels of iron than those used in the current study may have a detrimental effect even in a corn oil–containing diet. High stored iron levels could be of importance in the pathogenesis of coronary heart disease. Recent human epidemiological studies provided evidence that increased body iron stores as assessed by serum ferritin are a potential risk factor for acute myocardial infarction.[35-38] It has been proposed that iron depletion protects against ischemic heart disease.[39-41] If the same types of dietary nutrient interactions that have been reported here also occur in human populations, then major reconsideration should be taken when formulating dietary recommendations and advice.

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