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Effect of saturated versus unsaturated fat on the pathogenesis of copper deficiency in rats

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The type of dietary fat, saturated versus unsaturated, may be an important factor in modifying the pathogenesis of copper deficiency in rats fed fructose. We investigated if saturated fat such as beef tallow as compared with unsaturated fat such as corn oil will prevent abnormalities related to the combination of fructose feeding and copper deficiency. Rats were fed copper-deficient (0.6 μg Cu/g diet) or adequate (6.0 μg Cu/g) diets containing fructose or starch as the sole dietary carbohydrate and beef tallow or corn oil as their fat source. The "typical" pathologies associated with copper deficiency in rats fed fructose such as anemia, pancreatic atrophy, heart hypertrophy, and liver enlargements were not prevented by the consumption of saturated fat (beef tallow). Not only did it not prevent pathologies, but beef tallow raised hepatic iron and increased plasma cholesterol and triglycerides, however, only in rats fed fructose. Plasma triglycerides and cholesterol were also elevated by copper deficiency and fructose feeding. The effect of the interaction among dietary carbohydrate, level of copper, and degree of saturation of fat on excess hepatic iron deposition and on metabolic and biochemical indices associated with heart-related abnormalities needs further investigation. (J. Nutr. Biochem. 7:246–251, 1996.)

Keywords: fructose; starch; beef tallow; corn oil; copper

Introduction

During the last decade we repeatedly reported that the consumption of fructose in a diet that is low in copper is responsible for numerous pathologies that eventually lead to premature mortality of the laboratory rat.[1–3] The consumption of starch in copper-deficient diet did not produce such pathologies and the rats survived.[1–3] We hypothesized that the reasons for these dramatic differences between copper-deficient rats fed fructose and those fed starch were linked to metabolic pathways of fructose and glucose (upon the hydrolysis of starch).[4] Fructose is lipogenic and it induces fatty liver.[5–7] In addition, it produces the formation of aldehydes such as glyceraldehyde, a precursor of lipogenesis and a free radical generator.[8] Starch does not induce these changes.

The appearance of visible fat in liver cells implies either significant injury to the liver or some serious systemic disorder leading to overload of the liver with fat. Fatty infiltration in the liver has a major significance as an induction of serious hepatic cell injury and so is often accompanied or followed by necrosis of liver cells. We have recently reported that rats fed the diet containing fructose exhibited foci of individual cell necrosis detected by light microscopy.[9] Ultrastructurally, the livers showed some distortion of mitochrondria. Fat was present and cell necrosis of hepatocytes was evident in all sections.[9] These changes were more pronounced in copper-deficient rats. These hepatic lesions were similar to those induced by alcohol.[10]

The effects of consumption of copper-deficient diets containing fructose and of chronic alcohol administration on metabolic, physiological, and biochemical indices and damage to numerous organs are very similar.[4,11–15] We have recently shown that alcohol consumption by copper-deficient rats fed starch resulted in the exacerbation of the deficiency similar to that exerted by fructose.[16,17] If the pathology to the liver induced by fructose feeding could be

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prevented, the signs associated with copper deficiency might also be ameliorated or prevented. It has been reported that rats fed saturated fat such as beef tallow were protected against the development of alcoholic liver disease, whereas rats fed unsaturated fat such as corn oil were not and they developed liver pathology.[10,18,19] It has been suggested that unsaturated fat serves as an important source for lipids that accumulate in the livers of alcoholics, which results in pathological changes.[10,18,19] In all our past studies the rats were fed unsaturated fat in the form of corn oil. It may be that fat unsaturation affects metabolic processes associated with copper deficiency and fructose feeding, which are responsible for the pathologies associated with copper deficiency. This study was designed to determine if the type of dietary fat plays a role in the pathogenesis of copper deficiency in rats fed fructose.

Methods and material

Eighty weanling male Sprague-Dawley rats were randomly divided into eight dietary groups according to type of dietary carbohydrate, type of dietary fat, and level of copper. All diets contained 627 g/kg fructose or starch, deficient 0.6 μg Cu/g or adequate (6.0 μg Cu/g) in copper. Diets contained 95 g/kg fat as either corn oil or beef tallow. The diets also contained 200 g/kg egg white, 30 g/kg non-nutritive fiber (cellulose), 35 g/kg copper-free AIN-76 salt mix (formulated in our laboratory to omit cupric carbonate), and 10 g/kg AIN-76A vitamin mix[21] supplemented with 2 mg biotin and 2.7 g choline bitartrate. The copper-adequate diet was prepared by adding cupric carbonate to the copper-deficient mineral mixture to produce a final concentration of 6.0 μg Cu/g diet. All rats had free access to diet and to distilled deionized drinking water. During the 4th week, food intake was measured.

After consuming their respective diets for 5 weeks, rats were decapitated. Blood was collected for measurements of hematocrit, enzymes, and substrates. To assess liver and heart damage, plasma alanine aminotransferase (ALT) and lactic dehydrogenase (LDH) were determined. LDH is a valuable diagnostic and prognostic aid for assessing cardiac damage and other pathological states involving tissue necrosis. In addition, substrates associated with lipid and fructose metabolism such as triglycerides, cholesterol, and lactic acid were also measured in plasma. All enzymes and substrates were measured by the automated procedure of the Centrifichem using Sigma reagents. Livers were removed and aliquots were taken for measurements of copper and iron concentrations. Copper and iron in diets and liver were measured by a method combining dry heat and acid digestion.[22] Duplicate samples were analyzed by flame atomic absorption spectrophotometry. National Institute of Standards and Technology Reference Material was digested and analyzed along with samples to verify accuracy.

This study was designed to test the hypothesis that beef tallow may ameliorate the signs associated with copper deficiency in rats fed fructose. Therefore, statistical analyses were performed by using a 2 × 2 × 2 analysis of variance (ANOVA). Two levels of copper, (– Cu versus + Cu), two types of dietary carbohydrate (starch versus fructose), and two types of dietary fats (corn oil versus beef tallow). Main effects of copper (Cu), carbohydrate (CHO), and type of fat and the interactions between them of $P < 0.05$ were considered statistically significant.

Results

Body weight, relative organ sizes, and hematocrit are summarized in *Table 1*. All rats that had consumed beef tallow experienced reduced body weight compared with rats that consumed corn oil. This effect of beef tallow was independent of either copper or type of dietary carbohydrate. Fructose feeding, copper deficiency, and the combination of fructose feeding with copper deficiency caused a reduction in body weight. The combination of fructose feeding with copper deficiency reduced the size of the pancreas. The consumption of beef tallow and fructose combined with copper deficiency resulted in the smallest pancreata. Heart size was increased by fructose feeding. It was also increased by copper deficiency. The combination of fructose feeding with copper deficiency caused a greater heart hypertrophy compared with all other dietary treatment. The type of di-

Table 1 Body weight, relative organ sizes, and hematocrit

Diet	body weight (g)	Relative organ sizes g/100g bw			hematocrit (%)
		pancreas	heart	liver	
FR-Cu + CO	167 ± 4	0.45 ± 0.047	0.67 ± 0.04	4.89 ± 0.24	20 ± 2.5
FR-Cu + BT	155 ± 2	0.40 ± 0.051	0.62 ± 0.03	4.73 ± 0.14	21 ± 1.9
ST-Cu + CO	204 ± 6	0.61 ± 0.17	0.53 ± 0.02	3.33 ± 0.09	41 ± 0.1
ST-Cu + BT	185 ± 3	0.65 ± 0.046	0.54 ± 0.01	3.59 ± 0.08	36 ± 1.6
FR + Cu + CO	209 ± 5	0.70 ± 0.019	0.39 ± 0.01	3.57 ± 0.05	37 ± 0.1
FR + Cu + BT	188 ± 5	0.80 ± 0.033	0.42 ± 0.01	3.67 ± 0.06	42 ± 0.1
ST + Cu + CO	215 ± 5	0.67 ±0.034	0.36 ± 0.01	3.11 ± 0.08	40 ± 0.1
ST + Cu + BT	206 ± 3	0.63 ±0.019	0.38 ± 0.01	3.03 ± 0.06	40 ± 0.1
ANOVA		P Values			
Fat	0.0001	NS	NS	NS	NS
CHO	0.0001	0.0342	0.002	0.0001	0.0001
Cu	0.0001	0.0001	0.001	0.0001	0.0001
CHO × Cu	0.0012	0.0001	0.0206	0.0001	0.0001
CHO × fat	NS	NS	NS	NS	0.0315
Cu × fat	NS	NS	NS	NS	NS
CHO × Cu × fat	NS	0.0220	NS	NS	NS

Mean ± SEM of 10 observations/group.
FR = fructose, ST = starch, Cu = copper, BT = beef tallow, CO = corn oil, CHO = carbohydrate, NS = not significant, fat = type of fat.

etary fat had no effect on heart or liver size. Fructose feeding caused an increase in liver size. Liver size was also increased in copper deficient rats compared to their copper-adequate controls. The combination of fructose feeding with copper deficiency resulted in the largest liver size.

Copper deficiency and fructose feeding resulted in the lowest hematocrits. The consumption of beef tallow by starch fed rats resulted in the highest hematocrits.

Table 2 summarized data regarding food intake and feed efficiency. It was important to measure food intake because rats that had been fed beef tallow weighed less than rats that consumed corn oil. Rats fed fructose consumed less food than rats fed starch. Copper deficiency also caused a small, but significant, reduction in food intake. Similarly, when dietary intake was expressed per 100 g body weight, both fructose and copper deficiency were responsible for a lower food intake compared with starch and copper adequate rats. The consumption of saturated fat (beef tallow) did not affect food intake compared with the consumption of unsaturated fat (corn oil). The consumption of beef tallow caused a significant reduction in feed efficiency in all groups compared with the consumption of corn oil. Feed efficiency was lowered by the consumption of fructose and copper deficient diets.

Hepatic copper and iron concentrations are summarized in *Table 3*. As expected, rats that consumed copper-deficient diets exhibited lower hepatic copper concentrations than rats that consumed the adequate copper diet. Fructose-fed rats exhibited lower concentration of copper than starch-fed rats. Rats fed the copper-deficient diets containing fructose had the lowest hepatic copper concentrations. Copper-adequate rats that had been fed a starch-based diet, exhibited the highest hepatic copper concentration. In this dietary group beef tallow raised hepatic copper compared with corn oil (CHO × Cu × Fat, $P < 0.0252$). Although these effects achieved statistical significance they were of small magnitude.

Table 2 Daily dietary intake and feed efficiency

Diet	daily consumption (g diet/day)	daily consumption (g diet/100g bw)	feed efficiency (wt gain/Kcal consumed)
FR-Cu + CO	11.3 ± 0.54	15.1 ± 0.90	11.3 ± 0.46
FR-Cu + BT	12.1 ± 0.52	14.9 ± 0.75	8.4 ± 0.39
ST-Cu + CO	14.1 ± 0.46	20.6 ± 1.10	12.7 ± 0.34
ST-Cu + BT	14.8 ± 0.65	19.7 ± 0.86	10.8 ± 0.47
FR + Cu + CO	14.3 ± 0.43	20.5 ± 0.99	12.7 ± 0.27
FR + Cu + BT	13.9 ± 0.36	18.5 ± 0.83	10.4 ± 0.42
ST + Cu + CO	15.3 ± 0.35	23.6 ± 0.95	12.7 ± 0.36
ST + Cu + BT	16.0 ± 0.76	23.2 ± 1.11	11.5 ± 0.61
ANOVA	P Values		
Fat	NS	NS	0.0001
CHO	0.0001	0.0001	0.0002
Cu	0.0001	0.0001	0.0020
CHO × Cu	NS	NS	0.0178
CHO × fat	NS	NS	NS
Cu × fat	NS	NS	NS
CHO × Cu × fat	NS	NS	NS

Mean ± SEM of 10 observations/group.
FR = fructose, ST = starch, Cu = copper, BT-diets containing beef tallow, CO = diets containing corn oil, CHO = carbohydrate, Cu = copper, NS = not significant, fat = type of fat.

Table 3 Hepatic copper and iron concentrations

Diet	Hepatic Copper (μg Cu/g wet wt.)	Hepatic Iron (μg Fe/g wet wt.)
FR-Cu + CO	0.67 ± 0.04	164.2 ± 10.8
FR-Cu + BT	0.66 ± 0.05	241.7 ± 7.7
ST-Cu + CO	1.33 ± 0.08	240.8 ± 17.2
ST-Cu + BT	1.19 ± 0.05	251.4 ± 7.8
FR + Cu +CO	3.97 ± 0.11	99.7 ± 6.7
FR + Cu + BT	3.59 ± 0.13	213.0 ± 25.6
ST + Cu + CO	4.73 ± 0.17	106.5 ± 5.2
ST + Cu + BT	4.91 ± 0.11	129.7 ± 4.9
ANOVA	P Values	
Fat	NS	0.0001
CHO	0.0001	NS
Cu	0.0001	0.0001
CHO × Cu	0.0047	0.0001
CHO × fat	NS	0.0001
Cu × fat	NS	NS
CHO × Cu × fat	0.0252	NS

Mean ± SEM of 10 observations/group.
FR = fructose, ST = starch, Cu = copper, BT = diets containing beef tallow, CO = diets containing corn oil, CHO = carbohydrate, Cu = copper, NS = not significant, fat = type of fat.

Rats fed a low-copper diet displayed increases in hepatic iron concentration compared to rats fed the adequate copper diet. Rats that had been fed a copper-deficient diet containing starch exhibited the highest hepatic iron concentration. The consumption of beef tallow raised hepatic iron concentration in all groups. The magnitude of the increase by beef tallow in hepatic iron was greater in fructose-fed than in starch-fed rats.

ALT and LDH activities are constantly used to assess liver damage. Their activities were not affected by either the type of dietary carbohydrate, fat, or levels of copper (data not shown).

Triglycerides, cholesterol and lactic acid are presented in *Table 4*. Beef tallow was responsible for raising the concentration of triglycerides, in rats fed fructose. Regardless of beef tallow the combination of fructose feeding with copper deficiency resulted in the highest concentrations of plasma triglycerides. The combination of copper deficiency with fructose feeding resulted in higher levels of plasma cholesterol than all other combinations. Beef tallow raised cholesterol in fructose-fed rats, but not in starch fed rats. Copper-deficient rats fed fructose had the highest levels of plasma cholesterol when fed beef tallow. Lactic acid was elevated by the combination of copper deficiency and fructose. Beef tallow lowered lactic acid concentration, however, in copper-deficient rats.

Discussion

Data of the present study show that the degree of fat saturation plays a major role in the exacerbation of the signs associated with copper deficiency in rats fed fructose. Beef tallow raised hepatic iron, a potential initiator of lipid peroxidation and elevated blood risk factor metabolites asso-

Table 4 Triglycerides, cholesterol, and lactic acid in plasma

Diet	Triglycerides mmol/L	Cholesterol mmol/L	Lactic Acid mmol/L
FR-Cu + CO	0.49 ± 0.022	3.80 ± 0.10	5.08 ± 0.25
FR-Cu + BT	0.66 ± 0.045	4.36 ± 0.18	4.63 ± 0.19
ST-Cu + CO	0.26 ± 0.014	3.23 ± 0.15	4.09 ± 0.24
ST-Cu + BT	0.24 ± 0.013	3.21 ± 0.09	3.47 ± 0.23
FR + Cu + CO	0.22 ± 0.010	2.83 ± 0.11	2.62 ± 0.19
FR + Cu + BT	0.32 ± 0.030	4.00 ± 0.14	2.57 ± 0.22
ST + Cu + CO	0.30 ± 0.012	3.80 ± 0.18	2.46 ± 0.17
ST + Cu + BT	0.24 ± 0.011	2.96 ± 0.17	2.78 ± 0.11
ANOVA	P Values		
Fat	0.0067	0.0449	NS
CHO	0.0001	0.0001	0.0007
Cu	0.0001	0.0207	0.0001
CHO × Cu	0.0001	0.0002	0.0005
CHO × Fat	0.0001	0.0001	NS
Cu × Fat	NS	NS	0.0277
CHO × Cu × Fat	NS	0.0011	NS

Mean ± SEM of 10 observations/group.
FR = fructose, ST = starch, Cu = copper, BT = beef tallow, CO = corn oil, CHO = carbohydrate, NS = not significant, fat = type of fat.

ciated with heart disease such as cholesterol and triglycerides. The effects of beef tallow occurred only in fructose fed rats. Data show that the combination of beef tallow and fructose is responsible for these risk factors. Copper deficiency was also involved in this interaction, but played a secondary role.

It is well established that rats fed diets high in fructose exhibit many pathophysiological abnormalities associated with carbohydrate and lipid metabolism such as hyperinsulinemia, insulin resistance,[23–27] lipogenesis, and fatty liver.[5,6,23,24] Fatty liver eventually would lead to necrosis and fibrosis. Indeed, we have recently reported that fructose feeding caused hepatic lesions detected by light and electron microscopy.[9] These lesions consisted of lipid infiltration, cell necrosis, and distortion of mitochondria.[9] Hepatic lipogenesis could be responsible in part for the pathologies that developed as a result of fructose feeding and copper deficiency. In our past studies we could not prevent hepatic lipogenesis in rats fed fructose-based diets even by the administration of high doses of choline.[28] It is possible, however, to prevent liver necrosis by the consumption of beef tallow.[10,18,19] Indeed, this was the purpose of our study.

Beef tallow neither prevented liver damage nor prevented pathologies. Alanine aminotransferase activity, which is routinely used to assess liver disease, was not altered by the type of dietary fat. In the study where beef tallow prevented liver necrosis, the diets had been fed for 4 months.[19] We cannot prolong our studies beyond 5 to 6 weeks of dietary regimen due to premature mortality of fructose-fed, copper-deficient rats.

One of the most striking effects of beef tallow in the present study was on iron deposition in the liver. In general, copper-deficient rats exhibit hepatic iron overload due to the antagonistic properties between copper and iron.[29,30] The copper-deficient rats of the present study followed this pattern. Regardless of copper, beef tallow raised hepatic iron in all rats. However, in rats fed fructose the consumption of beef tallow doubled the concentration of hepatic iron in copper-adequate rats and in copper-deficient rats it caused a 50% increase in hepatic iron compared with corn oil. Iron has the potential to play a role in the pathogenesis of numerous diseases,[31,32] however, only under certain redox conditions.[33,34] The elevated levels of hepatic iron in copper-deficient rats fed fructose and beef tallow could induce formation of destructive free radicals,[35,36] which in turn could damaged cellular components. Although copper and iron are antagonistic to each other, it was surprising to see that the increases of hepatic iron by beef tallow did not induce changes of hepatic copper.

It has been reported that the consumption of beef tallow with fructose promoted iron utilization assessed by the increased hemoglobin levels.[37,38] The rats of those studied were adequate in all nutrients including copper.[37,38] In the present study, beef tallow did not promote iron utilization in copper-deficient rats fed fructose although hepatic iron concentration was of similar magnitude in copper-deficient rats fed fructose or starch. These data strongly suggest that iron utilization such as incorporating iron into hemoglobin is not simply related to increased concentration of hepatic iron.[35,36,39]

Other striking effects of beef tallow were on cholesterol and triglycerides, but only in rats fed fructose. It is well established that the type of dietary fat alters plasma lipid concentrations.[40–48] Beef tallow contains 18.9% stearic acid compared with 1.8% in corn oil, and it contains 109 mg/100 g cholesterol. Corn oil is free of cholesterol. However, beef tallow was hypercholesterolemic and hypertriglyceridemic only when it was consumed by rats fed diets containing fructose. The highest cholesterol and triglyceride levels were found in copper-deficient rats fed fructose. Thus, the combination of these three dietary factors—fructose, copper, and beef tallow contribute to dramatic elevations of blood risk factor metabolites associated with heart disease.

Plasma cholesterol and triglycerides have been reported to be elevated by copper deficiency. The diets that had been used to induce copper deficiency contained simple sugars such as sucrose or fructose but not starch.[1–3,14,49,50] When the diets contained starch, the elevations of both cholesterol and/or triglycerides were of a small magnitude.[1–3,51,52] In humans, blood lipids were elevated by the combination of fructose feeding and saturated fat compared with starch feeding.[53]

Beef tallow lowered plasma lactic acid only in copper deficient rats. The reasons for changes in lactic acid due to fat saturation are not fully understood. High lactate is a common finding in fructose consumption.[54]

Depressed body weight was noted in rats fed beef tallow. This observation is in agreement with studies reported by Johnson et al.[38] Depressed feed efficiency raises the question of adequacy of essential fatty acids in beef tallow.

In conclusion, fructose feeding in combination with either a saturated fat such as beef tallow or with copper deficiency is responsible for elevated levels of high risk factor metabolites and pathological changes. Beef tallow did not alleviate the signs associated with copper deficiency in rats fed fructose.

Diets rich in simple sugars are one of the consequences of use of fructose-rich sweeteners.[55] In addition, diets consumed by subjects living in industrialized societies are low

in copper.[56] Furthermore, high-fat and high-saturated diets are prevalent in many socioeconomic groups.[46,57] High-fat diet has been shown to be detrimental to the fructose × copper interaction.[58] If the same type of interactions that have been reported here also occur in humans then major considerations should be taken when formulating dietary recommendations and advice.

References

1 Fields M., Ferretti R.J., Reiser S., et al. (1984). The severity of copper deficiency is determined by the type of dietary carbohydrate. *Proc. Soc. Exp. Biol. Med.* **175,** 530–537

2 Reiser S., Ferretti R.J., Fields M., et al. (1983). Role of dietary fructose in the enhancement and mortality in rats fed sucrose or starch diets. *Am. J. Clin. Nutr.* **38,** 214–222

3 Fields M., Ferretti R.J., Smith J.C., et al. (1983). Effect of copper deficiency on metabolism and mortality in rats fed sucrose or starch diets. *J. Nutr.* **113,** 1335–1345

4 Fields M., Lewis C.G., Beal T. (1989). Accumulation of sorbitol in copper deficiency: Dependency on gender and type of dietary carbohydrate. *Metabolism* **38,** 371–375

5 Cohen A.M., Briller S., Shafrir E. (1972). Effect of long-term sucrose feeding on the activity of some enzymes regulating glycolysis, lipogenesis and gluconeogenesis in rat liver and adipose tissues. *Biochem. Biophys. Acta.* **279,** 129–138

6 Blakely S., Hallfrisch J., Reiser S., et al. (1982). Long-term effects of moderate fructose feeding on lipogenic parameters in Wistar rats. *Nutr. Rep. Int.* **25,** 674–685

7 Fields M., Ferretti R.J., Judge J.M., et al. (1985). Effect of different dietary carbohydrate on hepatic enzymes of copper-deficient rats. *Proc. Soc. Exp. Biol. Med.* **178,** 362–366

8 Thornalley P., Wolff S., Crabbe J., et al. (1984). The autoxidation of glyceraldehyde and other simple monosaccharides under physiological conditions catalyzed by buffer ions. *Biochem. Biophys. Acta.* **797,** 276–287

9 Fields M., Lewis C.G., Lure M.D., et al. (1995). Dietary ferric vs. ferrous iron in copper-deficient rats fed fructose-based diets. *J. Am. Coll. Nutr.* **14,** 399–403

10 Nanji A.A., Mendenhall C.L., French S.W. (1989). Beef fat prevents alcoholic liver disease in the rat. Alcoholism: *Clin. Exp. Res.* **13,** 15–19

11 Lieber C.S., DeCarli L.M. (1984). Metabolic effects of alcohol on the liver. In Metabolic Aspects of Alcoholism. p. 32–79, (Lieber C.S., ed.) Park Press, Baltimore University, MD

12 Mezey E. (1985). Metabolic effects of alcohol. *Fed. Proc.* **44,** 134–138

13 Redman R.S., Fields M., Reiser S., et al. (1988). Dietary fructose exacerbates the cardiac abnormalities of copper deficiency in rats. *Atherosclerosis* **74,** 203–214

14 Allen K.G.D., Klevay L.M. (1978). Cholesterolemia and cardiovascular abnormalities in rats caused by copper deficiency. *Atherosclerosis* **29,** 81–93

15 Adams M.A., Hirst M. (1986). Ethanol-induced cardiac hypertrophy: Correlation between development and the excretion of adrenal catecholamines. *Pharmacol. Biochem. Behav.* **24,** 33–38

16 Fields M., Lewis C.G. (1990). Alcohol consumption aggravates copper deficiency. *Metabolism* **39,** 610–613

17 Fields M., Lewis C.G., Lure M.D. (1984). Alcohol consumption mimics the effects of a high-fructose, low-copper diet in rats. *Alcohol* **11,** 17–23

18 Nanji A.A., French S.W. (1988). Effect of different dietary fats on ethanol metabolism. Implications for pathogenesis of experimental alcoholic liver disease. *Internat. J. Vet. Nutr. Res.* **58,** 475–476

19 Nanji A.A., French S.W. (1989). Dietary linoleic acid is required for development of experimentally induced alcoholic liver injury. *Life Science* **44,** 223–227

20 Anonymous. (1977). American Institute of Nutrition: Report of the AIN ad hoc committee for standards on nutritional studies. *J. Nutr.* **107,** 1340–1348

21 Anonymous. (1980). American Institute of Nutrition: Second Report of the AIN ad hoc committee on standards for nutritional studies. *J. Nutr.* **110,** 1726

22 Hill D., Patterson K.Y., Veillon C., et al. (1988). Digestion of biological materials for mineral analysis using a combination of wet and dry ashing. *Anal. Chem.* **58,** 2340–2342

23 Van den Berghe G. (1978). Metabolic effects of fructose in the liver. *Curr. Top. Cell. Regul.* **13,** 97–125

24 Henry R.R., Crapo P.A. (1991). Current issues in fructose metabolism. *Ann. Rev. Nutr.* **11,** 21–39

25 Pagliassotti M.J., Shahrokhi K.A., Moscarello H. (1994). Involvement of liver and skeletal muscle in sucrose-induced insulin resistance: dose response studies. *Am. J. Phys.* **35,** R1037–R1644

26 Zavaroni I., Sander S., Scott S., Reaven G.M. (1980). Effect of fructose feeding on insulin secretion and insulin action in the rat. *Metabolism* **29,** 970–973

27 Hwang I-S., Ho H., Hoffman B.B., Reaven G.M. (1987). Fructose-induced insulin and hypertension in rats. *Hypertension.* **10,** 512–516

28 Fields M., Lewis C.G., Beal T. (1988). High dietary choline and copper deficiency. *Nutr. Rep. Int.* **37,** 1281–1287

29 Williams D.M., Kennedy F.S., Green B.G. (1983). Hepatic iron accumulation in copper deficient rats. *Br. J. Nutr.* **50,** 653–660

30 Owen J.R. (1973). Effects of iron on copper metabolism and copper on iron metabolism in rats. *Am. J. Physiol.* **224,** 514–518

31 Halliwell B., Gutteridge J.M.C. (1994). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **219,** 1–14.

32 Cantoni O., Furmo M., Cattaberri F. (1989). Role of metal iron in oxidant cell injury. *Biol. Trace. Elem. Res.* **21,** 277–281

33 Braughler J.M., Duncan L.A., Chase R.L. (1986). The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation. *J. Biol. Chem.* **261,** 10282–10289

34 Rowley D., Halliwell B. (1982). Superoxide-dependent formation of hydroxyl radicals from NADH and NADPH in the presence of iron salts. *FEBS. Lett.* **142,** 39–41

35 Fields M., Lewis C.G., Lure M.D., et al. (1991). The severity of copper deficiency can be ameliorated by deferoxamine. *Metabolism* **40,** 105–109

36 Fields M., Lewis C.G., Lure M.D., et al. (1992). The influence of gender on developing copper deficiency and on free radical generation of rats fed a fructose diet. *Metabolism* **41,** 988–994

37 Amine E.K., Hegsted D.M. (1975). Effect of dietary carbohydrates and fats on inorganic iron absorption. *J. Agri. Food. Chem.* **23,** 204–208

38 Johnson P.E., Lukaski H.C., Korynta E.D. (1992). Effects of stearic acid and beef tallow on iron utilization by the rat. *Proc. Soc. Exp. Biol. Med.* **200,** 480–486

39 Fields M., Lewis C.G., Lure M.D., et al. (1993). Low dietary iron prevents free radical formation and heart pathology of copper-deficient rats fed fructose. *Proc. Soc. Exp. Biol. Med.* **202,** 225–232

40 Hegsted D.M., McGandy R.B., Myers M.L., et al. (1965). Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.* **17,** 281–295

41 Keys A., Anderson J.T., Grande F. (1965). Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. *Metabolism* **14,** 776–787

42 Mattson F.H., Hollenbach E.J., Klingman A.M. (1975). Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels of man. *Am. J. Clin. Nutr.* **28,** 726–731

43 Mattson F.H., Grundy S.M. (1985). Comparison of the effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid. Res.* **26,** 194–202

44 Grande F., Anderson J.T., Key A. (1970). Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. *Am. J. Clin. Nutr.* **23,** 1184–1193

45 Reiser R., Probstfield J.L., Silvers A., et al. (1985). Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil. (Published erratum appears in *Am. J. Clin. Nutr.* (1986). **43,** 978). *Am. J. Clin. Nutr.* **42,** 190–197

46 Schaefer E.J., Lichtenstein A.H., Lamon-Fava S., et al. (1995). Lipoproteins, nutrition, aging and atherosclerosis. *Am. J. Clin. Nutr.* **61(suppl),** 726S–740S

47 Denke M.A., Grundy S.M. (1991). Effects of fats high in stearic acid on lipid and lipoprotein concentrations in men. *Am. J. Clin. Nutr.* **54,** 1036–1040

48 Bonanome A., Grundy S.M. (1988). Effect of dietary stearic acid on

plasma cholesterol and lipoprotein levels. *N. Engl. J. Med.* **318,** 1244–1248
49 Allen K.G.D., Klevay L.M. (1980). Hyperlipoproteinemia in rats due to copper deficiency. *Nutr. Rep. Int.* **22,** 295–299
50 Allen K.G.D., Klevay L.M. (1978). Cholesterol metabolism in copper-deficient rats. *Life Sciences* **22,** 1691–1696
51 Sinthusek G., Magee A.C. (1984). Relationship of dietary zinc/copper ratio to plasma cholesterol and liver trace minerals deposition in young rats fed saturated and unsaturated fats. *Nutr. Res.* **4,** 841–851
52 Petering H.G., Murthy L., O'Flaherty E. (1977). Influence of dietary copper and zinc on rat lipid metabolism. *J. Agric. Food. Chem.* **25,** 1105–1109
53 Reiser S., Powell A.S., Scholfield D.J., et al. (1989). Blood lipids, lipoproteins, apoproteins and uric acid in men fed diets containing fructose or high-amylose cornstarch. *Am. J. Clin. Nutr.* **49,** 832–839
54 Hallfrisch J. (1990). Metabolic effects of dietary fructose. *FASEB. J.* **4,** 2652–2660
55 Sugar and Sweetener. Situation and Outlook Report. (1994). United States Department of Agriculture. *Economic Research Service.* SSSV19N2
56 Klevay L.M., Viestenz K.E. (1981). Abnormal electrocardiograms in rats deficient in copper. *Am. J. Physiol.* **240,** H185–H189
57 US Department of Health and Human Services (1988). Surgeon General's Report on Nutrition and Health, summary and recommendation. Washington DC: US Government Printing Office. (DHHS Publications (PHS) 88-50211)
58 Wapnir R.A., Devas G. (1995). Copper deficiency: interactions with high-fructose and high-fat diets in rats. *Am. J. Clin. Nutr.* **61,** 105–110