Copper Deficiency in Pregnancy: Effect on Maternal and Fetal Polyol Metabolites

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The present study was undertaken to determine whether the mortality of the fetus and the neonate of copper-deficient rats consuming fructose during pregnancy is associated with an aberration in carbohydrate metabolism. A total of 84 Sprague-Dawley rats were fed a copper-deficient or a copper-adequate diet containing fructose or starch for 19 or 21 days after conception. The consumption of a fructose-based diet during pregnancy resulted in higher concentrations of maternal blood fructose, sorbitol, triglyceride, and uric acid when compared with a starch diet. The placenta contained more than 10-fold the concentration of glucose and more than double the concentrations of fructose, triglycerides, and sorbitol when fructose was the dietary carbohydrate compared with starch. The livers of fetuses belonging to the fructose dietary group exhibited high concentrations of glucose and sorbitol. In addition, fetal blood contained higher concentrations of glucose, fructose, sorbitol, and triglycerides than the corresponding values from the starch dietary group. The consumption of a copper-deficient diet containing fructose during pregnancy resulted in massive subcutaneous hemorrhages of the fetus. In contrast, this pathology was rare in other dietary groups. The combination of copper deficiency with fructose feeding resulted in more than double the concentration of sorbitol in fetal liver, and higher concentrations of insulin and dopamine of fetal blood compared with the consumption of a copper-deficient diet containing starch. The results of the present study suggest that the combination of the aberration of carbohydrate metabolism due to fructose feeding together with copper deficiency may be responsible for the mortality of the fetus and the neonate rat when the copper-deficient diet consumed during pregnancy contains fructose.

IT IS WELL ESTABLISHED that a copper-deficient diet consumed during pregnancy can induce fetal abnormalities such as edema, subcutaneous hemorrhages, and neural abnormalities, fetal resorption, and mortality of the neonate rat.[1-8] However, recently it became apparent that copper deficiency by itself may not be sufficient to produce these abnormalities. In order for the resorption and malformations of the fetus to occur, the copper-deficient diet consumed during pregnancy must contain fructose[1,4,6-8] or lactose.[5] When the copper-deficient diet ingested during pregnancy contained starch, the rats gave birth to live pups.[4] Thus, the type of dietary carbohydrate eaten during pregnancy determines the outcome of gestation in copper deficiency.

The mammalian fetus normally contains high levels of fructose.[9-12] Fetal fructose formation is attributed to the conversion of maternal blood glucose to sorbitol and then to fructose by enzymes located in the placenta and fetal liver.[13-15] However, the utilization of fructose by the fetus is rather slow in comparison to glucose, and the production rate of CO_2 is approximately five times greater from fetal glucose carbon than fructose carbon.[16,17] Thus, the high concentration of fructose in fetal blood[9-11,18] may result from the slow utilization of fructose.

The feeding of fructose-based diets during pregnancy could raise the concentration of fructose in fetal tissues even higher than that considered to be normal. High concentration of fructose may be disadvantageous, since it may stimulate the polyol pathway.[18] Indeed, tissue levels of fructose and sorbitol have been shown to be elevated by fructose consumption as compared with starch and glucose consumption.[19,20] Copper deficiency further increased these concentrations.[20] This impaired carbohydrate metabolism when a fructose-based diet was consumed by young growing rats could play an important role in the pathology and mortality of copper deficiency.[21]

The objectives of the present study were (1) to determine whether the ingestion of diets containing fructose during gestation creates an aberrant environment of carbohydrate metabolism for the developing fetus, and (2) to establish whether dietary deprivation of copper magnifies the fructose effect.

The present study describes the metabolic effects of different dietary carbohydrates deficient or adequate in copper at days 19 and 21 of gestation on maternal and fetal metabolites and fetal hormones associated with carbohydrate metabolism. Days 19 and 21 were chosen because in previous studies fetal resorption occurred during the last 3 days of gestation.[4]

MATERIALS AND METHODS

Three separate studies were conducted in which a total of 84 timed pregnant Sprague-Dawley rats (Hilltop, Scottdale, PA) weighing approximately 200 g each were used.

Study I—19 Days of Gestation

Thirty-two pregnant rats were divided into four dietary groups according to the type of dietary carbohydrate and copper levels. Group 1: fructose, copper deficient; group 2: fructose, copper adequate; group 3: cornstarch, copper deficient; group 4: cornstarch, copper adequate. The copper-deficient diets contained (grams per kilogram diet) 622 carbohydrate as either fructose or cornstarch, 200 egg white, 95 corn oil, 30 nonnutritional fiber (cellulose), 35 AIN 76 salt mix (Tekland Diets, Madison, WI) prepared in our laboratory and formulated to omit cupric carbonate, 2.7 choline bitartrate, and 10 vitamin mix AIN 76A supplemented with 2

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mg/kg biotin. The copper-supplemented diets were prepared by adding copper carbonate to the copper-deficient mineral mixture to produce a final concentration of 6 μg Cu/g of supplemented diet. To assure accuracy, National Bureau of Standards Certified Reference Material, bovine liver, was analyzed along with the diets. The levels of dietary copper were 0.6 μg Cu/g diet for the deficient, and 6.0 μg Cu/g diet for the adequate diets as analyzed by atomic absorption spectrophotometry.[22]

The presence of a vaginal plug was denoted as gestational day 0. Pregnancy was terminated by decapitation on day 19 of gestation. The placenta and whole fetuses were quickly removed by cesarean section. Maternal livers, placentas, and fetal livers were placed on aluminum foil and were frozen on dry ice. Some tissues were transferred to separate preweighed tubes for freeze-drying and determinations of dry weight. The freeze-dried tissue was stored at −70°C until analyzed. Maternal and pooled fetal blood was collected in heparinized tubes on ice and plasma was obtained by centrifugation.

Freeze-dried tissues were homogenized in perchloric acid and neutralized with one-half volume of 2N KOH.[23] Fructose was measured in serum and tissues according to Roe.[24] Glucose was analyzed by the automated enzymatic procedure of the Centrifichem (Baker Instruments, Allentown, PA). Sorbitol was assayed enzymatically in the presence of sorbitol dehydrogenase.[23] Maternal, fetal carcass, and fetal liver copper was extracted from samples by a method combining dry heat and acid digestion[25] and measured by atomic absorption spectrophotometry.

Study II—21 Days of Gestation

This study consisted of two separate experiments in which a total of 52 pregnant rats were used. All the animals were divided into the four dietary groups as described in study I. The composition of the diet was the same as in study I. Pregnancy was terminated at day 21 of gestation. Triglycerides, cholesterol, and uric acid were analyzed by the automated enzymatic procedures of the centrifichem. Insulin was measured by the radioimmunoassay.[26] Blood catecholamines were measured by the high-performance liquid chromotography (HPLC) procedure.[27] Liver glycogen was measured according to Hansen et al[28] following tissue digestion by heating in boiling water for 30 minutes in the presence of alcoholic KOH.

Statistical Analysis

Analysis of Variance was performed using the SAS (Cary, NC) software system for data analysis (PROC GLM).[29] Main effects due to dietary copper and type of dietary carbohydrates and interactions between copper and type of dietary carbohydrates of .05 or less were considered statistically significant.

RESULTS

In both studies, all rats had litters that ranged between eight and 14 fetuses. Edema and massive hemorrhages in the upper part of their body were evident only in those fetuses of the copper-deficient fructose-fed dams. Also, many resorption sites were found. Fetuses of rats consuming the starch diet deficient in copper had only few hemorrhagic spots and none were resorbed. No visible pathology was detected in copper-adequate rats fed fructose or starch.

Study I

Maternal predelivery weight at gestational day 19, plasma glucose, fructose, and sorbitol, and hepatic copper concentrations are summarized in Table 1. Body weight before birth and weight gain were not affected by dietary carbohydrate or copper status. Hepatic copper concentration was lower in rats that ate the copper-deficient diet compared with copper-adequate controls. Fructose consumption reduced hepatic copper concentration when compared with starch consumption. The consumption of fructose during pregnancy resulted in significantly higher levels of serum fructose and sorbitol compared with feeding starch.

Fetal weights and the concentrations of glucose, fructose, and sorbitol in the placenta and livers of the 19-day-old fetuses are given in Table 2. More than 95% of the copper-deficient fetuses of the fructose dietary group exhibited massive subcutaneous hemorrhages and edema. In contrast, only a few fetuses exhibited small spots of subcutaneous hemorrhage in the other dietary groups. Fetal weight was neither affected by the nature of dietary carbohydrate nor by copper deficiency. As expected, dietary copper deprivation resulted in a reduced whole fetal and placental copper concentration compared with copper-adequate controls.

Fructose consumption during pregnancy increased the concentration of glucose in the placenta by more than 10-fold. Placental fructose was increased by more than twofold by the consumption of fructose during pregnancy when compared with the consumption of starch. Higher concentrations of placental fructose were found in copper-deficient compared with copper-adequate rats. Sorbitol was higher in the placenta of rats fed fructose than in those fed starch. The consumption of a diet containing fructose resulted in a 10-fold increase in hepatic glucose and in a

Table 1. Body Weight, Hepatic Copper and Blood Metabolites of 19-Day Pregnant Rats

	Fructose		Starch	
	−Cu	+Cu	−Cu	+Cu
Predelivery wt (g)	326 ± 7	312 ± 7	322 ± 12	308 ± 10
Weight gain (g)	139 ± 4	125 ± 7	130 ± 4	123 ± 7
Liver Cu (μg/g wet wt)	2.30 ± 0.23	4.66 ± 0.10	2.81 ± 0.25	5.29 ± 0.17*†
Maternal blood (mg/dL)				
Glucose	93.4 ± 3.3	89.6 ± 1.4	89.0 ± 2.7	87.6 ± 1.9
Fructose	10.4 ± 2.0	8.4 ± 0.9	5.3 ± 0.2	5.5 ± 0.5†
Sorbitol	7.5 ± 0.4	8.1 ± 0.5	6.8 ± 0.3	6.5 ± 0.1†

NOTE. Rats consumed their respective diets from conception to gestational day 19 when pregnancy was terminated. Values are means ± SEM of eight animals per group. A P value of .05 or less was considered statistically significant.

*Copper effect.

†Carbohydrate effect.

Table 2. Fetal Weight and Carcass Copper, and the Concentrations of Glucose, Fructose, and Sorbitol in Placentas and Livers of 19-Day-Old Fetuses

	Fructose		Starch	
	−Cu	+Cu	−Cu	+Cu
Fetal wet wt (g)	3.7 ± 0.4	3.8 ± 0.3	3.9 ± 0.3	3.9 ± 0.6
Carcass copper (μg/g wet wt)	0.5 ± 0.02	2.1 ± 0.2	0.6 ± 0.06	2.2 ± 0.3*†
Placenta				
Copper (μg/g)	0.85 ± 0.09	3.16 ± 0.26	1.12 ± 0.21	3.32 ± 0.21*†
Glucose (μmol/g dry wt)	27.4 ± 2.0	28.7 ± 3.0	1.4 ± 0.1	2.0 ± 0.2†
Fructose (μmol/g dry wt)	5.4 ± 0.5	4.0 ± 0.5	2.3 ± 0.2	2.1 ± 0.1*†
Sorbitol (μmol/g dry wt)	13.3 ± 2.3	11.6 ± 1.2	3.7 ± 0.4	2.3 ± 0.2†
Fetal liver				
Glucose (μmol/g dry wt)	183 ± 17	227 ± 18	18 ± 1.5	21 ± 1.2†
Fructose (μmol/g dry wt)	80.8 ± 11.7	67.8 ± 11.6	23.8 ± 1.6	26.8 ± 1.1†
Sorbitol (μmol/g dry wt)	5.5 ± 0.8	2.1 ± 0.2	2.1 ± 0.3	2.0 ± 0.1*†‡

NOTE. See Table 1 for description. Mean ± SEM of eight observations per group. Each observation consists of two to three pooled placentas and two pooled livers.

*Copper effect.

†Carbohydrate effect.

‡Copper-carbohydrate interaction.

threefold to fourfold increase in hepatic fructose concentration compared with the consumption of starch. The highest hepatic sorbitol concentration was found in the livers of fetuses of the fructose, copper-deficient dietary group.

Study II

Maternal weight, hepatic copper, and plasma metabolites at 21 days of pregnancy are presented in Table 3. Predelivery weight and weight gain were significantly reduced by fructose feeding in both copper-deficient and copper-adequate rats compared with starch feeding. As expected, liver copper concentration was reduced by the deficiency when compared with adequate controls. Plasma triglycerides were twofold higher in rats consuming the fructose diet as compared with starch. Some rats had triglyceride values of more than 1,000 mg/dL. Plasma cholesterol was not affected by either the type of dietary carbohydrate or copper deficiency. Uric acid was significantly higher in the fructose dietary group than the starch dietary group and was further increased by copper deficiency. As noted at day 19 of gestation, at day 21 numerous copper-deficient fetuses of the fructose dietary group exhibited massive hemorrhages. Blue hemorrhagic spots were scarce in all other fetuses.

Fetal body weight and blood concentrations of insulin, glucose, fructose, sorbitol, triglycerides, and catecholamines are presented in Table 4. Body weight was not affected by the dietary treatments. Blood insulin concentration was higher in the fructose dietary group compared with starch and was further increased by copper deficiency. Blood glucose, fructose, sorbitol, and triglycerides were increased by fructose feeding regardless of dietary copper levels. Dopamine concentration was the highest in copper-deficient fetuses from the fructose dietary group. Norepinephrine and epinephrine were not affected by the type of carbohydrate or copper deficiency.

Placenta and liver metabolites of 21-day-old fetuses are summarized in Table 5. Placental triglycerides were nearly twofold higher in the fructose dietary group compared with starch. However, liver triglyceride was not affected by either the type of dietary carbohydrate or copper status. Glycogen was significantly reduced by copper deficiency and fructose feeding compared with starch feeding and copper-adequate controls. As expected, hepatic copper concentration was reduced by dietary copper deprivation.

DISCUSSION

The data of the present study indicate that the type of dietary carbohydrates consumed during pregnancy by the copper-deficient rat determines the outcome of the pregnancy. The majority of the fetuses of dams fed the copper-

Table 3. Maternal Body Weight, Hepatic Copper, and Blood Metabolites at 21 Days of Pregnancy

	Fructose		Starch	
	−Cu	+Cu	−Cu	+Cu
Predelivery wt (g)	342 ± 10	339 ± 6	359 ± 8	378 ± 8†
Weight gain (g)	140 ± 7	137 ± 3	161 ± 7	176 ± 6†
Liver Cu (μg/g wet wt)	2.7 ± 0.2	5.2 ± 0.3	2.2 ± 0.07	4.7 ± 0.2*†
Maternal blood (mg/dL)				
Triglycerides	412 ± 67	396 ± 62	177 ± 14	151 ± 11†
Cholesterol	72 ± 5	73 ± 3	85 ± 3	71 ± 9
Uric acid	13.0 ± 2.3	8.0 ± 0.8	2.3 ± 0.3	2.3 ± 0.5†

NOTE. See Table 1 for description. Mean ± SEM of eight to 14 animals from two separate studies.

*Copper effect.

†Carbohydrate effect.

Table 4. Body Weight, Blood Metabolites, and Hormones of 21-Day-Old Fetus

	Fructose		Starch	
	−Cu	+Cu	−Cu	+Cu
Weight (g)	5.75 ± 0.22	5.78 ± 0.1	5.96 ± 0.16	5.90 ± 0.01
Fetal blood				
Insulin (μU/mL)	114.5 ± 6.5	86.6 ± 2.1	77.3 ± 1.1	76.2 ± 2.2*†
Glucose (mg/dL)	59.2 ± 2.1	54.0 ± 1.8	50 ± 0.2	49.2 ± 2.2†
Fructose (mg/dL)	19.3 ± 1.0	20.4 ± 2.4	15.6 ± 0.4	15.3 ± 0.2†
Sorbitol (μg/dL)	49.5 ± 4.1	53.1 ± 4.3	34.4 ± 2.4	27.4 ± 6.2†
Triglycerides (mg/dL)	8.2 ± 0.9	8.4 ± 0.8	4.6 ± 0.4	4.9 ± 0.5†
Catecholamines (pg/mL)				
Dopamine	141	67	72	55
Norepinephrine	910	710	670	942
Epinephrine	840	780	635	922

NOTE. See Table 1 for description. Mean ± SEM of 12 to 15 observations from two separate studies ± SEM. Values for catecholamines are one pooled sample from eight to 10 fetuses.

*Copper effect.

†Carbohydrate effect.

deficient diet containing fructose had massive hemorrhages of the head, neck and chest, and fetal resorption was confined only to this dietary group. In contrast, fetuses of the copper-deficient starch-fed group had only minor hemorrhagic spots scattered throughout their bodies. In addition, consuming the fructose or starch diet adequate in copper resulted in fetuses that were normal in appearance without any hemorrhagic lesions. The induction of an abnormal carbohydrate metabolism in the placenta and the fetus when the copper-deficient diet contains fructose as compared with the consumption of a diet containing starch may be responsible for these lesions. The elevated metabolites and hormones in fetal liver and fetal blood may have a deleterious effect on the prenatal development of the copper-deficient offspring.

Although the reasons for the elevated levels of glucose, fructose, and sorbitol of liver and blood are not fully understood, the consequences of such a phenomenon have been well documented. Any aberration in carbohydrate metabolism such as in diabetes[30-34] or in alcohol ingestion[35] can induce pathology in the fetus. High levels of glucose resulted in several morphological changes in cultured embryos.[36-39] In addition, high levels of sorbitol in the placenta and liver of offspring of diabetic rats were associated with malformations induced during fetal development.[32] However, decreased levels of sorbitol in the whole body of 11-day-old fetuses of diabetic rats by the administration of aldose reductase inhibitors did not reduce the incidence of resorptions and malformations.[32] Thus, the accumulation of sorbitol by itself may not be the only factor involved in the teratology of the fetus, but the combination of a high level of sorbitol with the deprivation of copper may contribute to the mortality of the offspring. A diminished availability of trace elements has been implicated as playing a role in the teratology of diabetes.[32,34,40] An interaction between sorbitol and copper may explain the results of our previous study[4] in which the mortality of the fetus or of the neonate rat was confined only to the fructose, copper-deficient group. Sorbitol, a strong chelator of copper,[41] may chelate the limited amount of copper in the deficient animal, making copper unavailable for utilization.

The high levels of fructose in the placenta and fetal liver when fructose-based diets were consumed during pregnancy could be derived from both maternal and fetal compartments. Maternal blood of rats consuming fructose-based diets contained higher levels of fructose than those consuming starch. It could be reasonably assumed that the high concentration of fructose in fetal liver originated at least in part from maternal circulation. However, fructose could be formed from the abundance of glucose found in the placenta and in fetal liver via the polyol pathway.[18] The utilization of fructose by the fetal liver is slower than the utilization of glucose.[16,17] Thus, it is not surprising to find higher levels of

Table 5. Placenta and Liver Metabolites of 21-Day-Old Fetuses

	Fructose		Starch	
	−Cu	+Cu	−Cu	+Cu
Placenta				
Triglycerides (nmol/g wet wt)	413 ± 40	428 ± 22	218 ± 21	217 ± 17†
Liver				
Triglycerides (nmol/g wet wt)	250 ± 26	245 ± 20	275 ± 18	230 ± 21
Glycogen (mg/g)	48.3 ± 3.4	62.9 ± 4.7	60.9 ± 3.9	61.3 ± 6.6*†‡
Copper (μg/g)	1.98 ± 0.22	22.4 ± 0.7	2.13 ± 0.22	22.7 ± 1.5*

NOTE. See Table 1 for description. Mean of total of 12 to 15 observations ± SEM.

*Copper effect.

†Carbohydrate effect.

‡Copper-carbohydrate interaction.

fructose in the fetal liver in the fructose dietary group as compared with the starch dietary group. Increased doses of dietary fructose results in a decreased clearance of fructose from blood.[42] High levels of fructose and glucose have been shown to have severe deleterious effects in numerous tissues due to the progressive accumulation of glycated proteins.[43-49] The levels of fructose have been shown to increase up to 23-fold in tissues of diabetic animals[50] where the sorbitol pathway is active. During the formation of glycated proteins from either glucose or fructose, free radicals are generated. In copper-deficient animals fed fructose, the protection against the accumulation of free radicals may be limited. In young growing rats the consumption of diets containing fructose result in low activities of the copper enzyme superoxide dismutase[51] and the selenoenzyme glutathione peroxidase.[52] Recently, it has been reported that the consumption of copper-deficient diets containing fructose results in the production of glycosylated hemoglobin.[52]

The placenta takes up glucose from maternal blood and releases it into the umbilical circulation.[53] If the magnitude of the gradient of maternal: fetal glucose is decreased either by maternal hypoglycemia or by fetal hyperglycemia, the flux of glucose into the umbilical circulation decreases.[53] Since in the present study, maternal blood glucose was not affected by the type of dietary carbohydrate or copper status, it could be assumed that the reason for the elevation of placental glucose may be due to abnormal glucose utilization by the placenta or the fetus.

The activities of hepatic gluconeogenic enzymes are low in the fetus.[53] In contrast, the enzymes of glycolysis are active in fetal liver.[54] Liver glycogen levels increase as the time of birth approaches, but it is not utilized as an energy source until birth or just prior to it.[55-58] The high concentration of glucose found in the 19-day-old fetal liver and in blood of the 21-day-old fetus (not shown) may reflect an inhibition of pathways of glucose utilization such as glycogenesis. The low concentration of glycogen in the liver of fetuses from fructose, copper-deficient dietary group supports this hypothesis.

Ingestion of fructose containing diets has been shown to induce a significant turnover of catecholamines[59,60] and insulin resistance.[61,62] Since catecholamine and insulin secretion is a critical event in permitting normal glucose homeostasis during gestation,[53] any aberration in hormone turnover, secretion, or resistance will affect fetal metabolic, hormonal, and biochemical processes. Only the copper-deficient fetus of dams consuming the fructose diet showed higher levels of dopamine compared with all other fetuses. This may be due to a reduction in the activity of the copper enzyme dopamine β-hydroxylase.[64]

In the rat, the placenta is impermeable to triglycerides.[53] The relative hypertriglyceridemia of the fetus when a fructose-based diet was consumed by the pregnant rat may have been induced by fetal fructose.

The enhancement of the sorbitol pathway shown in this study implicates sorbitol, glucose, and fructose in the pathogenesis of copper deficiency. The mortality of the copper-deficient fetus of the fructose dietary group may be due to both copper utilization and tissue fructose concentration. Sorbitol may bind with copper[41] in the copper-deficient fetus of rats eating fructose, thereby further decreasing its utilization. The integrity of the cardiovascular system of the fetus requires copper since the formation of collagen and elastin are dependent on the copper enzyme lysyl oxidase.[3,5] Both glucose and fructose in high concentrations can glycosylate proteins, hormones, and enzymes. However, fructose is much more potent than glucose in the nonenzymatic glycosylation process.[42-49] The fructated collagen may contribute to a reduction of tensile strength and to a faulty integrity of fetal tissues such as the cardiovascular system. Indeed, the edema and the massive hemorrhages of the copper-deficient fetus of the present study is consistent with this hypothesis. Although fetuses of pregnant rats consuming starch are as copper deficient as those fetuses of rats consuming fructose, they do not exhibit elevated levels of sorbitol, glucose, or fructose, and thus should be protected against the pathology and mortality of copper deficiency.

The data of this study are intriguing. Regardless of copper status, fructose feeding during pregnancy induces severe abnormalities of carbohydrate metabolism. In a copper-adequate animal, neither the fetus[1-8] nor the young growing rat[20,51,62] exhibits signs of pathology. However, once copper is omitted from the diet, mortality of the fetus, neonate, and the young rat occurs.[1-8,62] Although no known pathway in the conversion of glucose or fructose to sorbitol has been shown to be copper dependent, it appears that copper deficiency either promotes sorbitol synthesis or prevents its catabolism. In addition, sorbitol accumulation during copper deficiency appears to derive from a pathway in which fructose but not glucose supplies the initial source of dietary carbohydrate. The involvement of the copper-dependent step in the conversion of fructose to glucose, which then serves as the immediate precursor of sorbitol, is not excluded.

REFERENCES

1. Masters DG, Keen CL, Lonnerdal B, et al: Comparative aspects of dietary copper and zinc deficiencies in pregnant rats. J Nutr 113:1448-1451, 1983

2. Begin DP, Kincaid RL, Hargist AM: Fetal death in copper-deficient rats. Nutr Rep Int 31:991-999, 1985

3. Hurley LS, Keen CL, Lonnerdal B: Copper in fetal and neonatal development, in Excerpta Medica: Biological Roles of Copper. Ciba Foundation Symposium 79:227-245, 1980

4. Fields, M, Lewis CG, Beal T: Copper-carbohydrate interaction in maternal, fetal and neonate rat. Neurotoxic Teratol 10:555-562, 1988

5. O'Dell BL, Hardwick BC, Reynolds G: Mineral deficiencies of milk and congenital malformation in the rat. J Nutr 73:151-157, 1967

6. Keen CL, Lonnerdal B, Hurley LS: Teratogenic effects of copper deficiency and excess, in Sorenson JRJ (ed): Inflammatory Diseases of Copper. Clifton, NJ, Humana, 1982, pp 109-121

7. Carlton WW, Kelly WA: Neural lesions in the offsprings of female rats fed a copper deficient diet. J Nutr 97:42-52, 1969

8. Hurley LS: Teratogenic aspects of manganese, zinc and copper nutrition. Physiol Rev 61:249-295, 1981

9. Walker DG: Fructose in fetal fluids of the deer. Nature 173:309-311, 1954

10. Hitchcock MWS: Fructose in the sheep fetus. J Physiol 108:117-121, 1949

11. Comline RS, Silver M: The composition of fetal and maternal blood during parturition in the ewe. J Physiol 222:223-226, 1972

12. Andrews WHH, Button HG, Huggett ASG, et al: Fructose metabolism in the isolated liver of the fetal and newborn sheep. J Physiol 153:199-203, 1960

13. Hers HG: Presence of sorbitol in seminal vesicles and in fetal blood of sheep. Biochem J 66:30, 1957

14. Hers HG: Le mechanisme de la formation du fructose seminal et du fructose foetal. Biochem Biophys Acta 37:127-138, 1960

15. Clemens RS, Winegrad AI: Purification of alditol: NADP oxireductase from human placenta. Biochem Biophys Res Commun 47:1473-1479, 1972

16. Meznarich HK, Hay WW Jr, Sparks JW, et al: Fructose disposal and oxidation rates in the ovine fetus. Q J Exp Physiol 72:617-625, 1987

17. Hay WW Jr, Myers SA, Sparks JW, et al: Glucose and lactate oxidation rates in the fetal lamb. Proc Soc Exp Biol Med 173:555-563, 1983

18. Jeffry J, Jornvall H: Enzyme relationship in a sorbitol pathway that bypasses glycolysis and pentose phosphates in glucose metabolism. Proc Natl Acad Sci USA 80:901-905, 1983

19. Bellomo G, Comstock JP, Wen D, et al: Prolonged fructose feeding and aldose reductase inhibition: Effect on the polyol pathway in kidneys of normal rats. Proc Soc Expt Biol Med 186:348-354, 1987

20. Fields M, Lewis CG, Beal T: Accumulation of sorbitol in copper deficiency: Dependency on gender and type of dietary carbohydrate. Metabolism 38:371-375, 1989

21. Fields M, Ferretti RJ, Reiser S, et al: The severity of copper deficiency in rats is determined by the type of dietary carbohydrate. Proc Soc Exp Biol Med 175:530-537, 1984

22. Anonymous: Analytical Methods for Atomic Absorption Spectrophotometry. Norwalk, CT, Perkin-Elmer, 1984

23. Bergmeyer V: Methods of Enzymatic Analysis. Academic, San Diego, CA, 1974

24. Roe JH: A colorimetric method for the determination of fructose in blood and urine. J Biol Chem 107:15-22, 1934

25. Hill AD, Patterson KY, Veillon C, et al: Digestion of biological materials for mineral analysis using a combination of wet and dry ashing. Anal Chem 58:2340-2342, 1986

26. Hales CN, Randle PJ: Immunoassay of insulin with insulin antibody precipiate. Biochem J 88:137-140, 1963

27. Frayn KN, Maycock PF: Sample preparation with ion-exchange resin before liquid-chromatographic determination of plasma catecholamines. Clin Chem 29:1426-1428, 1983

28. Hansen RG, Rutter WJ, Craine EM: A nephelometric method for the determination of glycogen. J Biochem 195:127-132, 1952

29. SAS User's Guide Statistics (ed 5). Cary, NC, SAS Institute, 1985

30. Cockroft DL, Coppola PT: Teratogenic effects excess glucose on head-fold rat embryos in culture. Teratology 16:141-146, 1977

31. Horton WE, Sadler TW: Effects of maternal diabetes on early embryogenesis: Alterations in morphogenesis produced by the ketone body, beta hydroxybuturate. Diabetes 32:610-616, 1983

32. Eriksson UJ, Naeser P, Brolin SE: Increased accumulation of sorbitol in offsprings of manifest diabetic rats. Diabetes 35:1356-1363, 1986

33. Ornoy A, Cohen AM: Teratogenic effects of sucrose diet in diabetic and non-diabetic rats. Isr J Med Sci 16:789-791, 1980

34. Uriu-Hare JY, Stern JS, Reawen GM, et al: The effect of maternal diabetes on trace element status and fetal development in the rat. Diabetes 34:1031-1040, 1985

35. Witek-Janusek L: Maternal ethanol ingestion: Effect on maternal and neonatal glucose balance. Am J Physiol 251:E178-E184, 1986

36. Garnham EA, Beck F, Clarke CA, et al: Effects of glucose on rat embryo in culture. Diabetologia 25:291-295, 1983

37. Horton WE, Sadler TW, Hunter ES: Effects of hyperketonemia on mouse embryonic and fetal glucose metabolism in vitro. Teratology 31:227-233, 1985

38. Clawert A, Wolff-Querrot MJ, Buck P: Etude de l'action embryopathique du glucose in injection intraamniotique. CR Soc Biol 166:1789-1792, 1972

39. Hughes AF, Freeman RB, Faderm T: The teratogenic effects of sugar on the chick embryo. J Embryol Exp Morphol 32:661-674, 1974

40. Eriksson UJ: Diabetes in pregnancy: retarded fetal growth, congenital malformations and fetal-maternal concentrations of zinc, copper and manganese in the rat. J Nutr 114:477-484, 1984

41. Briggs J, Finch P, Matulewicz MC, et al: Complexes of copper (II) and other metal ions with carbohydrates: thin-layer ligand-exchange chromatography and determination of relative stability of complexes. Carbohydr Res 97:181-188, 1981

42. Kim HS, Paik HY, Lee KU, et al: Effect of several simple sugars on serum glucose and serum fructose levels in normal and diabetic subjects. Diabetes Res Clin Pract 4:281-287, 1988

43. Bunn HF, Higgins PJ: Reaction of monosaccharides with proteins: Possible evolutionary significance. Science 213:222-224, 1981

44. Suarez G, Rajaram R, Bhuyan KC, et al: Administration of an aldose reductase inhibitor induces a decrease of collagen fluorescence in diabetic rats. J Clin Invest 82:624-627, 1988

45. Stevens VJ, Vlassara H, Abati A, et al: Nonenzymatic glycosylation of hemoglobin. J Biol Chem 252:2998-3002, 1977

46. Walton DJ: Fructose mediated cross linking of proteins. NIH Conference on the Maillard Reaction in Aging, Diabetes and Nutrition, 1988, p 7 (abstr)

47. Taniguchi N, Arai K, Kinoshita N, et al: Glycation of Cu-Zn-superoxide dismutase in vitro and in vivo: Identification of the glycated sites which leads its inactivation, in Oxy-radicals in Molecular Biology and Pathology. New York, NY, Liss, 1988, pp 217-231

48. Sakurai T, Matsuyama M, Tsuchiya K: Glycation of erythrocyte superoxide dismutase reduces its activity. Chem Pharm Bull (Tokyo) 35:302-307, 1987

49. DoPhofer R, Wieland OH: Preparation and biological properties of glycosylated insulin. FEBS Lett 100:133-136, 1979

50. Gabbay KH, Kinoshita JH: Mechanisms of development and prevention of cataracts. Isr J Med Sci 8:1557-1561, 1972

51. Fields M, Ferretti RJ, Smith JC, et al: Interaction between dietary carbohydrate and copper nutriture on lipid peroxidation in rat tissues. Biol Trace Elem Res 6:379-391, 1984

52. Klevay LM: An increase in glycosylated hemoglobin in rats deficient in copper. Nutr Rep Int 26:329-334, 1982

53. Battaglia FC, Meschia G: Fetal nutrition. Ann Rev Nutr 8:43-61, 1988

54. Vernon RG, Walker DG: Changes in the activities of some enzymes involved in glucose utilization and formation in developing rat liver. Biochem J 106:321-329, 1968

55. Yeung D, Oliver IT: Development of gluconeogenesis in neonatal rat liver. Biochem J 105:1229-1233, 1967

56. Angel JF, Back DW: Weaning and metabolic regulation in the rat. Can J Physiol Pharmacol 63:538-545, 1985

57. Ballard FJ, Oliver IT: Glycogen metabolism in embryonic chick and neonatal rat liver. Biochem Biophys Acta 71:578-588, 1963

58. Khandelwahl RL: Glycogen metabolizing enzyme activities in the developing rat liver. Int J Biochem 14:1067-1073, 1982

59. Young JB, Landsberg L: Stimulation of the sympathetic nervous system during sucrose feeding. Nature 269:615-616, 1977

60. Landsberg L, Young JB: Effects of nutritional status on autonomic nervous system function. Am J Clin Nutr 35:1234-1240, 1982

61. Beck-Nielsen H, Pederson O, Lindskov HO: Impaired cellular insulin binding and insulin sensitivity induced by high-fructose feeding in normal subjects. Am J Clin Nutr 33:273-278, 1980

62. Fields M, Ferretti RJ, Smith JC, et al: Effect of copper deficiency on metabolism and mortality in rats fed sucrose or starch diets. J Nutr 113:1335-1345, 1983

63. Sperling MA, Ganguli S, Leslie N, et al: Fetal-perinatal catecholamines secretion: Role in perinatal glucose homeostasis. Am J Physiol 247:E69-E74, 1984

64. Hesketh JE, Watts A, Arbuthnott GW: A radioisotope and HPLC study of catecholamine synthesis in copper deficiency, in Mills GF, Chesters JK (eds): Trace Elements in Man and Animals 5. London, England, Commonwealth Agriculture Bureaux, 1984, pp 32-36