Effect of changing the type of dietary carbohydrate or copper level of copper-deficient, fructose-fed rats on tissue sorbitol concentrations

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This study was designed to examine the relationship between the fructose-copper interaction and tissue sorbitol concentrations. Weanling male rats were provided with a diet which contained 62.7% fructose and 0.6 μg copper/g (F − Cu) for 4 weeks. At this time, rats were changed to either a fructose diet which contained 6.0 μg copper/g or to a starch diet with or without copper for 2 weeks. When compared with the other dietary groups, it was found that rats fed the F − Cu diet grew poorly; had altered relative liver, pancreatic, heart, and kidney sizes; were anemic; and had higher tissue concentrations of pancreatic and heart glucose, liver, pancreatic, heart, and kidney fructose, and liver, pancreatic, and kidney sorbitol. When rats were changed from the F − Cu diet to one containing copper or to a starch diet with or without copper, weight gain, relative liver, pancreatic and heart sizes, and hematocrit improved significantly. In general, there was a reduction in pancreatic and heart glucose; liver, pancreatic, heart, and kidney fructose; and pancreatic and kidney sorbitol concentrations when rats were changed from the F − Cu diet to any of the other diets. We conclude that the fructose-copper interaction may have a common biochemical basis related to the metabolism of glucose, fructose, and sorbitol.

Keywords: Glucose; liver; heart; pancreas; kidney

Introduction

The severity of the signs of copper deficiency in the laboratory rat is determined to a large extent by the type of carbohydrate consumed with a copper-deficient diet.[1-4] Male rats eating a copper-deficient diet which contained starch as its only type of carbohydrate had low tissue levels of copper, low tissue superoxide dismutase (SOD) activity, and mild anemia. However, these rats did not exhibit the morbidity and only rarely did they exhibit the early mortality that is classically associated with copper deficiency.[1-4] Male rats eating a copper-deficient diet which contained fructose or a fructose-containing carbohydrate had low tissue levels of copper and low tissue SOD activity, and they developed anemia,[1-4] fatty liver,[2] hypercholesterolemia,[1-3] enhanced lipid peroxidation,[5] pancreatic[6] and thymic[7] atrophy, edema,[8] and cardiac biochemical abnormalities and dysfunction[1,2] which generally led to an early death. The causal factors responsible for the more pronounced morbidity and mortality of the copper-deficient male rat eating a fructose-containing diet are not known.

Recently, it has been demonstrated that feeding rats a diet containing fructose enhanced sorbitol metabolism. Feeding male rats a fructose-rich diet for 30 days led to significantly higher levels of kidney sorbitol compared with rats fed glucose- or corn starch-containing diets.[9] Copper-deficient male rats consuming a fructose-containing diet had higher levels of sorbitol in the liver and kidney when compared with those eating a starch-containing diet.[10] It has been suggested

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that some of the complications of diabetes[9,11,12] and, possibly, copper deficiency[10] are a consequence of sorbitol accumulation in tissues. In the present study, we have posed the following question: if sorbitol accumulates in tissues of copper-deficient male rats fed fructose-containing diets, then can we reverse this process by replacing fructose with starch and/or by adding copper to the diet?

Materials and Methods

Weanling male Sprague-Dawley rats were housed individually in hanging stainless steel, wire-meshed cages in a room maintained at 20°C with 12-hour light/dark cycles. Rats were randomly assigned to one of four diets which contained either 62.7% starch (S) or fructose (F), with 6.0 (+Cu) or 0.6 μg copper/g diet (−Cu). Each of the S+Cu, S−Cu, and F+Cu dietary groups contained eight rats, which were maintained on these diets for 6 weeks. The F−Cu dietary group contained 32 rats which were maintained on this diet for 4 weeks. At this time, 24 rats (eight per dietary group) were randomly changed to either the S+Cu, S−Cu, or F+Cu diet for the remaining 2 weeks of the study. All diets contained the following ingredients (g/kg diet): carbohydrate, 627; egg white solids, 200; corn oil, 95; nonnutritive fiber (cellulose), 30; copper-free AIN-76 salt mix[13] (formulated in our laboratory to omit cupric carbonate), 35; and AIN-76A vitamin mix[14] supplemented with 2 mg biotin and 2.7 g choline bitartrate, 10. All rats had free access to diet and to distilled deionized drinking water.

After 6 weeks, the rats were decapitated, blood was collected in capillary tubes, and the liver, heart, kidneys, and pancreas were removed quickly and placed on dry ice. Tissues were stored at −70°C until analyzed. For the determination of metabolites, frozen tissues were homogenized in five volumes of 1N perchloric acid and the homogenate was centrifuged at 15,000 × *g* for 10 minutes. The supernatant was neutralized with one-half volume of 2N KOH and centrifuged, and the supernatant was removed for analysis of glucose,[15] fructose,[16] and sorbitol.[17]

Liver and dietary copper were extracted from samples by a method combining dry heat and acid digestion.[18] Duplicate samples were analyzed by flame atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Norwalk, CT, USA).[19] National Bureau of Standards Reference Material, bovine liver 1577a, was digested and analyzed along with samples to verify accuracy.

Data were analyzed by computer using the SAS software system for data analysis.[20] Data from rats changed from the F−Cu diet to other diets were analyzed by analysis of variance (ANOVA) and Duncan's Multiple Range Test. Data from rats that had been changed to a S+Cu, S−Cu, or F+Cu diet for 2 weeks were compared with rats consuming the respective diet for 6 weeks by Students' *t* test. A probability value of 0.05 or less was considered statistically significant.

Results

The mean body weight of weanling male rats at the beginning of the study was 66 ± 5g (± SEM). After consuming the F−Cu diet for 4 weeks, the mean body weight was 211 ± 10 g. At this time, 75% of the F−Cu dietary group was randomly assigned to either a F+Cu, S+Cu, or S−Cu diet. Some rats were given their respective diet at weaning and were maintained on that diet during the entire 6-week experimental period. *Table 1* gives body weight and some copper status measurements for the various dietary groups. The final body weight achieved was significantly higher for rats changed to the S+Cu diet compared with those maintained on the F−Cu diet. However, body weight gain during the 2 weeks was significantly greater for all rats changed from the F−Cu diet to another diet. None of the rats changed to a different diet for 2 weeks achieved the final body weight as those rats that had been consuming their respective diet for the entire 6-week period, although the weight gain in the change-over S−Cu dietary groups was greater than the continuous S−Cu group during the last 2 weeks of the study. The liver copper level was significantly elevated by changing rats to a diet that contained adequate copper regardless of the type of dietary carbohydrate. There were no differences between liver copper concentrations of rats switched from a F−Cu diet to the other diets and the rats that were maintained on the respective diets for the entire study. The hematocrit of rats changed from the F−Cu diet to diets containing copper returned to normal regardless of the type of dietary carbohydrate. Changing rats from a fructose diet deficient in copper to one containing starch was sufficient to improve the hematocrit by about 50%. Plasma glucose, fructose, and sorbitol concentrations were measured in the continuous dietary groups at the end of the study. Plasma glucose concentration was not affected by dietary treatment (7.7 ± 0.4 mmol/l for fed rats). Rats consuming the fructose diets had significantly higher plasma fructose and sorbitol concentrations (0.52 ± 0.08 and 0.44 ± 0.02 mmol/l, respectively) compared with rats fed starch diets (0.30 ± 0.02 and 0.36 ± 0.01 mmol/l, respectively).

Relative organ sizes are shown in *Table 2*. Relative liver size was significantly reduced by changing rats from the F−Cu diet to ones containing starch, regardless of the level of dietary copper. Relative liver sizes were significantly higher in rats changed from the F−Cu diet to the other diets than in rats that were maintained on the respective diets for the entire study. Relative pancreatic size was significantly increased and heart size decreased after changing rats from the F−Cu diet to diets containing copper, regardless of the type of dietary carbohydrate. Relative kidney size was not affected by either the dietary treatment or the experimental design.

Tissue fructose concentrations are given in *Table 3*. Liver fructose levels were significantly lowered by changing rats from the F−Cu diet to a diet containing

Table 1 Body weight, liver copper concentration, and hematocrit of rats changed from a low-copper, high-fructose diet to one containing copper or to a starch diet with or without copper[1]

Diet	Change-over[2]	Continuous[3]
Body weights (g)		
F−Cu		244 ± 13[b]
F+Cu	264 ± 7[a,b]	308 ± 5***,†
S+Cu	280 ± 10[a]	313 ± 10*,†
S−Cu	262 ± 8[a,b]	298 ± 9*,†
Weight gain/2 weeks (g)		
F−Cu		43 ± 5[b]
F+Cu	72 ± 4[a]	83 ± 3†
S+Cu	84 ± 5[a]	76 ± 8†
S−Cu	76 ± 4[a]	60 ± 5*,†
Liver copper (μg/g wet wt)[4]		
F−Cu		1.18 ± 0.09[c]
F+Cu	4.45 ± 0.25[b]	4.81 ± 0.25†
S+Cu	4.99 ± 0.18[a]	4.93 ± 0.22†
S−Cu	1.31 ± 0.17[c]	1.49 ± 0.17
Hematocrit (%)		
F−Cu		19 ± 3[c]
F+Cu	41 ± 2[a]	45 ± 1†
S+Cu	41 ± 2[a]	44 ± 1†
S−Cu	29 ± 4[b]	38 ± 2*,†

[1] All rats were fed ad libitum and were provided with deionized distilled water. Diets were 62.7% by weight carbohydrate and contained either 6.0 ppm copper (+Cu) or 0.6 ppm copper (−Cu).
[2] Weanling male rats consumed the F−Cu diet for 4 weeks, at which time 75% were randomly changed to the diets shown. Rats then consumed their respective diets for 2 weeks, after which the study was terminated. Each value represents the mean ± SEM for eight rats. Data were analyzed by ANOVA and Duncan's Multiple Range Test using the SAS Software System for data analysis (SAS Institute Inc.). A probability value of 0.05 or less was considered statistically signficant. Values under the change-over column or in the F−Cu dietary group sharing a common superscript letter are not different.
[3] Weanling male rats consumed their respective diets for 6 weeks. Group means of values under the continuous column were compared with values under the change-over column by Student's *t* test. Within a row, $*P < 0.05$, $**P < 0.001$, and $***P < 0.0001$. Group means of values under the continuous column different from F−Cu at $P \leq 0.05$ by Student's *t* test are indicated by a dagger (†).
[4] Liver samples were digested by a method combining dry heat and acid digestion. National Bureau of Standards reference material, bovine liver, was digested and analyzed with samples to verify accuracy. Tissue copper was measured by flame atomic absorption spectrophotometry.

Table 2 Relative organ sizes of rats changed from a low-copper, high-fructose diet to one containing copper or to a starch diet with or without copper[1]

Diet	Change-over	Continuous
Relative liver size[2]		
F−Cu		6.45 ± 0.28[a]
F+Cu	5.97 ± 0.18[a,b]	5.08 ± 0.24*,†
S+Cu	4.39 ± 0.06[c]	4.06 ± 0.11*,†
S−Cu	5.38 ± 0.27[b]	4.68 ± 0.14*,†
Relative pancreatic size		
F−Cu		0.15 ± 0.03[b]
F+Cu	0.42 ± 0.04[a]	0.39 ± 0.04†
S+Cu	0.36 ± 0.02[a]	0.32 ± 0.02†
S−Cu	0.23 ± 0.02[b]	0.33 ± 0.02*,†
Relative heart size		
F−Cu		0.56 ± 0.02[a]
F+Cu	0.39 ± 0.01[b]	0.36 ± 0.01†
S+Cu	0.43 ± 0.03[b]	0.36 ± 0.01*,†
S−Cu	0.57 ± 0.03[a]	0.47 ± 0.02*,†
Relative kidney size		
F−Cu		0.92 ± 0.05[a]
F+Cu	0.92 ± 0.02[a]	0.93 ± 0.02
S+Cu	0.84 ± 0.02[a]	0.79 ± 0.03†
S−Cu	0.85 ± 0.03[a]	0.80 ± 0.02†

[1] See *Table 1* for description.
[2] Relative organ size = (organ weight) 100/body weight.

Table 3 Tissue fructose concentration of rats changed from a low-copper, high-fructose diet to one containing copper or to a starch diet with or without copper[1]

Diet	Change-over	Continuous
Liver fructose[2]		
F−Cu		12.78 ± 0.48[a]
F+Cu	12.20 ± 0.47[a,b]	9.34 ± 0.58**,†
S+Cu	10.39 ± 0.58[c]	8.21 ± 0.56*,†
S−Cu	11.09 ± 0.27[b,c]	10.68 ± 0.58†
Pancreatic fructose		
F−Cu		0.77 ± 0.11[a]
F+Cu	0.35 ± 0.02[b]	0.27 ± 0.06†
S+Cu	0.33 ± 0.03[b]	0.36 ± 0.04†
S−Cu	0.38 ± 0.08[b]	0.22 ± 0.02†
Heart fructose		
F−Cu		0.95 ± 0.07[a]
F+Cu	0.64 ± 0.06[b]	0.49 ± 0.07†
S+Cu	0.58 ± 0.06[b]	0.39 ± 0.04*,†
S−Cu	0.76 ± 0.05[b]	0.67 ± 0.06†
Kidney fructose		
F−Cu		0.69 ± 0.06[a]
F+Cu	0.64 ± 0.06[a,b]	0.40 ± 0.01**,†
S+Cu	0.51 ± 0.01[b,c]	0.44 ± 0.04†
S−Cu	0.46 ± 0.04[c]	0.52 ± 0.04†

[1] See *Table 1* for description.
[2] Values are expressed as μmol/g wet weight; after acid precipitation of tissue samples, fructose was measured from the supernatant by the resorcinol colorimetric method.

Table 4 Tissue glucose concentration of rats changed from a low-copper, high-fructose diet to one containing copper or to a starch diet with or without copper[1]

Diet	Change-over	Continuous
Liver glucose[2]		
F−Cu		26.27 ± 2.29[a]
F+Cu	26.87 ± 1.79[a]	25.55 ± 2.03
S+Cu	25.61 ± 1.04[a]	26.03 ± 2.01
S−Cu	24.36 ± 1.07[a]	25.96 ± 1.74
Pancreatic glucose		
F−Cu		1.60 ± 0.24[a]
F+Cu	0.62 ± 0.04[b]	0.53 ± 0.02*,†
S+Cu	0.74 ± 0.06[b]	0.53 ± 0.03*,†
S−Cu	0.87 ± 0.13[b]	0.56 ± 0.04*,†
Heart glucose		
F−Cu		2.61 ± 0.26[a]
F+Cu	1.53 ± 0.17[b]	1.32 ± 0.20†
S+Cu	1.83 ± 0.24[b]	1.46 ± 0.13†
S−Cu	2.01 ± 0.12[a,b]	1.81 ± 0.09†
Kidney glucose		
F−Cu		0.95 ± 0.08[a]
F+Cu	1.09 ± 0.13[a]	0.85 ± 0.05
S+Cu	0.88 ± 0.05[a]	0.94 ± 0.05
S−Cu	1.00 ± 0.06[a]	0.84 ± 0.06*

[1] See *Table 1* for description.
[2] Values are expressed as μmol/g wet weight; after acid precipitation of tissue samples, glucose was measured from the supernatant by an enzymatic method.

starch, regardless of the level of copper. Liver fructose was significantly less in rats fed the copper-supplemented diets continually compared with rats that had been changed from the F − Cu diet to the copper-supplemented diets. Pancreatic and heart fructose were significantly lowered after changing rats from the F − Cu diet to any of the other experimental diets. Kidney fructose levels were lower in rats that were changed from a F − Cu diet to diets that did not contain fructose.

Tissue glucose concentrations are given in *Table 4*. Neither liver nor kidney glucose concentrations were altered by changing rats from the F − Cu diet to any of the other experimental diets. Pancreatic and heart glu-

Table 5 Tissue sorbitol concentration of rats changed from a low-copper, high-fructose diet to one containing copper or to a starch diet with or without copper[1]

Diet	Change-over	Continuous
Liver sorbitol[2]		
F−Cu		1.14 ± 0.08[a]
F+Cu	1.03 ± 0.15[a]	0.08 ± 0.11†
S+Cu	0.75 ± 0.17[a,b]	0.52 ± 0.08†
S−Cu	0.50 ± 0.02[b]	0.76 ± 0.07*†
Pancreatic sorbitol		
F−Cu		1.76 ± 0.29[a]
F+Cu	0.59 ± 0.03[b]	0.73 ± 0.10†
S+Cu	0.50 ± 0.06[b]	0.64 ± 0.02*†
S−Cu	1.70 ± 0.13[a]	0.78 ± 0.06***†
Heart sorbitol		
F−Cu		1.24 ± 0.06[b]
F+Cu	1.17 ± 0.04[b]	1.18 ± 0.13
S+Cu	1.46 ± 0.06[a]	1.36 ± 0.06
S−Cu	1.22 ± 0.09[b]	1.27 ± 0.08
Kidney sorbitol		
F−Cu		2.12 ± 0.18[a]
F+Cu	1.24 ± 0.18[b]	1.74 ± 0.16*
S+Cu	1.47 ± 0.08[b]	1.39 ± 0.10†
S−Cu	1.46 ± 0.15[b]	1.49 ± 0.16†

[1] See *Table 1* for description.
[2] Values are expressed as μmol/g wet weight; after acid precipitation of tissue samples, sorbitol was measured from the supernatant by an enzymatic method.

cose concentrations were significantly reduced by changing rats from the F−Cu diet to any of the other experimental diets. After consuming their new diets for 2 weeks, pancreatic glucose concentrations were still higher than those seen in rats continuously fed their respective diets for 6 weeks.

Tissue sorbitol concentrations are given in *Table 5*. Liver sorbitol concentration was significantly lowered by changing rats from the F−Cu diet to the S−Cu diet. The sorbitol level achieved was also lower than that observed in rats consuming the S−Cu diet continuously. Pancreatic sorbitol concentration was reduced two thirds when rats were changed from the F−Cu diet to diets containing copper, regardless of the type of carbohydrate. Rats consuming the F−Cu diet and changed to the S−Cu diet had twofold more pancreatic sorbitol than rats consuming the S−Cu diet continuously. Heart sorbitol concentration was significantly elevated by changing rats from the F−Cu to the S+Cu diet. Kidney sorbitol concentration was significantly lowered by changing rats from the F−Cu diet to any of the other experimental diets.

Discussion

The unusually high liver glucose concentrations reported in *Table 4* deserve mention. These values are several-fold higher than we have reported previously[10] and are higher than expected for animals with plasma glucose of 7.7 mmol/l. Livers, and other tissues, were quickly frozen between dry ice blocks to preserve metabolities, and the extraction procedure does not result in glycogen hydrolysis. All tissue samples were assayed at the same time, and normal and high controls were included with each assay. The measured control values were within the acceptable range and the measured glucose values for nonliver samples were within the expected range, but repeat assay values of liver glucose were higher than expected. Since rats were killed in the fed state and all stomachs contained food, we propose that hepatic portal glucose concentration was elevated as a result of the fed state, which led to a concomitant elevation in liver glucose concentration.

Rats fed the F−Cu diet grew poorly, had altered tissue sizes, were anemic, and had high tissue fructose and sorbitol concentrations compared with rats fed a fructose diet with copper or a starch diet with or without copper. In general, when rats were changed from the high-fructose, low-copper diet to any of the other diets, weight gain, relative tissue sizes, and hematocrit improved significantly and, in most instances, these variables returned to normal when compared with rats that had been maintained on the respective diet for the entire experimental period. The mechanism(s) of the dietary fructose-copper interaction is (are) not known. We have recently demonstrated that male rats consuming the F−Cu diet had elevated tissue levels of glucose, fructose, and sorbitol,[10] and we speculated that the consequences of the fructose-copper interaction may be the result of the tissue accumulation of these metabolites.

The conceptualization of the meaning of data on tissue concentrations of metabolites is difficult because we do not know the fractional contribution of extracellular or intracellular metabolites. However, even rigorous estimation of intracellular metabolite concentrations involve a number of actual and poten-

tial errors which include local blood flow changes within the tissue, uneven or changing distribution of metabolites within the cell, changes in enzyme activities, changes in end-product storage concentrations, and/or the presence of concentration gradients in the interstitial spaces. In the present study, our objectives were to determine, in general, if dietary fructose consumption with copper deficiency would alter certain tissue metabolite concentrations, if these alterations could be reversed by changing the diet, and if any of the observed responses were tissue-specific. Therefore, our approach was to measure total organ metabolite concentrations, disregarding inhomogeneity of the organ or the distribution of metabolites between extracellular and intracellular spaces. Estimates of tissue metabolite concentrations should be regarded as provisional only, but it is possible that tissue concentrations mimic those in the intracellular or subcellular spaces even though the relative concentrations between the spaces may change with diet.

In the present study, rats fed the F – Cu diet had the highest liver, pancreatic, heart, and kidney fructose concentrations compared with the other dietary groups. Changing rats from the F – Cu diet to any of the other diets led to a significant reduction and a resultant return to normal values in pancreatic and heart fructose concentrations. Liver and kidney fructose concentrations were significantly reduced in rats switched to a starch diet with or without copper. A similar pattern was seen with pancreatic and heart glucose concentrations, but not with liver and kidney glucose concentrations. Heart hypertrophy and rupture at the apex of the heart[1-3] and atrophy of the pancreas[6] have been reported in male rats consuming a high-fructose, low-copper diet. The cause of these observations is not known, but decreased cardiac cytochrome oxidase and norepinephrine concentrations, anoxia, and decreased heart collagen and elastin content have been implicated in the cardiac hypertrophy of copper deficiency.[21] The data of the present study suggest that elevated tissue fructose and glucose concentrations could also provide a partial explanation. It has been demonstrated that monosaccharides autoxidize under physiologic conditions and generate free radicals.[22] It was suggested that glucose, and other monosaccharide autoxidation, may contribute to oxidative stress in the pathophysiology associated with several diseases and degenerative processes.[22] Superoxide dismutase is one of the enzymes that has evolved to scavenge free radicals generated during biologic processes. In the pancreas, copper deficiency lowered SOD activity dramatically in rats consuming a diet containing starch, but pancreatic SOD activity in copper-deficient rats eating a fructose-containing diet was only one half the value of starch-fed rats.[6] The combination of high tissue glucose and fructose concentrations and low SOD activity in copper-deficient, fructose-fed rats may provide sufficient tissue oxidative stress to cause the observed tissue lesions.

Elevated levels of glucose[12] and fructose[9,10] have been shown to result in the accumulation of tissue sorbitol. In the present study, rats fed the F – Cu diet had the highest liver, pancreatic, and kidney sorbitol concentrations. There was considerable tissue sorbitol variation in response to changing rats from the F – Cu diet to the other diets. In the liver, although sorbitol concentration was lowered as a result of changing diets, only the decrease in liver sorbitol in the S – Cu dietary group was statistically significant. In the pancreas, changing rats from the F – Cu diet to any of the diets containing copper significantly lowered sorbitol concentration to normal or lower than normal values. In the heart, changing rats from the F – Cu diet to the S + Cu diet led to an increase in heart sorbitol concentration. In the kidney, sorbitol concentration was significantly reduced to normal or lower than normal values when F – Cu fed rats were changed to any of the other diets. Sorbitol accumulation in tissues of diabetics has been implicated in much of the pathophysiology of diabetes.[12] Sorbitol penetrates cellular membranes poorly and its accumulation upsets osmoregulation, which alters membrane permeability and cellular metabolism. Thus, the accumulation of sorbitol in some of the tissues (liver, pancreas, kidneys) of rats fed the F – Cu diet provides another possible mechanism for the observed tissue lesions with this diet.

Since both glucose and fructose can be reduced to sorbitol by aldose reductase and sorbitol dehydrogenase, respectively, the source of tissue sorbitol in the F – Cu rats cannot be determined from the present study. Glucose concentrations were elevated in the pancreas and heart but not in the liver and kidneys of F – Cu rats; thus the source of sorbitol may be different for different tissues. In a recent study of sorbitol accumulation in the kidney of fructose-fed rats, Bellomo et al.[9] concluded that the fructose-induced accumulation of kidney sorbitol was a result of increased flux of glucose through the polyol pathway since treatment with the aldose reductase inhibitor tolrestat prevented sorbitol accumulation. A similar mechanism may be working in the copper-deficient, fructose-fed rat.

In conclusion, when rats fed a diet deficient in copper and containing fructose were changed to a fructose diet containing copper or to a starch diet with or without copper, weight gain, relative tissue sizes, and hematocrit improved significantly, with a concomitant reduction in tissue glucose, fructose, and sorbitol concentrations. The fructose-copper interaction may have a common biochemical basis related to the metabolism of these three metabolites.

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