Carbohydrate tolerance in humans as influenced by sex, age, and erythrocyte superoxide dismutase activity

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Glycemic response using an oral load of fructose, glucose, and sucrose was measured in men and in premenopausal and postmenopausal women after being grouped by differences in erythrocyte superoxide dismutase activity (SOD). Blood was drawn at 0, 1/2, 1, 2, and 3 hr postload. Indices of copper status, carbohydrate tolerance, lactate, and uric acid levels were determined. Premenopausal women had lower plasma lactate, glucose, and fructose levels at 1/2 hour after the carbohydrate loads compared to postmenopausal women and men. Men had higher plasma uric acid responses to the carbohydrate loads than women. The fructose load produced higher plasma lactate and uric acid levels compared to sucrose and glucose. Postmenopausal women had higher plasma lipids, ceruloplasmin, and plasma copper levels than premenopausal women and men. SOD was used to divide the three subject groups into those with low (<269 U/ml) and normal (494–598 U/ml) activity. Fasting plasma triglyceride and the insulin and uric acid responses after the carbohydrate loads were significantly higher in the normal SOD group compared to the low SOD group. SOD activity did not correlate with other parameters of copper nutriture such as ceruloplasmin and plasma copper or with dietary copper intake.

Keywords: insulin; glucose; uric acid; lactic acid; triglycerides; ceruloplasmin

Introduction

American diets have high levels of simple carbohydrates.[1] According to the 1989 U.S. Department of Agriculture Sugar and Sweetener Report, the per capita consumption of total caloric sweeteners averages 167 g/day and accounts for 19% of total caloric intake.[1] Evidence linking high dietary levels of sucrose with risk factors associated with heart disease and diabetes has led to concern about the high consumption of sugars in the American diet. The deleterious effects of feeding diets high in sucrose and especially fructose are impaired glycemic control,[2] elevated serum triglycerides,[3,4] increased uric acid levels,[4] and lactate production.[5]

It is well established also that American diets are marginal in copper.[6] The nationwide average copper intake for women was 14.8 μmol and 19.4 μmol for men from the Food and Drug Administration's Total Diet Study 1982–1986.[6] This dietary level of copper is considered to be below the estimated adequate and safe intake of 23.6 to 47.2 μmol/day.[7] However, a recent study by Turnland et al.[8] has shown that 12.4 μmol Cu/day is adequate to maintain copper status and balance[9] for 42 days in normal healthy men.

Most of our knowledge regarding the existence of an interaction between copper and carbohydrate has been derived from experimental animals such as the rat[10-12] and the pig.[13-15] Only one human study attempted to determine the effect of the type of dietary carbohydrate and level of copper intake on indices of copper status[16] and blood lipids.[17] A low copper diet containing 16.2 μmol Cu/day with 20% of calories as

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fructose, fed for 11 weeks to 24 men significantly decreased erythrocyte superoxide dismutase (SOD) levels.[16] Cardiac abnormalities occured in some subjects.[16] Moreover, artherogenic lipids were elevated while the protective HDL-cholesterol was reduced when subjects consumed fructose as compared to starch.[17]

Men, and premenopausal and postmenopausal women were selected as subjects since differences in glucose tolerance,[18] triglycerides,[19] and uric acid[20] have been observed as a function of aging and sex. Sex differences have also been noted when determining indices of copper status.[21,22] The hypotheses for the present study were: (a) glycemic responses to the three carbohydrates measured in men and premenopausal and postmenopausal women may not be the same; (b) the three carbohydrates selected, fructose, glucose, and sucrose, which are the usual components of caloric sweeteners, would vary in their effect on human metabolic responses after a load dose; (c) indices of copper status and plasma lipids measured would show differences between men and women; and (d) a possible interaction between the copper status of a person and the ability of the carbohydrate load ingested to influence various metabolites of blood would be observed.

Materials and methods

The study was approved by the U.S. Department of Agriculture Human Studies Review Committee and the University of Maryland Institutional Review Board for Human Subjects.

All subjects selected for the study were free of overt disease as determined by a medical evaluation. Fifty-four subjects were chosen and divided into three groups of 18 men, 18 premenopausal women, and 18 postmenopausal women. All women participants had their blood analyzed for follicle stimulating hormone and luteinizing hormone levels to establish menopausal status. Premenopausal women were not using oral contraceptives and postmenopausal women were not using an estrogen supplement since exogenous estrogen supplements increase plasma copper.[23] Each subject was administered three tolerance tests two weeks apart, which consisted of 1 g/kg body weight sucrose, fructose, or glucose in a randomized order. Volunteers were screened to assess copper status using a 7-day diet record to determine dietary copper intake. Plasma copper concentration, ceruloplasmin oxidase activity, and erythrocyte SOD were used to assess copper status. Only erythrocyte SOD activity varied over a wide range of levels encompassing both low levels (< 269 U/ml) and normal levels (494–598 U/ml).[24] Each subject group was further divided into two subgroups of nine each based on low and normal SOD activity. The two subgroups were matched as closely as possible for weight, height, age, and sex.

For screening, fasting blood was collected in a mineral-free blood tube. Follicle stimulating hormone[25] and luteinizing hormone[26] concentrations were measured by a solid phase immunoradiometric assay which quantitates the intact human hormone molecule (ICN Biomedicals, Inc., Carson, CA). The volume of red blood cells was measured to express SOD activity/ml packed red cells. Red blood cell SOD activity was determined by the photochemical augmentation assay by Misra and Fridovich.[27] Measurement of ceruloplasmin was based on its oxidase activity.[28] Plasma copper was analyzed using flame atomic absorption spectrophotometry.[29] Human serum (Standard reference material, 909, National Institute of Standards and Technology, U.S. Dept. of Commerce, Gaithersburg, MD) was analyzed at the same time as blood samples to verify accuracy of analyses. For the 7-day dietary copper analysis, dietary intake record forms were used to note daily food intake by the method described by Hallfrisch et al.[30] Any subject taking a vitamin/mineral supplement was asked to include the label from the bottle. The copper content of diets was calculated using a recently compiled food copper database.[31]

Sucrose (Domino pure cane, Amstar Sugar Corp., NY) glucose (D (+)-Glucose, Sigma, St. Louis, MO), and fructose (Krystalline Fructose, A.E. Staley Mfg. Co., Decatur, IL) were used for the tolerance tests. Blood was drawn before and at 1/2, 1, 2, and 3 hour time points after the sugar drink. From the fasting blood sample at each tolerance test, plasma triglyceride[32] and cholesterol[33] were measured using an automated Centrifichem System 500 (Baker Instruments Co., Allentown, PA). Plasma glucose,[34] lactate,[35] and uric acid[36] concentrations at each time point were measured using the Centrifichem. Insulin was measured by radioimmunoassay (Radioassay Systems Laboratories, Inc., Carson, CA).[37] Plasma fructose was determined using a modification of the colorimetric method by Roe[38] after a solid phase extraction procedure using a cartridge (Supelco Inc., Bellefonte, PA) to isolate fructose.

Statistical analysis included analysis of variance and Duncan's multiple range test.[39] A logarithmic transformation was used for plasma insulin, glucose, and triglyceride levels since the data did not meet the requirement for homogeneity of variance.[40] Area under the curve was calculated using the summation of trapezoids method for insulin, glucose, fructose, uric acid, and lactic acid concentrations. Pearson correlation coefficients were determined between the indices of copper status. Differences between values with $P < 0.05$ were considered statistically significant.

Results

Table 1 presents the summation (1/2–3 hr) of insulin, glucose, fructose, uric acid, and lactic acid responses. Plasma insulin response was lower for men compared to women. Premenopausal women showed a significant lower area under the plasma uric acid response curve compared to postmenopausal women ($P < 0.026$) and men ($P < 0.0002$). This difference was significant at each time point. Premenopausal women tended to have a lower summation of lactic acid, glu-

Table 1 Summation of plasma insulin, glucose, fructose, uric acid, and lactic acid responses to an oral load of various carbohydrates for subject groups

Subject groups	Insulin (pmol/L)	Glucose (mmol/L)	Fructose (mmol/L)	Uric acid (μmol/L)	Lactic acid (mmol/L)
Men	1125[a]	21	0.46	851	4.9
Premenopausal women	1137	19	0.42	636	4.4
Postmenopausal women	1190	20	0.52	753	5.2
SE	±31	±4	±0.03	±36	±0.3
ANOVA[b] group	ns	ns	ns	$P < 0.008$	ns

Abbreviation: ns = nonsignificant.
[a] Each mean represents the average of 18 subjects.
[b] Analysis of variance $P < 0.05$ significant.

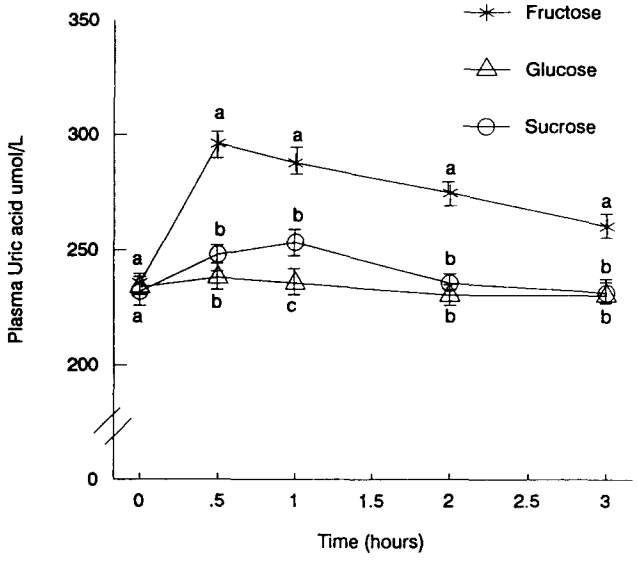

Figure 1 Plasma uric acid concentrations before and during the carbohydrate tolerance tests. Vertical lines represent mean ± SE. Means at each time point with different superscript letters are significantly different from each other ($P < 0.05$), as determined by Duncan's multiple range test

cose, and fructose responses than postmenopausal women and men. This difference was significant at ½ hr ($P < 0.01$) for lactic acid, glucose, and fructose levels and at 1 hr ($P < 0.02$) for lactic acid levels.

The levels of blood metabolites after an oral load of the three carbohydrates were also examined independent of the age and sex of the subjects. Plasma uric acid concentrations were significantly higher ($P < 0.0001$) at all time points postload during the fructose tolerance compared to the sucrose and glucose tolerance tests and at 1 hr after the sucrose as compared to the glucose tolerance test (*Figure 1*). Plasma lactate concentrations were significantly higher at 1/2 hr ($P < 0.001$) and 1 hr ($P < 0.008$) after the fructose and sucrose loads compared to the glucose load (*Figure 2*).

The fasting plasma lipid concentrations for the three subject groups are shown in *Table 2*. Postmenopausal women had significantly higher plasma cholesterol concentrations compared to premenopausal women ($P < 0.001$) and men ($P < 0.003$). Nine (three men, one premenopausal woman, five postmenopausal women) of the 54 subjects had elevated cholesterol levels compared to the normal range for age-adjusted individuals (men, < 5.85 mmol/L; premenopausal women, < 5.2–5.85 mmol/L; postmenopausal women, < 6.85 mmol/L.[41] Plasma triglycerides were significantly higher ($P < 0.01$) for postmenopausal women compared to premenopausal women. Men had higher plasma triglycerides than premenopausal women but lower levels than postmenopausal women. However, neither difference was significant. Nine (two men, one

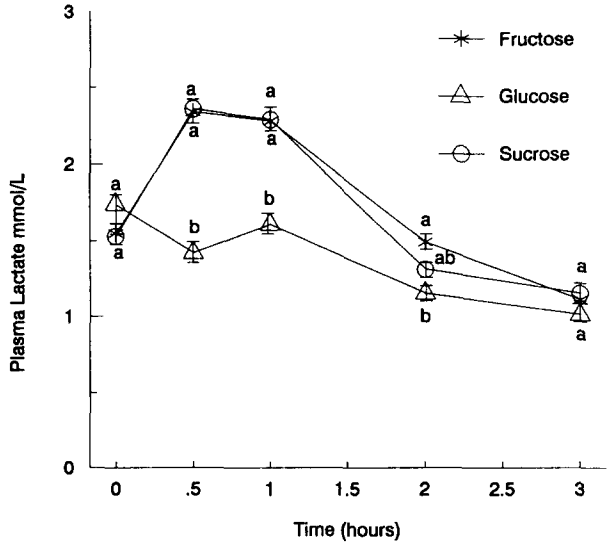

Figure 2 Plasma lactate concentrations before and during the carbohydrate tolerance tests. Vertical lines represent mean ± SE. Means at each time point with different superscript letters are significantly different from each other ($P < 0.05$), as determined by Duncan's multiple range test

TABLE 2 Fasting plasma cholesterol, triglyceride, copper concentrations, erythrocyte SOD activity, ceruloplasmin activity, and dietary copper intake for subject groups

Subject groups	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Plasma copper (μmol/L)	Erythrocyte SOD (U/ml)	Ceruloplasmin (mg/L)	Diet copper[a] (μmol/day)
Men	5.1[b]	0.98	17	371	284	39.4
Premenopausal women	4.6	0.86	20	355	341	22.0
Postmenopausal women	6.4	1.30	21	354	378	29.9
SE	±0.3	±0.04	±1.0	±14	±17	±6.3
ANOVA[c] group	$P < 0.001$	$P < 0.033$	$P < 0.001$	ns	$P < 0.001$	$P < 0.003$

Abbreviation: ns = nonsignificant.
[a] Dietary copper intake from 7-day diet records includes mineral supplements containing copper taken by the subjects.
[b] Each mean represents the average of 18 subjects.
[c] Analysis of variance $P < 0.05$ significant.

premenopausal woman, six postmenopausal women) out of 54 subjects had high plasma triglyceride levels compared to the normal range of 0.38–1.87 mmol/L.[32] Three subjects (one man, two postmenopausal women) had both elevated plasma cholesterol and triglyceride concentrations.

ANOVA revealed a significant group effect (men, premenopausal and postmenopausal women) on dietary copper intake, plasma copper levels, and ceruloplasmin oxidase activity but not for erythrocyte SOD activity (*Table 2*). The dietary copper intake for men was significantly higher ($P < 0.003$) than copper intake for premenopausal women. Copper intake for postmenopausal women was not, however, significantly different from either men or premenopausal women. Plasma copper concentrations and ceruloplasmin oxidase activity were significantly higher ($P < 0.001$) for postmenopausal and premenopausal women compared to men. Postmenopausal women tended to have higher plasma copper concentrations and ceruloplasmin activity than premenopausal women, but this difference was not statistically significant.

No significant correlation was found between SOD activity and plasma copper or ceruloplasmin oxidase activity. However, plasma copper was correlated positively ($R = 0.81$, $P < 0.03$) with ceruloplasmin.

When subjects were divided on the basis of SOD activity, the group with normal SOD activity showed significantly higher plasma insulin and uric acid responses to the tolerance tests and higher fasting triglycerides than subjects with low SOD activity (data not shown). No significant differences in blood glucose, fructose, and lactate levels were noted. Further investigation into the individual responses of these parameters for each subject revealed that of the six subjects that were classified as being hyperinsulinemic,[42] four had normal SOD activity and two displayed low SOD activity. ANOVA run without these six subjects showed no significant differences in plasma insulin, uric acid, and triglyceride levels based on SOD activity.

Discussion

Three different subject groups were selected to participate in this study. Although the average age for men (36 years) and premenopausal women (31 years) was lower than for postmenopausal women (59 years), there were four older men aged 56–61 years in the mens' subject group. When these four older male subjects were deleted from the data analysis, the fasting plasma cholesterol and triglycerides were not significantly lower as compared to values for all 18 men. The plasma insulin, glucose, fructose, and lactate responses also did not change.

Glucose intolerance associated with aging was noted in 17 elderly adults by Fink et al.[18] They found the total glucose and insulin responses following an oral glucose load were increased by 24% and 127%, respectively, in the elderly compared with the young adults. In our study, an age effect on carbohydrate tolerance was evident only for female subjects. The premenopausal women had lower insulin, glucose, and fructose responses compared to postmenopausal women.

Premenopausal women also had lower uric acid and lactate responses compared to postmenopausal women and men, indicating both an age and sex effect. It is generally accepted that males have higher fasting uric acid levels than females. Men had the highest summation of plasma uric acid response compared to women. A similar sex effect on plasma uric acid levels has been shown by Solyst et al.,[20] however, plasma lactate levels for men did not differ significantly from those for women before and after a 2 g/kg body weight sucrose load.

Fructose-induced hyperuricemia has been observed when fructose and sucrose, but not glucose, were administered orally to male volunteers by MacDonald et al.[43] Similar results were obtained in our study, suggesting the consumption of fructose and sucrose in large amounts in the diet can have deleterious effects by increasing the concentration of uric acid. Since

high levels of uric acid are associated with a number of disease states, these high levels of blood fructose are metabolically undesirable.

The type of carbohydrate used for the tolerance test in our study also influenced plasma lactate concentrations after a load dose. Serum lactate concentrations also have been shown to be elevated after sucrose and fructose loads in studies by Solyst et al.[20] and Crapo and Kolterman,[44] but not after glucose ingestion.[44] Therefore, another potential problem with fructose feeding is lactic acidemia.

Postmenopausal women in our study had the highest plasma triglyceride concentrations. An increase in plasma triglyceride with age in man has been shown by Greenfield et al.[19] Premenopausal women had the lowest plasma lipid levels compared to postmenopausal women and men. MacDonald[45] has shown a protective effect of the ovarian hormones on lipid levels in premenopausal women compared to postmenopausal women fed a high carbohydrate diet. Elevated plasma triglycerides are a risk factor for coronary artery disease.[46] The data from this study suggest that men and postmenopausal women appear to be at greater risk for the development of heart disease compared to premenopausal women on the basis of their higher triglyceride concentrations.

Over 75% of total serum copper is distributed as ceruloplasmin.[47] Therefore, one would expect a significant correlation between plasma copper and ceruloplasmin as was found in our study. Estrogens increase ceruloplasmin production in the liver.[23] The higher plasma copper levels for premenopausal women in our study compared to men probably reflects the increase in ceruloplasmin levels due to estrogen. However, a fall in ceruloplasmin would be expected at menopause. In contrast, we found postmenopausal women and four older men aged 56 to 61 years had higher ceruloplasmin oxidase activity than younger subjects. Other factors must influence ceruloplasmin synthesis during the aging process.[48] Ceruloplasmin is an acute phase reactive protein and levels increase during arthritis.[49] It also acts as an antioxidant and inhibits lipid peroxidation, known to be higher after menopause.[48]

Screening of volunteers for indices that reflect copper status indicated that only SOD activity was significantly different among subjects. Most of the copper (60%) in red cells is associated with the enzyme Cu-Zn SOD.[50] Copper occupies the site of enzymatic activity in SOD.[51] Since SOD activity has been used as a reliable indicator of copper status in several human studies,[16,52-54] this index was used to divide subjects in the present study into six subgroups. However, since there was no correlation between SOD activity and the other direct indices of copper status measured, or with dietary copper intake, the subjects with low SOD activity were not considered to be copper deficient or to exhibit inadequate copper status. The terms "low SOD activity" and "normal SOD activity" were used to describe the subject subgroups in an attempt to correlate SOD with other indirect "nonspecific" variables associated with changes in copper status and processes related to carbohydrate tolerance. These variables include plasma lipid levels and the glycemic response after a carbohydrate load.

The normal SOD activity subject group showed higher fasting plasma triglyceride, insulin, and uric acid responses after the carbohydrate loads than did the low SOD activity subject group. This indicated there were more hypertriglyceridemic, hyperinsulinemic, and hyperuricemic persons in this group compared to the low SOD activity subject group. Hyperinsulinemic individuals are known to have some risk factors associated with heart disease and diabetes.[46] These include elevated plasma glucose and insulin levels after an oral glucose challenge, elevated fasting plasma triglyceride levels, elevated blood pressure, and lower HDL-cholesterol levels.[46] Elevated serum uric acid is also a feature of hyperinsulinemia and insulin resistance.[55] Therefore, the higher plasma insulin, triglyceride, and uric acid levels in the normal SOD activity subject group could be due to the four hyperinsulinemic subjects in that group rather than to the SOD activity of these subjects. This was substantiated when these individuals were eliminated from subsequent analysis of experimental data.

Conclusion

The results of this study, when considered with respect to the hypotheses stated in the introduction, indicate that in human subjects, carbohydrate tolerance is influenced to a certain extent by the sex and age of a person, but probably not SOD activity. Aging was associated with higher insulin, glucose, fructose, lactate, and uric acid levels. Also, these results with regard to uric acid and lactate responses are in accord with the concern about intake of sucrose and fructose containing carbohydrates as compared to glucose-based carbohydrates.

The difference in SOD activity of these subjects could be due to the intake of nutrients other than copper in the diet, such as calcium and phosphorus (unpublished data) which are known to impair copper absorption and decrease SOD activity. Further research with humans is needed before any speculation about a mechanism on the copper-carbohydrate interaction can be made.

Note

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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