# Severity of copper deficiency in rats fed fructose is not solely dependent on hepatic copper concentration: effects of adrenalectomy

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*The present study was undertaken in order to establish whether an increase in hepatic copper concentration will ameliorate the severity of copper deficiency when a fructose based diet is consumed. Eighty weanling male Sprague-Dawley rats were divided randomly into four dietary groups and were fed a copper deficient (0.6 μg Cu/g) or adequate (6.0 μg Cu/g) diet containing 62% carbohydrate as either starch or fructose. Half of the rats underwent bilateral adrenalectomy and the rest were sham operated. Adrenalectomy was successful in increasing the concentration of hepatic copper in rats fed the copper deficient diet containing fructose compared to sham operated controls. However, the severity of copper deficiency was not ameliorated by these increases. The data suggest that the severity of copper deficiency in rats fed fructose may not be solely dependent on hepatic copper concentration but rather may be due to a combination of subsequent metabolites of fructose superimposed upon copper deficiency.*

**Keywords:** glucocorticoid; corticosterone; starch; iron

## Introduction

Recent studies have shown that the type of dietary carbohydrate consumed by experimental animals determines the severity of copper deficiency.[1-5] When the diet contains simple sugars such as sucrose or fructose, the signs associated with the deficiency are aggravated.[1-5] In contrast, when starch is provided as the sole carbohydrate moiety of the diet, the deficiency is ameliorated and the animals are protected against the toxic effects of copper deficiency.[1-5]

The reasons for the dramatic differences in the expression of copper deficiency between rats fed starch and those fed fructose could be due to differences in copper status. Specifically, if fructose feeding causes a reduction in copper content of tissues, then less copper will be available for utilization when fructose is consumed than when starch is consumed.

It is well established that the liver plays a major role in copper homeostasis.[6] For that reason, hepatic copper concentration is commonly used to assess copper status in experimental animals. In the majority of studies where dietary fructose and starch were fed to experimental animals, hepatic copper concentrations of rats fed fructose were lower than the corresponding values from rats fed starch.[1,3,4] This may have a deleterious effect on the well being of the fructose fed animal. However, when the whole body content of copper was measured, rather than of individual tissues, no differences in total copper concentrations could be found between the two experimental groups.[7] It has been reported that the ingestion of fructose containing diets increases the levels of glucocortical hormones.[8] It has also been shown that glucocorticoids regulate copper metabolism and homeostasis.[9] The administration of glucocorticoids decreased hepatic copper content.[9] In contrast, rats that underwent adrenalectomy exhibited higher hepatic copper concentration than

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sham operated controls.[9] If fructose feeding increases the synthesis of adrenocortical hormones, which in turn reduce hepatic copper stores, then by removing the adrenals more copper should be deposited in the liver for subsequent utilization. This effect of adrenalectomy should be beneficial to the copper deficient rat consuming fructose.

The purpose of this study was to attempt to increase the copper content of the liver of copper deficient rats fed fructose by adrenalectomy and to then determine if the increase in hepatic copper will result in the amelioration of the signs associated with the deficiency.

## Materials and methods

Eighty weanling male Sprague-Dawley rats, weighing approximately 40–45 g each were divided randomly into eight groups according to a 2 × 2 × 2 factorial design, which differed in the levels of dietary copper, type of dietary carbohydrate, and type of operation.

Group 1 Fructose-Cu, Adrenalectomy (ADX)
Group 2 Fructose-Cu, Sham
Group 3 Starch-Cu, ADX
Group 4 Starch-Cu, Sham
Group 5 Fructose + Cu, ADX
Group 6 Fructose + Cu, Sham
Group 7 Starch + Cu, ADX
Group 8 Starch + Cu, Sham

All rats were fed from a diet that consisted of the following ingredients (g/kg diet): 627 carbohydrate as either starch or fructose, 200 egg white, 95 corn oil, 30 non-nutritive fiber (cellulose), 35 copper-free AIN76A salt mix[10] formulated in our laboratory to omit copper carbonate, 10 AIN76A vitamin mix[11] supplemented with 2 mg biotin, and 2.7 g choline bitartrate. The concentrations of copper in the deficient and adequate diets were 0.6 μg Cu/g and 6.0 μg Cu/g, respectively, as measured by flame atomic absorption spectrophotometry.

Upon arrival, half of the rats underwent bilateral adrenalectomy under ether anesthesia. Adrenalectomy was performed via a dorsolumbar approach. In the remaining rats, both adrenal glands were identified, left in situ, and the incision closed. All adrenalectomized rats were allowed free access to salt solution (0.9% NaCl). Sham operated rats did not receive saline since a pilot study showed that drinking distilled water or saline did not influence the parameters measured in this study. The study was terminated at the end of the sixth week of dietary regimen. All rats were decapitated between 08:00 and 11:00 hours and 2 hours after diet had been removed from their cages. To minimize stress-induced release of glucocorticoids in sham operated rats, all rats (ADX and sham) were "quiescent walked" for 10 to 14 days before killing. Between 08:00 and 11:00 hours, rats were removed from their cages, handled, and carried to a guillotine for "familiarization." Blood samples were drawn into heparinized microhematocrit tubes for the measurement of hematocrit following the decapitation of the rats. Hearts, pancreas, epididymal fat pads, adrenals, and livers were removed and weighed. Total copper and iron concentrations were determined in liver by flame atomic absorption spectrophotometry[12] following their digestion according to Hill et al.[13] Total plasma glucocorticoids[14] and the maximal output of glucocorticoids from the adrenal glands following an ACTH challenge in vitro were measured.[14]

All data were subjected to analysis of variance (ANOVA).[15] A value of $P < 0.05$ was considered statistically significant.

## Results

By the end of the study, two of the ten adrenalectomized copper deficient rats fed fructose had died. The remaining copper deficient rats fed fructose exhibited heart hypertrophy with gross pathological changes. The data in *Table 1* illustrate the changes in body weight and relative organ sizes in bilaterally adrenalectomized and sham operated rats consuming copper deficient or adequate diets containing fructose or starch for 6 weeks. Body weight was reduced by adrenalectomy. It was also reduced by copper deficiency and by fructose feeding. Liver size increased by fructose feeding compared to starch. Adrenalectomy reduced liver size only in rats fed fructose compared to non-adrenalectomized animals. Adrenalectomy increased heart size in all animals. The largest relative heart size was found in copper deficient rats fed fructose. The pancreas was atrophied only in rats fed the copper deficient diet containing fructose. Adrenalectomy had no effect on pancreas size. Epididymal fat pad size was significantly reduced by copper deficiency in rats fed fructose compared to all other animals. Adrenalectomy reduced epididymal fat pad size compared to sham operated controls.

*Table 2* summarizes data pertaining to hepatic copper and iron concentrations. Copper deficiency was verified by the reduced hepatic copper concentrations compared with copper adequate controls. Only in copper deficient rats fed fructose did adrenalectomy cause nearly a three-fold increase in hepatic copper concentration compared to sham operated controls. Except for copper deficient rats fed starch, all other adrenalectomized animals exhibited moderate increases in the concentration of copper compared to non-adrenalectomized animals. All copper deficient rats exhibited a two-fold increase in hepatic iron concentrations compared to copper adequate controls. Adrenalectomy had no effect on iron concentrations in the liver.

*Table 3* summarizes the concentrations of glucocorticoids in plasma and the output of glucocorticoids from adrenal gland upon the stimulatory effect of ACTH. Adrenalectomy was verified by the absence of the adrenals and by significantly reduced concentrations of glucocorticoids in plasma compared to sham operated controls. Sham operated copper deficient rats that consumed fructose exhibited a two-fold increase in plasma glucocorticoids compared to all other control animals. When ACTH stimulated the adrenal

**Table 1** Body weight and relative organ sizes in adrenalectomized (ADX) and sham operated, copper deficient (−Cu) and adequate (+Cu) rats fed fructose (FR) or starch (ST)

| | Body wt (g) | Relative organ sizes | | | |
|---|---|---|---|---|---|
| | | Liver | Heart (g/100g bw) | Pancreas | Epididymal fat pad |
| FR − Cu + ADX | 238 ± 9 | 4.3 ± 0.3 | 0.60 ± 0.06 | 0.28 ± 0.04 | 0.46 ± 0.07 |
| FR − Cu + Sham | 265 ± 8 | 6.0 ± 0.1 | 0.55 ± 0.02 | 0.28 ± 0.02 | 0.59 ± 0.03 |
| ST − Cu + ADX | 300 ± 11 | 4.7 ± 0.1 | 0.51 ± 0.03 | 0.49 ± 0.03 | 0.80 ± 0.06 |
| ST − Cu + Sham | 300 ± 4 | 4.3 ± 0.1 | 0.42 ± 0.01 | 0.54 ± 0.03 | 1.08 ± 0.06 |
| FR + Cu + ADX | 258 ± 10 | 5.2 ± 0.1 | 0.43 ± 0.01 | 0.54 ± 0.04 | 0.70 ± 0.05 |
| FR + Cu + Sham | 292 ± 5 | 5.5 ± 0.1 | 0.36 ± 0.01 | 0.56 ± 0.03 | 1.10 ± 0.07 |
| ST + Cu + ADX | 291 ± 10 | 4.2 ± 0.1 | 0.42 ± 0.02 | 0.53 ± 0.03 | 0.80 ± 0.08 |
| ST + Cu + Sham | 323 ± 5 | 4.1 ± 0.1 | 0.35 ± 0.01 | 0.59 ± 0.02 | 1.15 ± 0.06 |
| | | ANOVA | | | |
| Carbohydrate (CHO) | S | S | S | S | S |
| Copper (Cu) | S | NS | S | S | S |
| CHO × Cu | NS | S | S | S | S |
| ADX | S | S | S | NS | S |
| CHO × ADX | NS | S | NS | NS | NS |
| Cu × ADX | NS | S | NS | NS | S |
| CHO × Cu × ADX | NS | S | NS | NS | NS |

Mean ± SEM of 10 rats per group except for FR − Cu + ADX which had 8. A 2 × 2 × 2 Analysis of Variance. Effects and interaction significant (S) $P < 0.05$; non-significant (NS).

**Table 2** Hepatic copper and iron concentrations in adrenalectomized (ADX) and sham operated, copper deficient (−Cu) and adequate (+Cu) rats fed fructose (FR) or starch (ST) diets

| | Copper μg/g wet wt. | Iron |
|---|---|---|
| FR − Cu + ADX | 1.52 ± 0.18 | 134 ± 10 |
| FR − Cu + Sham | 0.55 ± 0.10 | 111 ± 11 |
| ST − Cu + ADX | 1.03 ± 0.09 | 122 ± 13 |
| ST − Cu + Sham | 1.19 ± 0.10 | 135 ± 16 |
| FR + Cu + ADX | 3.77 ± 0.26 | 62 ± 5 |
| FR + Cu + Sham | 3.49 ± 0.12 | 59 ± 5 |
| ST + Cu + ADX | 3.67 ± 0.12 | 57 ± 10 |
| ST + Cu + Sham | 3.39 ± 0.10 | 67 ± 4 |
| | ANOVA | |
| CHO | NS | NS |
| Cu | S | S |
| CHO × Cu | NS | NS |
| ADX | S | NS |
| CHO × ADX | S | S |
| Cu × ADX | NS | NS |
| CHO × Cu × ADX | S | NS |

Mean ± SEM of 10 rats per group except for FR − Cu + ADX that had 8.

**Table 3** Plasma and adrenal glucocorticoids in adrenalectomized (ADX) and sham operated, copper deficient (−Cu) and adequate (+CU) rats fed fructose (FR) or starch (ST) diets

| | Plasma glucocorticoids nmol/l | Adrenal glucocorticoids ng/mg/hr |
|---|---|---|
| FR − Cu + ADX | 171.3 ± 30.2 | |
| FR − Cu + Sham | 1467.2 ± 277.2 | 567 ± 187 |
| ST − Cu + ADX | 232.4 ± 41.2 | |
| ST − Cu + Sham | 703.0 ± 137.2 | 305 ± 52 |
| FR + Cu + ADX | 140.5 ± 22.4 | |
| FR + Cu + Sham | 660.8 ± 84.0 | 258 ± 39 |
| ST + Cu + ADX | 243.9 ± 72.2 | |
| ST + Cu + Sham | 677.6 ± 86.8 | 250 ± 45 |
| | ANOVA | |
| CHO | NS | NS |
| Cu | S | NS |
| ADX | S | NS |
| CHO × Cu | S | NS |
| CHO × ADX | S | NS |
| Cu × ADX | S | NS |
| CHO × Cu × ADX | S | NS |

Mean ± SEM of 10 observations/group except for FR − Cu + ADX that had 8.

gland in vitro, the output of glucocorticoids tended to be higher in copper deficient rats fed fructose compared to all other rats.

## Discussion

The results of the present study confirm previous observations that the type of dietary carbohydrate plays a major role in the exacerbation of copper deficiency.[1-5] Fructose feeding, but not starch feeding, in combination with copper deficiency resulted in growth retardation, heart hypertrophy with gross pathology, pancreatic atrophy, and a reduction of extrahepatic lipogenesis reflected by a reduced epididymal fat pad size. In addition, two rats died of the deficiency.

The severity of copper deficiency in rats fed fructose was not ameliorated by adrenalectomy, although adrenalectomy increased hepatic copper concentration by nearly three-fold compared to fructose, copper deficient, sham operated controls. In addition, copper deficient, adrenalectomized rats fed fructose had 50% more hepatic copper compared to rats fed starch, and yet copper deficient rats fed starch did not develop

any signs of pathology. Furthermore, although significantly more copper was deposited in the livers of copper deficient rats fed fructose, adrenalectomized rats exhibited the same deleterious signs associated with copper deficiency as those of non-adrenalectomized animals. Thus, the data presented here strongly imply that the severity of copper deficiency in rats fed fructose is not solely due to copper status as reflected by hepatic copper stores. It is suggested that the enhanced glucocorticoid output when fructose is fed creates a unique subcellular environment. The combination of this specific environment with copper deficiency is responsible for the aggravation and pathology of copper deficiency when fructose is consumed.[16,17]

The metabolism of copper has been shown to be regulated by glucocorticoids.[9] More copper is retained by the liver of adrenalectomized rats than by the liver of sham operated animals,[9] and the rate of elimination of the excess copper from the liver of copper injected rats is reduced by adrenalectomy.[9] In addition, the injection of glucocorticoids to adrenalectomized rats resulted in a reduction of hepatic copper concentrations compared to non-treated animals.[9] The data presented here confirm the contention that adrenal hormones play a role in copper homeostasis. Adrenalectomy elicited some increases in hepatic copper concentrations in all groups of rats regardless of copper deficiency. However, only copper deficient rats fed fructose ,exhibited nearly three-fold increases of hepatic copper as a result of the adrenalectomy compared to nonadrenalectomized controls (*Table 2*). This increased hepatic copper concentration in adrenalectomized rats was inversely correlated with the reduced concentration of glucocorticoids in plasma.

A daily variation in the pattern of adrenocortical hormone secretion has been well documented. The lowest concentration of corticosteroids are found between 6 a.m. and noon.[18-20] Adrenalectomized rats revealed plasma steroid concentrations of 138–276 nmol/l (5–10 μg/100 ml).[18-20] These values are comparable with the results of the present study and with the time of the day this study was conducted.

The highest plasma glucocorticoid concentration was found in sham operated, copper deficient rats fed fructose. Since all rats had been handled daily and "quiescent walked" for 10–14 days prior to terminating the study, it is highly unlikely that only one group of the 8 groups was stressed. In addition, all rats were killed at a time when plasma glucocorticoids are at their lowest levels.[19,20] Thus, other factors play a role in this enhanced glucocorticoid output when copper deficient rats are fed fructose.

It is well established that the major regulator of adrenocortical secretory activity is the anterior pituitary hormone ACTH.[21] Although all adrenals of the present study were incubated with the same concentration of ACTH and were treated the same, the adrenals of copper deficient rats fed fructose secreted a higher concentration of glucocorticoids than all other adrenals. Likewise the concentration of glucocorticoids in plasma of copper deficient rats fed fructose was two-fold the corresponding values from all other rats. The reasons for the increased output of glucocorticoids in copper deficient rats consuming fructose is not fully understood. However, it cannot be due to copper deficiency per se or to fructose consumption, since both copper deficient rats fed starch and copper adequate rats fed fructose do not secrete high levels of glucocorticoid. Only when fructose feeding is combined with copper deficiency is the secretion of glucocorticoid enhanced. Increased cyclic AMP content, an increase in steroid synthesis, and greater sensitivity to ACTH have been shown to play a role in glucocorticoid secretion.[21] The increased secretion of glucocorticoid in copper deficient rats consuming fructose could be due to stress or to the morbidity of the animals. It has been observed that plasma cortisol concentrations are generally elevated in both stress and in dying patients.[21] However, the design of this study does not allow us to differentiate between the numerous factors that could induce a hypersecretion of glucocorticoids in copper deficient rats fed fructose.

Many of the typical signs associated with copper deficiency when fructose containing diets are consumed are similar to those reported in hypersecretion of corticoids.[21] These include weight loss,[1,3] depletion of adipose tissue due to mobilization of fat,[3] involution of the thymus,[22] and impaired glucose tolerance.[23] Regardless of the factors that contribute to the elevation in plasma corticoids in rats consuming the copper deficient diet containing fructose, the high glucocorticoid secretion may be responsible for the "typical" signs associated with copper deficiency in rats fed fructose.

## Summary

The data of the present study show that copper deficiency per se is not sufficient to cause pathology to the rats since a) copper deficient rats fed starch are protected against heart pathology and mortality, and b) even when hepatic copper is increased, rats fed fructose die of the deficiency. Therefore, it is suggested that the induction of an enhanced glucocorticoid output when fructose is fed may provide a different subcellular environment than starch. The combination of copper deficiency with the increased secretion of glucocorticoids and the specific metabolic pathways of fructose may create a toxic environment which eventually will cause the pathology seen in copper deficient rats fed fructose.

## References

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

2 Lewis, C.G., Fields, M., Craft, N., Yang, C-Y, Reiser, S. (1988). Changes in pancreatic enzyme specific activities of rats fed a high-fructose low copper diet. *J. Am. Coll. Nutr.* **7**, 27–34

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

4 Reiser, S., Ferretti, R.J., Fields, M., Smith, J.C. (1983). Role of dietary fructose in the enhancement of mortality and biochemical changes associated with copper deficiency in the rat. *Am. J. Clin. Nutr.* 38, 214–222

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

6 Ettinger, M.J. (1984). Copper metabolism and diseases of copper metabolism. In *Copper proteins and copper enzymes.* (R. Lonti, ed.) Vol. 3, pp. 176–229, CRC Press Inc., Boca Raton, FL

7 Failla, M.L., Seidel, K.E. (1988). Total body content of copper and other essential metals in rats fed fructose or starch. *Nutr. Res.* **8,** 1379–1389

8 Yudkin, J. and Szanto, S. (1972). Increased levels of plasma insulin and eleven hydrocoticosteroid induced by sucrose and their reduction by phenformin. *Hom. Metab. Res.* **4,** 417–421

9 Gregoriadis, G. and Sourkes T.L. (1970). Regulation of hepatic copper in the rat by adrenal gland. *Can. J. Biochem.* **48,** 160–163

10 American Institute of Nutrition. (1977). Report of the AIN Ad Hoc Committee on Standards for Nutritional Studies. *J. Nutr.* **107,** 1340–1348

11 American Institute of Nutrition. (1980). Second Report of the AIN Ad Hoc Committee on Standards for Nutritional Studies. *J. Nutr.* **110,** 1726

12 Perkin-Elmer Corporation. (1976). *Analytical Methods for Atomic Absorption Spectrophotometry.* Norwalk, CT

13 Hill, A.D., Patterson, K.Y., Veillon, C., Morris, E.R. (1986). Digestion of biological materials for mineral analysis using a combination of wet and dry ashing. *Anal. Chem.* **58,** 2340–2342

14 Sayers, G., Beall, R.J., Seelig, S., Cummins, K. Isolation of adrenal cortex cells—hormone response. In *Methods of Enzymology* vol **32,** 673–693

15 SAS Institute, Inc. (1985). SAS User's Guide: Statistics, 5th ed. Cary, NC

16 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

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

18 Kitay, J.I. (1961). Sex differences in adrenal corticol secretion in the rat. *Endocrinology* **68,** 818–824

19 Cheifetz, P., Gaffund N., Dingman, J.F. (1968). Effects of bilateral adrenalectomy and continuous light in the circadian rhythm of corticotropin in female rats. *Endocrinology* **82,** 1117–1124

20 Shimizu, K., Amagaya, S. and Ogihara, Y. (1983). Analysis of corticosterone in the serum of mice and rats using high-performance liquid chromatography. *J. Chromatography* **272,** 170–175

21 The Adrenals. In: *Textbook of Endocrinology.* (1981). (R.H. Williams, ed.) pp. 249–293, W.B. Saunders Company, Philadelphia

22 Failla, M.L., Babu, U., Seidel, K.E. (1988). Use of immunoresponsiveness to demonstrate that the dietary requirement for copper in young rats is greater with dietary fructose than dietary starch. *J. Nutr.* **118,** 487–496

23 Fields, M., Ferretti, R.J., Smith, J.C., Reiser, S. (1984). Impairment of glucose tolerance in copper deficient rats: Dependency on the type of dietary carbohydrate. *J. Nutr.* **114,** 393–397