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Effect of Copper or Insulin in Diabetic Copper-Deficient Rats (41621)

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Abstract. The effects of copper and insulin on lipogenesis and glucose tolerance were studied using diabetic, copper-deficient rats. Diabetes was induced by intraperitoneal injection of 50 mg streptozotocin/kg body weight to rats fed a sucrose–copper deficient diet for 7 weeks. Five days later the rats were injected intraperitoneally with [^{14}C]glucose with either saline, insulin, copper, or copper plus insulin. The disappearance of serum [^{14}C]glucose at 30, 60, and 120 min postinjection and the incorporation of [^{14}C]glucose into lipid of epididymal fat 2 hr after administration were determined. The combined effect of copper and insulin significantly decreased peak blood glucose at 30 min and increased the incorporation of [^{14}C]glucose into lipid in the epididymal fat pad when compared to either copper or insulin alone. The enhancement of glucose utilization may be due to a formation of a more stable complex which will increase insulin binding and/or decrease its degradation.

It has been demonstrated that copper stimulates lipogenesis in adipose tissue *in vitro*, even in the absence of insulin (1, 2). However, lipogenesis in the presence of copper has been reduced in adipose tissue from streptozotocin-diabetic rats as compared to control animals, and it has been suggested that insulin is required for the stimulatory effect of copper on lipogenesis (1). Adipose tissue from animals fed normal laboratory ration contains endogenous insulin and copper. Therefore, it seemed appropriate to prepare animals that were deficient in endogenous copper and insulin, in order to study both the individual and combined effects of copper and insulin. The present study reports the *in vivo* effects of copper and insulin on glucose tolerance and lipogenesis using streptozotocin-diabetic, copper-deficient rats.

Materials and Methods. Weanling, male Sprague–Dawley rats weighing 40–45 g each (Harlan–Sprague-Dawley, Indianapolis, Ind.) were housed individually in stainless steel cages and provided with distilled, deionized water *ad libitum*. All rats were fed a sucrose copper-deficient diet according to Klevay *et al.* (3). The diet contained a copper concentration of 0.9 μg/g according to analysis by atomic absorption spectrophotometry. After being fed the diet for 7 weeks, animals were injected intraperitoneally (ip) with 50 mg streptozotocin/kg body weight in fresh citrate buffer (pH 4.5). The animals continued to be fed the copper-deficient diet for 5 days postinjection. The diabetic rats developed polyuria and glycosuria within 2 days after the streptozotocin injections. Plasma ceruloplasmin activity, as determined by the method of Schosinsky *et al.* (4), was used as an index of plasma copper concentration. On the fifth day after the streptozotocin injection, a glucose tolerance test was performed following an overnight fast. Each rat received by ip injection 250 mg glucose/100 g body weight containing 2 μCi [U-^{14}C]glucose with or without either insulin (200 mU/kg), $CuCl_2 \cdot 2H_2O$, or NaCl according to the experimental design shown in Table I.

Blood was collected for determining glucose and insulin levels from the tip of the tail at 0, 30, 60, and 120 min postinjection. Serum glucose was determined by using the glucose-oxidase–peroxidase method (as adapted by Sigma) and insulin was analyzed by a double antibody method (5). After 120 min, animals were sacrificed by decapitation, the liver was removed, weighed, and stored at −20°C and later analyzed for copper content using atomic absorption spectrophotometry. The epididymal fat pad was removed, weighed, and the lipid extracted according to Folch *et al.* (6).

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TABLE I. EXPERIMENTAL DESIGN

Number of animals	Glucose load[a]	Control[b]	Copper[c]	Insulin[d]
5	+	+	−	−
5	+	−	+	−
5	+	+	−	+
5	+	−	+	+

[a] 250 mg/100 g body weight + 2 μCi [U-^{14}C]glucose.
[b] 3.3 μliter/g body weight, 2×10^{-2} *M* NaCl (saline).
[c] 3.3 μliter/g body weight, 2×10^{-2} *M* $CuCl_2 \cdot 2\ H_2O$ (copper).
[d] Insulin 200 mU/kg.

The washed extracts were evaporated to dryness, taken up in a known volume of chloroform, and aliquots were removed. The radioactivity was quantitated using a Beckman scintillation counter. Data were statistically analyzed by Duncan's multiple range test (Multiple Comparison Tests, Package 196-1032, 1/3/8, Wang Laboratories, Lowell, Mass.), and a $P < 0.05$ or less was considered as significant.

Results. The decreased plasma ceruloplasmin and liver copper concentrations established that the animals were copper deficient. Ceruloplasmin levels were <0.01 IU/ml (normal range 0.07 ± 0.009 IU/ml). The mean copper liver concentration after the rats were fed the copper-deficient diet for 8 weeks, was 1.9 ± 0.2 μg/g liver (wet weight). This was significantly lower from the liver copper concentration of 6.2 ± 0.2 μg/g from controls obtained from the same supplier, kept under the same conditions, and fed the same diet supplemented with copper. Serum insulin concentrations were not increased following the glucose load in the streptozotocin-diabetic rats injected with either saline or copper and averaged 26.0 ± 0.7 μU/ml. The administration of insulin to the streptozotocin-diabetic, copper-deficient rats significantly lowered the serum glucose levels at 30 and 60 min as compared to the control (NaCl) (Table II). The combination of copper plus insulin further decreased ($P < 0.05$) serum glucose levels at 30 min postinjection compared to insulin treatment alone. In addition, at 60 min there was also a decrease (40 mg%) in the serum glucose levels of rats receiving copper plus insulin compared to insulin alone. Although this decrease was not statistically significant at $P < 0.05$, a trend is evident. Copper alone improved serum glucose concentration as compared to saline but the difference was significant only at 60 min. Lipogenesis was significantly increased in animals where insulin was administered together with copper as compared to lipogenesis after all other treatments (Table III). Copper alone did not stimulate lipogenesis above that of the control (NaCl alone).

Discussion. The results reported in the present study demonstrate that a combination of insulin with copper significantly decreases blood glucose *in vivo*, at the time peak of concentration and increases lipogenesis when compared with either copper or insulin alone. Also, the results support the contention that there was an impairment of the glucose tolerance associated with the copper deficiency, suggesting that copper plays a role in glucose utilization (1, 7).

Streptozotocin selectively destroys the β cells of the pancreas, producing a diabetic-like condition characterized by hypoinsulinemia (8). In streptozotocin-diabetic rats, copper supplementation alone significantly improved the impaired glucose tolerance as compared to saline at 60 min postload. Under all experimental conditions, the highest blood glucose level was achieved at 30 min following the ip glucose load (Table II). The lowest

TABLE II. BLOOD GLUCOSE FOLLOWING AN INTRAPERITONEAL GLUCOSE LOAD

Treatment	0 Min	30 Min	60 Min	120 Min
Control (saline)	120 ± 16[a]	457 ± 45[a]	354 ± 8[a]	242 ± 48[a]
Copper	113 ± 15[a]	426 ± 38[a]	280 ± 30[b]	198 ± 25[a]
Insulin + saline	112 ± 9[a]	332 ± 7[b]	244 ± 32[b]	149 ± 14[a]
Insulin + copper	127 ± 23[a]	300 ± 8[c]	204 ± 35[b]	158 ± 19[a]

Note. Results are expressed as mg% glucose in blood and as mean ± SEM (five samples). Values in the same column with different superscript letters are significantly different ($P < 0.05$).

TABLE III. THE EFFECT OF COPPER AND INSULIN ON LIPOGENESIS

Control	Copper	Insulin + saline	Insulin + copper
1.4 ± 0.3^a	1.7 ± 0.5^a	2.2 ± 0.1^b	3.5 ± 0.6^c

Note. Results are expressed as mean ± SEM of five samples, μmole glucose incorporated/g fat pad/2 hr. Mean values with a different superscript letter are significantly different ($P < 0.05$).

peak, 300 mg%, at 30 min was achieved following the administration of copper together with insulin when compared to blood glucose levels of all other treatments, $P < 0.05$.

Copper has been shown to have an insulin-like action in promoting lipogenesis by the adipose tissue *in vitro,* even in the absence of exogenous insulin (1, 2). When adipose tissue from streptozotocin-diabetic rats was incubated in the presence of copper alone, lipogenesis was decreased as compared to the effect of copper on lipogenesis from control nondiabetic rats (1). However, when adipose tissue from streptozotocin-diabetic rats was preincubated for 1 hr with insulin prior to the addition of copper, the combined effect of copper and insulin on lipogenesis was greater than that of either insulin or copper alone (unpublished results).

The present study demonstrates that *in vivo* lipogenesis was significantly increased when copper was administered together with insulin, exceeding that observed in adipose tissue from rats receiving either copper or insulin alone. Studies have shown that, both *in vivo* and *in vitro,* in the presence of zinc, insulin binding was increased and its degradation was inhibited by the rat liver plasma membrane (9). In addition, zinc in *in vitro* preparations of adipose tissue has been shown to increase insulin-induced lipogenesis (10, 11). Recently, we have demonstrated that copper and zinc increase insulin binding and stimulate deoxyglucose transport by isolated adipocytes (unpublished results). These findings suggest that copper, like zinc, may interact with insulin to form a complex which has greater affinity for a receptor site than does insulin alone and/or increases the stability (decreases the degradation) of insulin. However, our results do not rule out the possibility of the formation of such an insulin–copper complex in the solution prior to injection. Nevertheless, the present data demonstrate that although copper and insulin alone improve glucose utilization, the effects are significantly greater when copper and insulin are combined.

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