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Developmental Changes of Selected Minerals in Zucker Rats[1] (42803)

ROBERT E. SERFASS, KYEUNG-EUN PARK, AND MURRAY L. KAPLAN

Department of Food and Nutrition, Iowa State University, Ames, Iowa 50011

Abstract. Effects of obesity and age on copper, iron, zinc, sodium, potassium, and protein were compared in liver, kidney, brain, and muscle of obese (*fa/fa*) and nonobese (non-*fa/fa*) male Zucker rats. Blood plasma cerulopasmin, copper, zinc, sodium, and potassium were also determined. Mean brain weight of *fa/fa* rats was less than that of non-*fa/fa* rats at 12 weeks of age; mean brain protein concentration was greater in *fa/fa* than in non-*fa/fa* at 5 and 12 weeks of age. At 18–19 days of age, mean sodium concentration (mg/g protein) in liver of *fa/fa* was less than that of non-*fa/fa*. At 5 weeks of age, mean copper concentration (μg/g protein) in kidney was greater in *fa/fa*. Mean total copper, iron, zinc, sodium, and potassium in liver and kidney were greater in *fa/fa* than in non-*fa/fa* at 5 weeks because of the larger livers and kidneys of *fa/fa*. Mean concentrations of copper, zinc, sodium, and potassium per gram of brain protein were slightly (6–10%) less in *fa/fa* than in non-*fa/fa* at 5 weeks. By 12 weeks, mean concentrations of copper in liver, kidney, (tibialis) muscle, and blood plasma, ceruloplasmin in blood plasma, zinc in liver and muscle, iron in muscle, and sodium in liver were greater in *fa/fa* than in non-*fa/fa*. However, total amount of each mineral in muscle at 12 weeks was less in *fa/fa* than in non-*fa/fa* because of the smaller mean muscle weight of *fa/fa*. Mean concentrations of copper and zinc in brain and of iron in liver and brain were less in *fa/fa* than in non-*fa/fa* at 12 weeks. The major age-related changes in *fa/fa* that were not observed in non-*fa/fa* were large increases in liver and kidney copper between 5 and 12 weeks of age. It seems that the abnormal mineral metabolism is a consequence of the obesity, but the mechanisms are not identified. © 1988 Society for Experimental Biology and Medicine.

Even though little is known about the influence of obese conditions on the tissue status and metabolism of essential minerals, literature suggests that metabolism of trace metals is altered in animal models of obesity. Begin-Heick and associates suggested a maldistribution of zinc in the tissues of the obese mouse. They reported that zinc concentrations in liver, muscle, and brown adipose tissue were significantly higher, but that zinc concentrations in bone and pancreas were significantly lower, in obese than in lean mice (1). Kennedy and associates reported that the concentrations of copper, zinc, and manganese were significantly lower in several tissues of adult obese mice than in their lean controls (2). Donaldson *et al.* (3) found that 14-week-old obese male Zucker rats had significantly lesser hepatic zinc and copper and significantly greater muscle and femur zinc concentrations than lean controls.

Developmental studies of tissue accumulation of minerals in young obese rats have not been reported. We have been interested particularly in the time course of development of abnormalities in mineral metabolism in tissues predominantly responsible for energy expenditure compared with the time of onset of decrease in oxygen consumption and increase in food intake. Accordingly, the purposes of this study were to determine copper, iron, zinc, sodium, potassium, and protein content of liver, kidney, tibialis anterior muscle, and brain in lean and obese male Zucker rats at various ages and to compare the effects of obesity and age on mineral content of these tissues. Blood plasma ceruloplasmin, copper, zinc, sodium, and potassium were also determined.

Materials and Methods. *Animals and diet.* Male Zucker rats were obtained from the breeding colony of the Food and Nutrition Department of Iowa State University. All lean and obese animals that were to be sacrificed at any one specified age were obtained

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within 2 days of each other and were processed together from start to finish as an experimental unit. The animals were raised in a temperature- and humidity-controlled room (24 ± 1°C and 50% relative humidity) with a 12-hr light–dark lighting schedule. They were observed on a daily basis for unusual signs by trained laboratory animal personnel. Litter size was adjusted to seven to nine pups per litter on the second day of life. All animals were healthy, and none were removed from the experiments.

When sacrifice was intended to be at 18 days of age, male pups were taken from the dams at 14–15 days of age, and oxygen consumption was measured (4). Pups were designated as either *fa/fa* or non-*fa/fa* on the basis of their oxygen consumption. Pups were marked by coded clipping of toes and were returned to their dams until 18–19 days of age, at which time they were retrieved, blood (anticoagulated with ammonium heparin) was withdrawn by cardiac puncture, and the animals were killed by exsanguination under ether anesthesia. Livers and kidneys were excised from the preweanling animals and weighed, and the samples were stored at −20°C.

For the rats to be sacrificed at 5 and 12 weeks of age, weanling male Zucker rats were fed stock diet (rat/mouse diet 1526, Simonsen Mill, Quimby, IA 51049) in the breeding colony until 4 weeks of age, at which time they were identified as obese or lean by visual appearance. Then the rats were housed individually in stainless-steel cages and were fed *ad libitum* AIN-76 semipurified diet (5) prepared in our facility from commercially available ingredients (ICN Nutritional Biochemicals, Cleveland, OH) modified by replacement of the 50% sucrose by additional cornstarch. Dietary fiber included at 5% by weight was α-cellulose (Sigma Chemical Co., St. Louis, MO). Animals were allowed free access to tap water. Individual body weights and food intakes were measured weekly. At 5 or 12 weeks of age, blood anticoagulated with ammonium heparin was withdrawn by cardiac puncture, and the rats were killed by exsanguination under sodium pentobarbital anesthesia (50 mg/kg body wt, ip). Liver, brain (excluding the olfactory lobes), kidneys, and tibialis anterior muscles were excised from each animal and weighed, and the samples were stored at −20°C.

Sample preparation and analysis. All aqueous solutions were prepared by using Type I water (MilliQ Water System, Millipore Corp., Bedford, MA). All labware was leached with reagent grade HCl. Concentrated nitric and perchloric acids were redistilled in quartz stills before use (6). Whole brain (excluding olfactory lobes), both kidneys, both tibialis anterior muscles, and the main lobe of the liver of each animal (liver and kidneys only of preweanling animals) were homogenized in deionized water by using an acid-washed ground-glass tissue grinder (Pyrex brand, Fisher Scientific Co., Fair Lawn, NJ) to yield 25% (w/w) homogenates. Blanks consisted of aliquots of deionized water that were processed in the same manner as the tissues. Aliquots of homogenates (1 ml) were evaporated to dryness at 85°C in Teflon PFA vials (33 × 21 mm, Cole-Parmer Instrument Co., Chicago, IL) by using a controlled-temperature heater (Reacti-Therm III, Pierce Chemical Co., Rockford, IL) fitted with aluminum blocks (Reacti-Block E-1, Pierce Chemical Co., Rockford, IL). Lyophilized bovine liver (Standard Reference Material 1577a, National Bureau of Standards, Gaithersburg, MD) and diet samples were powders that did not require homogenization; they were weighed directly into the Teflon PFA vials.

Samples were predigested overnight in 2 ml of redistilled nitric acid at room temperature, followed by heating for 1 hr at 120°C. Redistilled perchloric acid (1 ml) was added, and digestion was considered complete when the block temperature reached 200°C. The clear, nearly colorless digestate was further heated to drive off excess perchloric acid. Mineral residue was brought to volume with 0.7% aqueous nitric acid. Aliquots of digestates of organs, diets, and standard reference material were diluted with 0.7% nitric acid for copper, iron, and zinc determinations or with 4 m*M* CsCl for sodium and potassium determinations (7). Blood plasma was diluted with an equal volume of deionized water for copper determination and with 4 m*M* CsCl for sodium and potassium determinations. Copper, iron, zinc, sodium, and potassium were determined by using a flame

atomic absorption spectrophotometer (IL Video 12, Allied Analytical Systems, Waltham, MA). Mean recoveries of minerals in standard reference material were: iron, 90%; copper, 90%; zinc, 94%; sodium, 100%; potassium, 99%.

Tissue protein was determined on 40-μl aliquots of homogenate by the biuret method (8) modified by the presence of 0.2% sodium lauryl sulfate (Sigma Chemical Co., St. Louis, MO) in the final volume and by centrifugation at 900g for 15 min. Mineral content of tissues was expressed per organ and per milligram of protein.

Plasma ceruloplasmin oxidase activity was determined on thawed, undiluted, 100-μl aliquots of blood plasma by the manual procedure of Smith and Wright (9) by using p-phenylenediamine dihydrochloride as substrate with an optimal buffer pH of 5.9 and a final reaction volume of 3.0 ml. Samples of pooled rat plasma were used as controls to monitor for day-to-day variations in assay conditions. Results are expressed in terms of change in absorbance per unit volume of plasma per unit time, specifically as Δ milliabsorbance per milliliter plasma per minute.

Statistical analysis. Oxygen consumption by the preweanling animals, food consumption of the older animals, body and organ weights, organ protein, results for each mineral in each tissue, and plasma copper, sodium, potassium, and ceruloplasmin were analyzed by using the general linear model (Statistical Analysis System, Cary, NC). Within this model, two-way analysis of variance was conducted to determine phenotype-dependent, age-dependent, and interactive effects. Then, comparisons were made between different phenotypes within each age and between different ages within each phenotype by repeated t tests for unpaired data and unequal sample sizes (10). Statistical significance was accepted at the 1% level ($P < 0.01$).

Results. *Dietary mineral content.* Results of analysis (Table I) of the stock diet were very close to values specified by the manufacturer (personal communication, Simonsen Mill, Inc.) with the exception of the high iron content and, for the AIN-76 diet, were almost identical to literature values (5) with the exception of a slightly lower potassium content.

TABLE I. METAL CONTENT OF DIETS, AS ANALYZED

Element	Stock diet[a] (μg/g diet)	AIN-76 diet[b] (μg/g diet)
Potassium	7670 ± 520[c]	2570 ± 100[c]
Sodium	4590 ± 610	930 ± 110
Iron	675 ± 30	33.6 ± 1.4
Zinc	142 ± 1	31.7 ± 0.8
Copper	13.4 ± 0.3	4.3 ± 0.2

[a] Rat/mouse diet no. 1526, Simonsen Mill, Inc., Quimby, IA 51049.
[b] See Ref. (5) for diet composition.
[c] Values are means ± SD (n = 3).

Phenotype-related differences. The effect of phenotype was significant for 35 of the 59 dependent variables. The greatest number of significant phenotypic effects was found in muscle (10 effects), followed by liver and brain (8 effects each), and kidney (4 effects). All of the following phenotypic differences within each age are statistically significant ($P < 0.01$). Previously reported differences between means for lean and obese phenotypes were confirmed for body, liver, and muscle weights, oxygen and food consumption (Table II), liver protein concentration, total liver protein, muscle (tibialis anterior) protein concentration, and total muscle (tibialis anterior) protein (Table III). In addition, mean brain weights were less in obese rats at 12 weeks (Table II). At 5 and 12 weeks of age, mean brain protein concentration was greater in the obese than in the lean group (Table III).

By 12 weeks of age, mean liver copper (Table IV) concentration (μg/g protein) of the obese group was 3.4-fold, and mean total liver copper was 4-fold, that of the lean group. At 5 weeks of age, mean kidney copper concentration (μg/g protein) of the obese group was 2.2-fold, and mean total kidney copper was 3.4-fold, that of lean rats. In the obese group at 12 weeks, mean kidney copper concentration (μg/g protein) was 3.4-fold, and mean total kidney copper was 3.0-fold, that of the lean. At 5 weeks of age, mean brain copper concentration (μg/g protein) was less in obese than in lean rats, but mean total brain copper did not differ. By 12 weeks, mean brain copper concentration

TABLE II. BODY WEIGHT, ORGAN WEIGHTS, OXYGEN CONSUMPTION, AND FOOD INTAKE OF MALE ZUCKER RATS

Group								
Age (days)	Type	Body weight (g)	Liver weight (g)	Kidney weight (g)	Brain weight (g)	Tibialis muscle weight (g)	Oxygen consumption (ml/hr · g body wt)	Cumulative food intake (g)
18	Lean	39.1 ± 3.1	1.26 ± 0.10	0.41 ± 0.04	N.D.	N.D.	2.98 ± 0.32	N.D.
18	Obese	39.5 ± 1.8	1.24 ± 0.13	0.41 ± 0.05	N.D.	N.D.	1.51 ± 0.12[c]	N.D.
35	Lean	111 ± 15[a]	5.79 ± 1.08[a]	1.08 ± 0.17[a]	1.69 ± 0.12	0.46 ± 0.09	N.D.	79 ± 7
35	Obese	144 ± 15[a,c]	8.83 ± 1.09[a,c]	1.42 ± 0.21[a]	1.66 ± 0.12	0.46 ± 0.06	N.D.	136 ± 12[c]
84	Lean	313 ± 20[a,b]	11.55 ± 1.62[a,b]	2.32 ± 0.27[a,b]	1.93 ± 0.13[b]	1.36 ± 0.17[b]	N.D.	923 ± 52[b]
84	Obese	350 ± 22[a,b]	19.92 ± 0.77[a,b,c]	2.14 ± 0.15[a,b]	1.71 ± 0.07[c]	0.83 ± 0.08[b,c]	N.D.	1021 ± 47[b,c]
				Analysis of variance, P value[d]				
Phenotype (P)		<0.0001	<0.0001	N.S.	0.01	<0.0001	<0.0001	<0.0001
Age (A)		<0.0001	<0.0001	<0.0001	<0.01	<0.0001	N.D.	<0.0001
$P * A$		<0.005	<0.0001	<0.001	N.S.	<0.0001	N.D.	N.S.

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. N.D., not determined.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

(μg/g protein) was 15% less and mean total brain copper was 16% less in the obese than in the lean group. In the tibialis anterior muscle by 12 weeks, mean copper concentration (μg/g protein) of the obese group was 40% greater, but mean total muscle copper was 30% less than that of the lean group because of the smaller muscle size of the obese animals.

At 5 weeks of age, mean total kidney iron (Table V) was 60% greater in the obese group. In the brain, by 12 weeks, mean iron

TABLE III. ORGAN PROTEIN OF MALE ZUCKER RATS

Group		Liver		Kidney		Brain		Tibialis muscle	
Age (days)	Type	(mg/g wet wt)	(mg/organ)	(mg/g wet wt)	(mg/organ)	(mg/g wet wt)	(mg/organ)	(mg/g wet wt)	(mg/organ)
18	Lean	183 ± 6	231 ± 23	137 ± 9	56 ± 7	N.D.	N.D.	N.D.	N.D.
18	Obese	183 ± 6	227 ± 22	143 ± 6	58 ± 6	N.D.	N.D.	N.D.	N.D.
35	Lean	174 ± 11	997 ± 146[a]	114 ± 11[a]	123 ± 18[a]	105 ± 0	178 ± 13	192 ± 22	89 ± 26
35	Obese	147 ± 3[a,c]	1297 ± 156[a,c]	132 ± 22	185 ± 13[a,c]	117 ± 3[c]	195 ± 11	179 ± 13	82 ± 9
84	Lean	179 ± 6	2065 ± 304[a,b]	153 ± 5[a,b]	354 ± 44[a,b]	101 ± 3[b]	194 ± 11	205 ± 3	280 ± 38[b]
84	Obese	119 ± 3[a,b,c]	2367 ± 107[a,b]	151 ± 18	325 ± 56[a,b]	111 ± 1[b,c]	190 ± 8	174 ± 10[c]	145 ± 21[b,c]
				Analysis of variance, P value[d]					
Phenotype (P)		<0.0001	<0.0005	N.S.	N.S.	<0.0001	N.S.	<0.001	<0.0001
Age (A)		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N.S.	N.S.	<0.0001
$P * A$		<0.0001	<0.01	N.S.	<0.005	N.S.	N.S.	N.S.	<0.0001

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. N.D., not determined.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

TABLE IV. ORGAN COPPER OF MALE ZUCKER RATS

Group	Liver		Kidney		Brain		Tibialis muscle	
	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)
18 L	104 ± 43	23.7 ± 9.6	21.6 ± 3.3	1.2 ± 0.3	N.D.	N.D.	N.D.	N.D.
18 O	93 ± 27	21.4 ± 7.9	19.4 ± 1.9	1.1 ± 0.2	N.D.	N.D.	N.D.	N.D.
35 L	21.6 ± 2.1[a]	21.4 ± 3.1	45 ± 18[a]	5.3 ± 1.5	13.9 ± 0.4	2.5 ± 0.2	7.0 ± 1.6	0.6 ± 0.1
35 O	20.4 ± 0.5[a]	26.5 ± 3.3	99 ± 24[a,c]	18.1 ± 3.3[a,c]	12.5 ± 0.9[c]	2.4 ± 0.2	7.8 ± 1.1	0.6 ± 0.1
84 L	19.3 ± 2.6[a]	39.3 ± 2.5[a,b]	58 ± 15[a]	20.3 ± 5.0[a,b]	19.0 ± 2.0[b]	3.7 ± 0.3[b]	7.2 ± 0.7	2.0 ± 0.3[b]
84 O	66 ± 23[b,c]	156 ± 59[a,b,c]	195 ± 40[a,b,c]	61.8 ± 8.0[a,b,c]	16.2 ± 0.5[b,c]	3.1 ± 0.2[b,c]	10.1 ± 1.0[b,c]	1.4 ± 0.2[b,c]
	Analysis of variance, *P* value[d]							
Phenotype (*P*)	N.S.	<0.0001	<0.0001	<0.0001	<0.0005	<0.005	<0.001	<0.005
Age (*A*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N.S.	<0.0001
P * *A*	N.S.	<0.0001	<0.0001	<0.0001	N.S.	<0.001	N.S.	<0.0005

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. N.D., not determined; L, lean; O, obese.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

concentration (μg/g protein) was 13% less in obese than in lean rats. In the tibialis anterior muscle, by 12 weeks, mean iron concentration (μg/g protein) of the obese group was 45% greater.

Mean total zinc (Table VI) in liver at 5 and 12 weeks of age was 34% greater in the obese groups than in lean rats. At 5 weeks of age, mean total kidney zinc was 65% greater in the obese group. At 5 weeks, mean brain zinc concentration (μg/g protein) was less in obese than in lean rats. In the tibialis anterior muscle, by 12 weeks, mean muscle zinc concentration (μg/g protein) of the obese group was 60% greater than that of lean rats.

Mean sodium (Table VII) concentration (mg/g protein) in liver was 10% less in obese than in lean rats at 18–19 days of age. By 12

TABLE V. ORGAN IRON OF MALE ZUCKER RATS

Group	Liver		Kidney		Brain		Tibialis muscle	
	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)
18 L	101 ± 10	23.3 ± 3.1	94 ± 16	5.3 ± 1.1	N.D.	N.D.	N.D.	N.D.
18 O	97 ± 10	22.1 ± 3.7	102 ± 17	5.9 ± 1.1	N.D.	N.D.	N.D.	N.D.
35 L	410 ± 100[a]	420 ± 140[a]	290 ± 25[a]	35.7 ± 6.3[a]	108.1 ± 9.4	19.3 ± 2.9	44.9 ± 6.3	3.9 ± 1.0
35 O	354 ± 99[a]	450 ± 120[a]	307 ± 37[a]	56.7 ± 7.9[a,c]	107.9 ± 3.4	21.0 ± 1.6	42.7 ± 4.1	3.5 ± 0.2
84 L	490 ± 130[a]	1010 ± 280[a,b]	354 ± 68[a]	127 ± 37[a,b]	175 ± 11[b]	34.0 ± 3.6[b]	43.6 ± 4.7	12.3 ± 2.7[b]
84 O	314 ± 58[a]	740 ± 120[a,b]	405 ± 55[a,b]	130 ± 23[a,b]	152 ± 11[b,c]	28.8 ± 3.2[b]	63.0 ± 8.5[b,c]	9.0 ± 1.2[b]
	Analysis of variance, *P* value[d]							
Phenotype (*P*)	<0.01	N.S.	N.S.	N.S.	<0.005	N.S.	<0.005	<0.01
Age (*A*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.005	<0.0001
P * *A*	N.S.	N.S.	N.S.	N.S.	<0.01	<0.01	<0.0005	N.S.

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. N.D., not determined; L, lean; O, obese.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

TABLE VI. ORGAN ZINC OF MALE ZUCKER RATS

Group	Liver		Kidney		Brain		Tibialis muscle	
	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)	(μg/g protein)	(μg/organ)
18 L	189 ± 24	43.4 ± 5.7	163 ± 13	9.1 ± 1.1	N.D.	N.D.	N.D.	N.D.
18 O	189 ± 24	42.8 ± 5.3	158 ± 8	9.2 ± 1.1	N.D.	N.D.	N.D.	N.D.
35 L	116.3 ± 5.1[a]	115 ± 14[a]	153 ± 14	18.7 ± 2.1[a]	100.5 ± 3.0	17.9 ± 1.1	45 ± 15	3.7 ± 0.5
35 O	119.2 ± 4.5[a]	155 ± 20[a,c]	167 ± 14	30.9 ± 3.8[a,c]	94.3 ± 2.8[c]	18.3 ± 0.9	47 ± 10	3.8 ± 0.7
84 L	106.6 ± 8.5[a]	219 ± 23[a,b]	126 ± 11[a,b]	44.3 ± 2.7[a,b]	113.3 ± 4.5[b]	22.0 ± 1.2[b]	32.6 ± 4.9	9.2 ± 2.5[b]
84 O	124 ± 11[a]	292 ± 22[a,b,c]	129 ± 18[a,b]	41.0 ± 2.9[a,b]	104.8 ± 6.4[b]	19.9 ± 1.6	52.1 ± 3.7[c]	7.5 ± 1.1[b]
				Analysis of variance, *P* value[d]				
Phenotype (*P*)	N.S.	<0.0001	N.S.	<0.001	<0.001	N.S.	<0.01	N.S.
Age (*A*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	N.S.	<0.0001
P * *A*	N.S.	<0.0001	N.S.	<0.0001	N.S.	N.S.	N.S.	N.S.

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. N.D., not determined; L, lean; O, obese.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

weeks, mean liver sodium concentration was 21% greater and mean total liver sodium was 39% greater in the obese than in the lean group. Mean total kidney sodium was 55% greater in the obese group at 5 weeks. Mean brain sodium concentration of the obese group was 10% less than that of the lean group at 5 weeks. In the tibialis anterior muscle, by 12 weeks, mean muscle sodium concentration was 47% less and mean total muscle sodium was 73% less in the obese than in lean rats.

At 5 weeks of age mean total kidney potassium (Table VIII) was 44% greater in the

TABLE VII. ORGAN SODIUM OF MALE ZUCKER RATS

Group	Liver		Kidney		Brain		Tibialis muscle	
	(mg/g protein)	(mg/organ)	(mg/g protein)	(mg/organ)	(mg/g protein)	(mg/organ)	(mg/g protein)	(mg/organ)
18 L	4.22 ± 0.32	0.97 ± 0.10	10.54 ± 1.32	0.59 ± 0.10	N.D.	N.D.	N.D.	N.D.
18 O	3.79 ± 0.22[c]	0.86 ± 0.10	9.31 ± 1.36	0.54 ± 0.10	N.D.	N.D.	N.D.	N.D.
35 L	2.98 ± 0.25[a]	2.99 ± 0.61[a]	8.59 ± 0.47[a]	1.05 ± 0.15[a]	8.71 ± 0.35	1.55 ± 0.16	2.83 ± 0.46	0.25 ± 0.08
35 O	2.85 ± 0.27[a]	3.69 ± 0.42[a]	8.92 ± 1.53	1.63 ± 0.19[a,c]	7.81 ± 0.37[c]	1.52 ± 0.10	3.17 ± 0.72	0.26 ± 0.07
84 L	2.72 ± 0.38[a]	5.61 ± 1.17[a,b]	6.27 ± 1.08[a,b]	2.23 ± 0.52[a,b]	9.70 ± 0.66[b]	1.89 ± 0.20	5.00 ± 1.62	1.41 ± 0.52[b]
84 O	3.29 ± 0.22[a,b,c]	7.80 ± 0.79[a,b,c]	7.20 ± 1.07[a]	2.39 ± 0.72[a,b]	9.01 ± 0.39[b]	1.71 ± 0.10[b]	2.66 ± 0.55[c]	0.38 ± 0.08[c]
				Analysis of variance, *P* value[d]				
Phenotype (*P*)	N.S.	<0.0001	N.S.	N.S.	<0.0005	N.S.	N.S.	<0.0001
Age (*A*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0005	N.S.	<0.0001
P * *A*	<0.0001	<0.0001	N.S.	N.S.	N.S.	N.S.	<0.005	<0.0001

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. N.D., not determined; L, lean; O, obese.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

TABLE VIII. ORGAN POTASSIUM OF MALE ZUCKER RATS

Group	Liver (mg/g protein)	Liver (mg/organ)	Kidney (mg/g protein)	Kidney (mg/organ)	Brain (mg/g protein)	Brain (mg/organ)	Tibialis muscle (mg/g protein)	Tibialis muscle (mg/organ)
18 L	19.2 ± 1.4	4.43 ± 0.55	21.7 ± 1.8	1.21 ± 0.15	N.D.	N.D.	N.D.	N.D.
18 O	18.3 ± 0.8	4.17 ± 0.54	20.7 ± 1.2	1.20 ± 0.14	N.D.	N.D.	N.D.	N.D.
35 L	18.4 ± 0.7	18.3 ± 2.6[a]	19.7 ± 0.7	2.43 ± 0.41[a]	29.8 ± 0.7	5.30 ± 0.41	13.7 ± 1.3	1.23 ± 0.38
35 O	17.2 ± 1.3	22.2 ± 2.4[a]	19.0 ± 1.4	3.50 ± 0.17[a,c]	27.5 ± 1.2[c]	5.35 ± 0.31	13.7 ± 0.8	1.12 ± 0.16
84 L	15.7 ± 0.8[a,b]	32.4 ± 4.6[a,b]	12.6 ± 1.3[a,b]	4.44 ± 0.26[a,b]	31.9 ± 1.7	6.18 ± 0.44[b]	12.4 ± 1.3	3.46 ± 0.48[b]
84 O	16.7 ± 1.5	39.4 ± 4.1[a,b]	13.4 ± 3.0[a,b]	4.22 ± 0.39[a,b]	30.3 ± 0.6[b]	5.74 ± 0.26	13.9 ± 1.3	2.00 ± 0.29[b,c]
Analysis of variance, P value[d]								
Phenotype (P)	N.S.	<0.001	N.S.	<0.01	<0.0005	N.S.	N.S.	<0.0001
Age (A)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0005	N.S.	<0.0001
$P * A$	N.S.	<0.005	N.S.	<0.0001	N.S.	N.S.	N.S.	<0.0001

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. N.D., not determined; L, lean; O, obese.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

obese group. Mean brain potassium concentration (mg/g protein) at 5 weeks was 8% less in the obese than in the lean group. In the tibialis anterior muscle, by 12 weeks, mean total muscle potassium was 42% less in the obese group.

Mean plasma copper and ceruloplasmin (Table IX) were greater in the obese than in the lean group at 12 weeks. The means are not shown for plasma sodium and potassium, or for plasma zinc at 5 and 12 weeks (not enough plasma remained to determine plasma zinc at 18 days), because none of the means differed significantly. Mean plasma zinc concentrations were normal in the lean and obese groups at 5 and 12 weeks.

Age-related differences. The effect of age was significant for every dependent variable except total brain protein, plasma zinc, sodium, and potassium, and tibialis muscle protein, copper, zinc, sodium, and potassium concentrations. The mixed effect of phenotype and age was significant for 27 of the 58 dependent variables determinable. The significant ($P < 0.01$) means comparisons within each phenotype that follow are presented to make evident the mixed effects of phenotype and age.

Among the lean groups, mean liver copper (Table IV) concentration (μg/g protein) at 18–19 days was 4.8-fold that at 5 weeks of age and 5.4-fold that at 12 weeks of age. Within the obese phenotype, mean liver

TABLE IX. PLASMA COPPER AND CERULOPLASMIN

Group	Copper concentration (μg/ml)	Ceruloplasmin activity (mA/min · ml)
18 L	0.72 ± 0.22	48.8 ± 12.7
18 O	0.74 ± 0.22	48.0 ± 15.3
35 L	0.83 ± 0.11	61.9 ± 12.5
35 O	0.92 ± 0.12	64.3 ± 8.3
84 L	1.20 ± 0.12[a,b]	117.9 ± 21.2[a,b]
84 O	2.04 ± 0.22[a,b,c]	230.0 ± 33.1[a,b,c]
Analysis of variance, P value[d]		
P	<0.0001	<0.0001
A	<0.0001	<0.0001
$P * A$	<0.0001	<0.0001

Note. Values are means ± SD for each group, $n = 9$ for 18-day-old groups, $n = 6$ for 35- and 84-day-old groups. L, lean; O, obese.

[a] Significantly different from corresponding phenotype at 18 days of age in same column, $P < 0.01$.

[b] Significantly different from corresponding phenotype at 35 days of age in same column, $P < 0.01$.

[c] Significantly different from lean phenotype of same age in same column, $P < 0.01$.

[d] N.S., not significant; $P > 0.01$.

copper concentration (μg/g protein) at 18–19 days was 4.6-fold that at 5 weeks and increased 3.2-fold between 5 and 12 weeks of age. Mean kidney copper concentration increased 2.1-fold between 18–19 days and 5 weeks of age in lean Zucker rats. Mean kidney copper concentration increased 5.1-fold between 18–19 days and 5 weeks of age and doubled between 5 and 12 weeks of age in obese Zuckers. In lean Zucker rats, mean brain copper concentration (μg/g protein) was 37% greater and mean total brain copper was 48% greater at 12 weeks than at 5 weeks of age. In obese Zuckers, mean copper concentration (μg/g protein) and mean total copper in brain were approximately 30% greater at 12 weeks than at 5 weeks of age.

Within the lean groups, mean liver iron (Table V) concentration (μg/g protein) at 5 weeks was 4.1-fold and at 12 weeks was 4.9-fold that at 18–19 days of age. Within the obese phenotype, mean liver iron concentration (μg/g protein) increased 3.7-fold between 18–19 days and 5 weeks of age and did not change significantly between 5 and 12 weeks of age. Between 5 and 12 weeks of age, mean kidney iron concentration (μg/g protein) increased 32% in the obese group. In lean male Zucker rats, mean brain iron concentration (μg/g protein) was 62% greater and mean total brain iron was 76% greater at 12 weeks than at 5 weeks of age. In the obese Zucker, mean brain iron concentration (μg/g protein) was 41% greater and mean total brain iron was 37% greater at 12 weeks than at 5 weeks of age. In tibialis anterior muscle of lean rats at 12 weeks, mean total iron was 3.2 times the 5-week value. In the obese Zucker, mean muscle iron concentration (μg/g protein) at 12 weeks was 48% greater than, and mean total muscle iron was 2.6 times the 5-week value.

Among the lean groups, mean liver zinc (Table VI) concentration (μg/g protein) was 60% greater at 18–19 days than at 5 weeks of age and 80% greater at 18–19 days than at 12 weeks of age. Within the obese phenotype, mean liver zinc concentration was 59% greater at 18–19 days than at 5 weeks of age, and 53% greater at 18–19 days than at 12 weeks of age.

Among the lean groups, mean liver sodium (Table VII) concentration (mg/g protein) at 18–19 days was 40% greater than at 5 weeks of age and 60% greater than at 12 weeks of age. Within the obese phenotype, mean liver sodium concentration (mg/g protein) at 18–19 days was 33% greater than at 5 weeks of age and 15% greater than at 12 weeks of age. Mean liver sodium concentration (mg/g protein) of the obese group increased 15% between 5 and 12 weeks of age. Within the lean phenotype, mean kidney sodium concentration (mg/g protein) was 23% greater at 18–19 days than at 5 weeks of age and 37% greater at 5 weeks than at 12 weeks of age. Within the obese phenotype, mean kidney sodium concentration (mg/g protein) was 29% greater at 18–19 days than at 12 weeks of age. In lean Zucker rats, mean brain sodium concentration (mg/g protein) was 11% greater at 12 weeks than at 5 weeks of age. In obese Zucker rats, mean brain sodium concentration (mg/g protein) was 15% greater at 12 weeks than at 5 weeks of age, and mean total brain sodium was 13% greater at 12 weeks of age. In tibialis anterior muscle of lean rats, mean total muscle sodium at 12 weeks was 5.6-fold that at 5 weeks, whereas the corresponding value for obese rats did not increase significantly.

Plasma copper concentration and ceruloplasmin activity (Table IX) were greater at 12 weeks than at 5 weeks or at 18–19 days within each phenotype. No significant differences in plasma zinc, sodium, or potassium between ages within phenotypes were found (data not shown).

Discussion. Diverse aspects of development of lean and obese Zucker rats have been described and reviewed (11, 12). The data of Tables II and III with respect to body weight, organ weights, food consumption, oxygen consumption, and tissue protein are consistent with previous reports (4, 13, 14). To our knowledge, the significant effects of phenotype upon brain weight and brain protein concentration are new findings.

The content and concentrations of protein, iron, copper, zinc, sodium, and potassium in tissues of lean Zucker rats as a function of age that are reported in Tables III through IX are comparable to corresponding values from the literature (15–25) for other strains of rats and are suggestive of a normal pattern of development.

Many of the significant phenotypic differences of Tables II through IX are small ones that may be accounted for by factors such as the different food consumption of the lean and obese groups or by the well-known differences in metabolism between tissues of lean and obese Zucker rats. For example, accumulation of fat in liver and the obese phenotype (11) is associated with a greater organ size than in lean counterparts by 5 weeks of age; hepatic protein concentration of the obese phenotype is less than in the lean phenotype by 12 weeks of age (Table III), but total tissue protein is not significantly different. A decrease in protein synthesis in muscle of the obese phenotype that is accompanied by no change in rates of protein degradation (12) can be associated with the smaller increment in size of tibialis anterior muscle in obese than in lean Zuckers between 5 and 12 weeks of age (Table II) and with lower total muscle (tibialis anterior) copper, iron, sodium, and potassium in the obese than in the lean phenotype at 12 weeks of age.

Because of these pronouced phenotypic differences in fat content and/or organ size, we have chosen to express the mineral concentrations of Tables IV through VIII per gram of protein rather than per gram wet weight and to provide the total organ content of each mineral as well. As can be seen from the analysis of variance in Tables II and III, there are fewer significant effects of phenotype and age upon organ protein content than upon organ size. The mineral concentrations per gram of tissue wet weight may be derived by using the data provided in Table II or III for organ size or organ protein concentration if the reader so chooses.

This distinction of the basis of expression of results is an important one inasmuch as it affects the interpretation of results. Donaldson *et al.* (3) expressed tissue zinc and copper concentrations of 14-week-old male Zucker rats on a dry-weight basis, reported significantly lower hepatic zinc and copper concentrations for the obese phenotype, and attributed the lower results at least in part to a greater contribution of lipid to the hepatic dry weights of the obese animals.

The most salient phenotypic differences are difficult to ascribe completely to considerations such as differences in food consumption or organ size. Specifically, the much greater copper concentration and copper content of kidney of the obese phenotype that is already evident at 5 weeks of age; the greater copper, iron, and zinc (and lesser sodium) concentrations in muscle of the obese at 12 weeks; the greater total liver zinc of the obese at 5 and 12 weeks; and the greater total liver copper, plasma copper, and ceruloplasmin activity of the obese at 12 weeks all require alternative explanations. Beyond 3 weeks of age, obese Zucker rats exhibit hyperinsulinemia, hypothyroidism, and diminished pituitary responsiveness to corticotropin-releasing factor as well as other endocrinological abnormalities (11, 26). It is likely that accumulation of copper in kidney, liver, and plasma, and along with zinc in muscle of obese Zucker rats after 18 days of age is due to one or more of these hormonal influences.

Some insight into hormonal effects on copper and zinc metabolism in rats has been gained through the investigations of Failla and his colleagues (24, 27, 28). They have shown that hepatic copper and metallothionein increase during streptozotocin-induced diabetes in rats. The increases occurred shortly after development of hyperglycemia and were reversed by administration of insulin. However, streptozotocin induction results in hyperglucagonemia and hypercorticosteronemia concomitant with the hypoinsulinemia (29). Glucagon has a regulatory effect on hepatic metallothionein and has been shown to stimulate zinc uptake by liver parenchymal cells. The latter effect is additive when combined with that of glucocorticoids and is not blocked by insulin (30).

Zucker fatty rats, like streptozotocin-diabetic rats, exhibit elevated serum glucagon and corticosterone. The similar abnormalities in mineral distribution of the Zucker fatty rat and the streptozotocin-diabetic rat might be attributable largely to these hormonal similarities. The hyperinsulinemia of the Zucker fatty, in contrast to the hypoinsulinemia of the streptozotocin-diabetic rat, superimposed upon the hormonal similarities might be responsible for some of the differences between the two models in mineral, especially zinc, metabolism. For exam-

ple, plasma zinc is elevated in the streptozotocin-diabetic rat (24, 31), whereas we have found plasma zinc in the *ad libitum*-fed adult male Zucker fatty to be normal.

The *ob/ob* mouse is the congenitally obese rodent most thoroughly characterized with respect to altered tissue trace metal metabolism (1, 2, 32, 33). Kennedy *et al.* found lower concentrations (corrected for lipid content), but not necessarily lower content, of iron, manganese, zinc, and copper in several organs of the adult *ob/ob* mouse. Differences were small or nonexistent at 5–6 weeks of age. Plasma zinc concentrations were higher in adult *ob/ob* than in controls (2). Absorption, retention, and tissue distribution of iron and zinc, and constitutive levels of zinc-metallothionein in selected tissues of adult *ob/ob* mice and their lean counterparts have been compared. Alterations in several characteristics of iron and zinc metabolism were reported (32, 33).

Our observations of hypercupremia and hyperceruloplasminemia in adult male Zucker fatty rats are in agreement with what has been reported for the adult *ob/ob* mouse. Our other findings, particularly with respect to tissue copper concentrations, are in contrast. These two animal models of obesity differ in many ways beyond the species difference. For examples, hyperinsulinemia, hyperphagia, hyperglycemia, and excess body weight become more marked in adult *ob/ob* than in adult Zucker fatty rats.

The differential and synergistic effects of various hormones on copper metabolism and distribution in plasma, tissues, and isolated hepatocytes of various species have been reviewed (30). For rats, the hormonal effects are many and diverse. Several hormones (thyroid hormone, testosterone, epinephrine, growth hormone) may be involved in addition to those already mentioned as affecting zinc uptake, and effects may be mediated through alterations in ceruloplasmin secretion and in hepatic copper excretion via bile, as well as through stimulation of hepatic and renal metallothionein synthesis.

It seems likely that the pronounced accumulation of copper that we have observed in various compartments of obese male Zucker rats is due to hormonal induction of renal and hepatic metallothionein synthesis. The much greater accumulation of copper than of zinc, particularly in the kidney, may be mediated by hormones that stimulate hepatic (and renal) uptake of copper and hepatic secretion of ceruloplasmin without having similar effects on zinc transport. Effect of endocrine status on absorption and excretion of the minerals of interest, such as have been documented in the *ob/ob* mouse and the streptozotocin diabetic rat (31–33), might be contributory. Elucidation of the effects depends upon more specific experiments concerning copper, metallothionein, and ceruloplasmin metabolism in obese Zucker rats.

At the outset of this investigation we were interested in the possibility that the depressed whole body oxygen consumption seen as early as 14–15 days of age in the Zucker fatty rat would coincide with development of altered tissue mineral distribution and decreased activity of copper- or iron-containing respiratory enzymes. Since then it has been shown, at least for hepatocytes, that oxygen consumption of isolated cells and mitochondria, and hepatic activities of mitochondrial enzymes (e.g., succinate dehydrogenase, cytochrome oxidase) are similar in lean and obese Zucker rats at 6 weeks of age (14). It is clear from the present report that differences between lean and obese rats in basal metabolic rate precede development of major differences in mineral concentrations of the tissues studied. Our results support the concept, already developed by investigators of other animal models of obesity (2), that abnormal tissue trace mineral status is a general consequence in congenitally obese rodents. We are concerned that chronic accumulation of copper in various tissues may have toxic implications for the obese Zucker rat, but the significance, if any, of the altered mineral concentrations is unknown at this time.

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