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Management of dietary essential metals (iron, copper, zinc, chromium and manganese) by Wistar and Zucker obese rats fed a self-selected high-energy diet

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The balances and content of essential elements (iron, copper, zinc, chromium and manganese) in the body of Wistar, Zucker lean and Zucker obese rats fed a reference or cafeteria diet from day 30 to 60 after birth have been studied. Intestinal iron absorption compensated for low iron content of the cafeteria diet and the extra needs of growth and fat deposition. It can be assumed that the altered energy regulation processes that afflict the genetically obese rat are not directly related to altered iron metabolism. Obese Zucker rats had lower copper tissue concentrations than lean rats, but when fed a cafeteria diet the differences between Zucker rats strains disappear. This cannot be traced to large differences in diet copper concentration. A low diet availability of zinc—such as that of cafeteria-fed fa/fa rats—is easily compensated for by increasing absorption. So, as a consequence, we can conclude that genetic obesity did not impair zinc absorption. There was no deficit of zinc in any of the groups studied; the rats have enough capacity to extract zinc within a wide range of dietary concentrations. The absorption of dietary chromium was inversely proportional to its concentration. The ability to extract chromium from the diet and the very low urinary losses are a consequence of its scarcity in most dietary items. Despite wide variations in the manganese of the diets, the absorption rates were practically unchanged except for obese rats fed the cafeteria diet. It seems that this low absorptive capacity is enough to supply the rat with the manganese it needs, since a sizeable—but subjected to 8-fold-span variations—proportion is lost in the urine. This alone points towards a considerable excess of manganese in both diets studied. Obesity does not have a significant effect on the abilities to absorb and retain minerals, since these processes were more related to dietary availability. Management of essential metals by obese rats depends whether this condition is genetic or induced by diet. Most of the differences observed can be related to differences in diet concentration, to the excess fat content or different metabolic attitude to use substrates of obese animals. The data presented show that the cafeteria diet used adequately serves the mineral needs of the rat, since the rat adapts its absorbing and retaining strategies to match the dietary availability of these minerals.

Keywords: cafeteria diet, high-energy diet, obesity, Zucker fa/fa rat

Introduction

The essential metals are a qualitatively important but quantitatively small—often minuscule—part of the diet. They are needed for structural and catalytic roles in association with proteins and other cell components. Micronutrients in general, and especially some metals, are usually found in low concentrations in the diet, with ample variations in their levels depending on the type of food. In some cases, the chemical form is of paramount importance for their absorption and eventual incorporation to living tissue (Sandström 1988). As a consequence of this wide variability, animals have devised powerful homeostatic systems to absorb, retain and use the essential metals within a range for use in their specific metabolic functions. This is accomplished

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essentially by careful balancing of extraction/absorption from dietary sources by the intestine depending on the bioavailability of the materials in the diet. This is complemented by a tight control of excretion/losses, essentially through urine and intestine (Underwood & Mertz 1987), although the losses of some elements with hair, sweat and scales may be significant.

Scarcity and low bioavailability of metals in the diet strongly affects absorption and excretion. In some cases, high absorptive efficiency may result in unwanted accumulation, as may be the case with iron (Charlton & Bothwell 1983). The capacity of the mammalian organism to cope with a wide range of concentrations of most essential metals in the diet is affected, however, by physiological state—like pregnancy and lactation (Kochanowski & Sherman 1983)—as well as by the relative concentration and special needs, such as the need for more chromium in diets heavily loaded with carbohydrate (Anderson & Mertz 1977). The scarcity of a given element is often compounded by the relative abundance of another, as is the case with zinc and copper (Ogiso *et al.* 1974), which partly share a common mechanism for intestinal absorption (Oestreicher & Cousins 1985).

Iron is essential for oxygen transport and oxidative capacity (Morris 1987). Anemia affects thermogenesis (Lukaski *et al.* 1990) and thermoregulation (Beard *et al.* 1984). We have studied the iron balance because of its obvious correlation with oxidative energy disposal and because of its purported scarcity in human diets (Pilch & Senti 1984), comparable to our cafeteria diet (Sclafani & Springer 1976). Copper is necessary for iron metabolism (Roeser *et al.* 1970) and for overall oxidative capacity in mitochondria (Prohaska 1988). Zinc has been included in this study because it plays an important role in insulin secretion and action (Grodsky and Bennet 1966), and is an essential cofactor for many metabolic processes (Cousins 1985). We included manganese because of its direct relationship with oxidative energy metabolism (Body *et al.* 1968). Chromium is an essential part of the biologically active chromium or glucose tolerance factor (Mertz 1969) that strongly influences glucose disposal (Anderson 1981) and insulin action (Anderson *et al.* 1987). We included the study of chromium balances because of the direct relationship that exists between chromium availability and glucose intolerance (Hopkins *et al.* 1987), a condition encountered in many models of obesity (Bray & York 1979).

The alterations in mineral management induced by obesity have been studied only sparsely (Serfass *et al.* 1988, Folder *et al.* 1992), since obesity is often related to abundant nutrient intake (Bray & York 1979) and most studies deal with deficiencies. The self-selected high-energy 'cafeteria' diets have been described as defective for essential metal content (Moore 1987), in spite of their ability to promote growth (Rolls *et al.* 1980). We have studied the interaction of this diet with two different strains of rats (Wistar and Zucker) as well with the genetic obesity shown by Zucker fa/fa rats, with regard to the management of dietary essential micronutrient metals: iron, copper, zinc, chromium and manganese.

Materials and methods

Animals

Three groups of rats (*Rattus norvegicus*), all female and aged 30 days (weaned at day 22), were used in this study: (i) Wistar rats (bred at the University of Barcelona Animal Service from Charles River France stock), (ii) lean Zucker (Fa/?) rats and (iii) obese Zucker (fa/fa) rats; all Zucker rats were bred in the same service, from heterozygous parents (Harlan-Ollac, Oxon, UK). The fa/fa rats were identified on weaning by their aspect and a rapid fall in their rectal temperature during a short exposure to the cold.

The rats were housed individually, either in polypropylene-bottomed cages with wood shavings as absorbent material, or in polycarbonate metabolic cages (Tecniplast Gazzada, Guguggiate, Italy) which allowed the daily estimation of the consumption of individual food items. The cages were maintained in a light (on from 08:00 to 20:00), humidity (70–80% relative humidity) and temperature (21–22 °C) controlled environment.

Dietary treatments

Reference rats were fed a commercial reference diet (type A04 from Panlab, Barcelona, Spain) and tap water *ad libitum*. This diet contained 58.7% digestible carbohydrate, 3.0% lipid and 17.0% protein. All studies involving the measurement of food intake were carried out with the rats kept in metabolic cages. The rats given the cafeteria diet were presented daily with a fresh offering of biscuits spread with liver pâté, bacon, banana, chow pellets (as indicated above), tap water and whole milk complemented with 333 $g\,l^{-1}$ sucrose plus 10 $g\,l^{-1}$ of a mineral and vitamin supplement (Gevral, Cyanamid Ibérica, Madrid, Spain) (Esteve *et al.* 1992). The mineral composition of the aliments can be seen in Table 1. All diet components were previously weighed and presented in excess (~ 20–30% higher than the expected consumption). At 24 h later, the remaining debris was isolated, identified and weighed. The drying of food leftovers was corrected for by determining the amount of water lost in 1 day from known weight food samples left in a cage with no

Table 1. Essential metal concentrations of the dietary components given to Wistar and Zucker rats

Diet component	Iron (mg/kg)	Copper (mg/kg)	Zinc (mg/kg)	Chromium (mg/kg)	Manganese (mg/kg)
Chow pellet	354 ± 38	25.0 ± 5.0	82.4 ± 3.5	0.6 ± 3.8	68.2 ± 3.8
Biscuit	< 0.05	0.8 ± 0.1	11.5 ± 8.6	10.7 ± 4.3	3.6 ± 0.3
Sugar-enriched milk	40.5 ± 15.1	22.4 ± 4.5	14.0 ± 2.0	< 0.05	0.7 ± 0.2
Banana	12.6 ± 2.7	7.6 ± 2.3	2.2 ± 0.6	2.0 ± 1.6	1.3 ± 0.1
Bacon	4.0 ± 5.2	3.8 ± 2.7	16.4 ± 2.5	5.7 ± 2.6	0.2 ± 0.0
Liver pâté	29.5 ± 6.6	9.4 ± 2.8	20.0 ± 1.0	7.9 ± 4.8	1.0 ± 0.1
Water	< 0.01	0.05 ± 0.03	< 0.01	0.03 ± 0.03	0.2 ± 0.02

The data are the mean ± SEM of 10 different measures for each food component.

rats. This diet is a simplified version of an earlier cafeteria diet developed and studied by us (Prats *et al.* 1989), scaled down by selecting only the items actually consumed in significant portions. Droppings and urine were recovered separately. Urine evaporation was prevented with a 1 ml octyl alcohol layer.

Experimental set-up

The three rat stocks defined, Wistar, lean Zucker and obese Zucker, were studied under two dietary conditions: controls (fed rat chow pellets and water) and cafeteria (receiving the cafeteria diet). For each group two sets (five to seven rats each) of animals were studied, being killed at 30 (0 days of dietary treatment) or 60 (30 days of treatment) days of age. The rats belonging to the 60 day group were used for daily individual diet intake analysis, and dropping and urine collection (metabolic cages) during the whole 30 days of the study.

On days 30 or 60, the allotted groups of rats were weighed and immediately decapitated. Their corpses were again weighed (the difference being the net loss of blood and fluids) and then dissected. The content of their intestine was carefully removed and weighed. The weights recorded and used for calculations were the empty body weights. The remaining carcass was then minced and ground with a blender, and stored at −23 °C until processing. Droppings were weighed daily and stored frozen. Urine emissions were also recovered every day, measured and frozen.

Analytical procedures

The ground carcasses were sampled following a previously tested protocol (Esteve *et al.* 1992). The samples were frozen in liquid nitrogen, and then ground with a ceramic mortar and pestle. The finely powdered samples were mixed and used for the analysis of total nitrogen. Triplicate aliquots of about 0.2 g of the powder were mineralized in long pyrex tubes with 20 ml of reagent quality 80% nitric acid (Merck, Darmstadt, Germany) in a heating block. The tubes were kept for 12 h under reflux at 70 °C and 1 h at 120 °C, then concentrated down—at 150 °C, no reflux—to about 5 ml. An aliquot of 5 ml of reagent quality 70% perchloric acid was then added and the mixture was incubated for 30 min at 220 °C under reflux. The tubes were evaporated down to 1 ml—240 °C, no reflux—and pure (Milli-Q Millipore quality, 15 $M\Omega\,cm^{-1}$ resistivity) water was added to every tube to bring the final volume up to 10 ml. The droppings were also frozen in liquid nitrogen and powdered, aliquots of 0.2 g being mineralized in the same way. Urine samples of 0.5 ml were mineralized with 3 ml of nitric acid, incubated for 2 h at 70 °C and 2 h at 120 °C under reflux, evaporated to dryness—130 °C, no reflux—and then brought to 10 ml with 1% nitric acid.

The blood of a series of animals was collected in dry heparinized beakers and subjected to the tissue analytical procedures outlined above. The constituents of the diets given to the animals were ground and mineralized in the same way. In all types of samples, series of standards of the elements studied were added to random samples in order to determine the effectiveness of the measurements. No matrix effect was observed because of the very high temperature of the argon plasma.

The metals content of the acidic digests was measured with a Polyscan 61E ICAP spectrometer (Thermo Jasell Ash, Franklin, MA) fitted with a polychromator optical system, which allowed the simultaneous estimation of all indicated metals from the digests.

The final data on element content of the 'live' rat was a composite of the content of the paste plus that of the blood assumedly lost. The total nitrogen content of all carcass samples was measured by using an elemental nitrogen analyzer (NA-1500; Carlo Erba, Italy).

Calculations and statistics

The analyses of body composition of the series of rats killed on days 30 and 60 were used for the calculation of the differences in essential element content (i.e. the materials accrued in 1 month).

The composition of the food items was used to establish the amount of iron, copper, zinc, chromium and manganese ingested by each rat for each day. The tabulated data for each diet component were combined in order to determine the amount of each element ingested every day,

after correcting by the proportion of each component purportedly absorbed.

Statistical comparisons between the groups were carried out with standard analysis of variance (ANOVA) programs.

Results

Wistar rats fed the reference diet weighed 89.6 ± 2.2 g (mean ± SEM) on day 30 and 189.8 ± 4.2 g on day 60; the corresponding data for Zucker lean rats were 68.5 ± 1.9 and 165.9 ± 3.9 g, and those for obese rats were 82.1 ± 3.1 and 279.2 ± 6.3 g. When fed the cafeteria diet the weights were, respectively, 248.2 ± 4.9 g for Wistar rats, 209.1 ± 7.6, for lean Zucker and 350.4 ± 7.3 g for Zucker fa/fa 60-day rats. The weight increases shown in 1 month by all rats receiving the cafeteria diet were higher than those of the rats eating the reference diet. Obese rats increased their weight even more.

The iron balances for Wistar and Zucker rats are presented in Table 2. Since chow pellets contained at least 10 times as much iron as did the other components of the cafeteria diet, the rats eating the reference diet ingested more iron than the animals on the cafeteria diet. The proportion of iron absorbed, however, was much higher in cafeteria-fed rats, with low absorption percentages in reference-fed rats, irrespective of strain. The amount of iron accrued was higher for obese rats and also higher for cafeteria-fed rats—which also had higher body weights. The concentration of iron ($mg\,kg^{-1}$) showed only small differences between reference and cafeteria-fed rats, but obese rats had lower concentrations. These differences were much less marked when expressed per unit of protein weight. No measurable ($< 0.2\,mg\,kg^{-1}$) iron was found in the urine of all animals studied.

The copper status and balances are shown in Table 3. The amount of copper ingested was fairly similar for all groups of rats, being highest in reference-fed obese rats. The proportion of copper absorbed by cafeteria-fed rats was higher for Wistar and obese Zuckers, but was lower for lean Zucker rats on the same diet. Urinary losses amounted to a moderate proportion of the copper absorbed, being higher for all reference-fed groups. As a consequence, the copper accrued was higher for cafeteria-fed rats except for lean Zuckers. Copper concentration of the animals did changed little with age; the highest dietary differences were observed in Zucker lean rats, with lower copper content in cafeteria-fed animals; Wistar and obese Zucker rats showed a reversed pattern, with much closer copper concentration values in either dietary group.

The iron:copper concentration ratio in the pellet diet was 14.2; the cafeteria diet selected by Wistar rats gave a ratio of 5.1, that of lean Zuckers was 5.2 and obese rats 5.5. The iron:copper ratios in 30-day rats were similar, and showed little change 1 month later in animals fed the reference diet, decreasing in Wistar and obese Zucker rats and increasing in lean Zucker rats when subjected to the cafeteria diet.

The zinc balances are presented in Table 4. The intake of zinc was higher in rats fed the reference diet; the intestinal absorption was inversely proportional to the amount of zinc ingested, with highest/lowest absorption rates in obese Zucker rats fed the cafeteria/reference diets. The losses of zinc in urine were small, less than 1% of absorbed zinc, except for Wistar rats fed the reference diet. The zinc accrued was highest in all cafeteria groups, obese rats showing the highest rates of accumulation and 8 mg of zinc retained. There were some differences between strains: in Wistar rats, the concentration of zinc practically did not change either with age or diet; 60-day lean Zucker rats contained more zinc than either the 30-day rats or those cafeteria-fed. This was not true for obese Zucker rats, where the individual variability was considerable.

The zinc:copper concentration ratio for pellet diet was 3.3, 1.6 for the cafeteria diet selected by lean animals and 1.8 for the cafeteria diet selected by obese rats. The 30-day rats had similar zinc:copper ratios, which increased in all Zucker rats fed the cafeteria diet.

Table 5 shows the balances for chromium. In this instance, the rats fed the cafeteria diet ingested much more chromium than the animals on the reference diet. Wistar rats absorbed a similar proportion of chromium in both dietary groups, although Zucker lean and obese rats absorbed much more chromium from the less concentrated chow pellets than from the cafeteria diet. Urinary losses of chromium were practically undetectable.

Obese 30-day rats contained more chromium than both lean groups on a $mg\,kg^{-1}$ basis, but their content was practically uniform when expressed per gram of protein. In 60-day rats, however, feeding the cafeteria diet resulted in higher chromium content in all strains, the differences between both dietary groups being lower in obese Zucker rats.

The manganese content and distribution in the rat groups studied are presented in Table 6. The rats receiving the reference diet ingested about three times more manganese than those cafeteria-fed. The percentage of ingested manganese absorbed was

Table 2. Iron content and distribution in young Wistar and Zucker rats fed a reference and cafeteria diet

Parameter	Wistar control	Wistar cafeteria	Zucker Fa/? control	Zucker Fa/? cafeteria	Zucker fa/fa control	Zucker fa/fa cafeteria	
Fe ingested (mg/30 day)	173 ± 2	82 ± 5	161 ± 5	70 ± 4	269 ± 6	88 ± 8	**B/B_R/B_C/D/D_W/D_L/D_O**
Fe absorbed (% of ingested)	5.60 ± 0.22	14.24 ± 1.25	5.74 ± 0.28	14.68 ± 0.62	5.17 ± 0.11	19.48 ± 1.43	B/**B_C**/D/D_W/D_L/D_O
Fe accrued (mg/30 day)	9.68 ± 0.38	11.36 ± 0.39	9.23 ± 0.39	10.22 ± 0.88	13.92 ± 0.27	16.67 ± 0.22	**B/B_R/B_C/D/D_W/D_L/D_O**
(μg/mg per day)[a]	26.3	28.3	30.6	32.0	35.6	38.3	
30-day rat							
Fe mass (mg)	7.57 ± 0.23		5.49 ± 0.28		6.13 ± 0.32		**B**
[Fe] (mg/kg)	56.8 ± 5.4		53.4 ± 5.3		46.4 ± 2.8		
[Fe] (μg/g protein)	368 ± 29		334 ± 32		318 ± 24		
60-day rat							
Fe mass (mg)	17.01 ± 0.36	19.18 ± 0.38	14.62 ± 0.34	15.80 ± 0.93	19.96 ± 0.45	22.89 ± 0.47	**B/B_R/B_C/D/D_W**/D_L/**D_O**
[Fe] (mg/kg)	57.9 ± 4.6	53.2 ± 4.4	60.6 ± 6.2	53.7 ± 3.8	43.1 ± 3.3	40.6 ± 2.0	B/B_R
[Fe] (μg/g protein)	330 ± 30	348 ± 37	313 ± 35	311 ± 15	301 ± 23	365 ± 22	

The data are the mean ± SEM of five to seven different animals. Statistical comparison between groups (ANOVA): B = significant overall effect of breed; B_R, B_C = significant effect of breed for reference and cafeteria diets; D = significant overall effect of diet; D_W, D_L, D_O = significant effect of diet for Wistar, Zucker lean and Zucker obese rats, respectively. The level of significance is $P < 0.05$; bold symbols (**B**, **B_R**, **B_C**, **D**, **D_W**, **D_L**, **D_O**) represent a higher significance of $P < 0.001$.

[a]Micrograms of iron incorporated per day with respect to the mass of iron in the rat in milligrams. This mass has been taken as the arithmetic mean of the mass at 30 and at 60 days.

Lean Zucker rats are represented by the genotype Fa/? (either Fa/Fa or Fa/fa), and obese Zucker rats by the genotype fa/fa.

Table 3. Copper content and distribution in young Wistar and Zucker rats fed a reference and cafeteria diet

Parameter	Wistar control	Wistar cafeteria	Zucker Fa/? control	Zucker Fa/? cafeteria	Zucker fa/fa control	Zucker fa/fa cafeteria	
Cu ingested (mg/30 day)	12.2 ± 0.2	16.0 ± 0.6	11.2 ± 0.3	13.5 ± 0.7	18.7 ± 0.4	15.9 ± 0.7	**B/B_R/B_C/D/D_W/D_L/D_O**
Cu absorbed (% of ingested)	2.96 ± 0.16	3.42 ± 0.19	3.43 ± 0.15	2.09 ± 0.11	2.53 ± 0.08	3.79 ± 0.17	B/**B_R**/**B_C**/D_W/**D_L**/**D_O**
Urinary Cu (% of absorbed)	22.8 ± 2.2	8.0 ± 0.9	25.1 ± 1.2	17.0 ± 1.0	25.5 ± 2.1	9.9 ± 0.4	B/**B_C**/**D**/**D_W**/**D_L**/**D_O**
Cu accrued (mg/30 day)	0.28 ± 0.01	0.50 ± 0.01	0.29 ± 0.01	0.23 ± 0.02	0.35 ± 0.01	0.54 ± 0.01	**B/B_R/B_C/D/D_W/D_L/D_O**
(μg/mg per day)[a]	27.9	37.0	33.0	28.6	34.8	41.7	
30-day rat							
Cu mass (μg)	199 ± 7		151 ± 8		162 ± 9		B
[Cu] (mg/kg)	2.24 ± 0.36		2.26 ± 0.21		1.92 ± 0.10		
[Cu] (μg/g protein)	14.6 ± 2.4		14.2 ± 1.2		13.7 ± 1.6		
60-day rat							
Cu mass (μg)	470 ± 10	702 ± 13	435 ± 10	385 ± 23	509 ± 11	701 ± 15	**B/B_R/B_C/D/D_W**/D_L/**D_O**
[Cu] (mg/kg)	2.40 ± 0.32	2.90 ± 0.38	2.67 ± 0.14	1.97 ± 0.10	1.83 ± 0.13	2.03 ± 0.40	B/B_C
[Cu] (μg/g protein)	13.6 ± 1.4	18.8 ± 2.5	13.7 ± 0.8	11.4 ± 0.7	12.8 ± 0.8	17.9 ± 3.3	B_C
[Fe]/[Cu] ratio							
30-day rats	38		36		38		
60-day rats	36	27	34	41	39	33	

The data are the mean ± SEM of five to seven different animals. Statistical comparison between groups (ANOVA): B = significant overall effect of breed; B_R, B_C = significant effect of breed for reference and cafeteria diets; D = significant overall effect of diet; D_W, D_L, D_O = significant effect of diet for Wistar, Zucker lean and Zucker obese rats, respectively. The level of significance is $P < 0.05$; bold symbols (**B**, **B_R**, **B_C**, **D**, **D_W**, **D_L**, **D_O**) represent a higher significance: $P < 0.001$.

[a]Micrograms of copper incorporated per day with respect to the mass of copper in the rat in milligrams. This mass has been taken as the arithmetic mean of the mass at 30 and at 60 days.

Table 4. Zinc content and distribution in young Wistar and Zucker rats fed a reference and cafeteria diet

Parameter	Wistar control	Wistar cafeteria	Zucker Fa/? control	Zucker Fa/? cafeteria	Zucker fa/fa control	Zucker fa/fa cafeteria	
Zn ingested (mg/30 day)	40.2 + 0.5	24.9 ± 1.0	36.9 ± 1.1	22.2 ± 0.9	61.6 ± 1.4	29.0 ± 1.7	**B/B_R/B_C/D/D_W/D_L/D_O**
Zn absorbed (% of ingested)	9.33 ± 0.59	18.65 ± 1.21	9.13 ± 0.44	17.33 ± 0.55	7.63 ± 0.16	34.75 ± 1.92	**B/B_C/D/D_W/D_L/D_O**
Urinary Zn (% of absorbed)	3.07 ± 2.98	< 0.1	0.91 ± 0.12	0.35 ± 0.16	0.65 ± 0.26	0.14 ± 0.04	**B/B_R/B_C/D/D_W/D_L/D_O**
Zn accrued (mg/30 day)	3.60 ± 0.13	4.58 ± 0.14	3.34 ± 0.14	3.82 ± 0.32	4.66 ± 0.09	9.90 ± 0.15	**B/B_R/B_C/D/D_W/D_L/D_O**
(μg/mg per day)[a]	29.7	33.2	31.9	33.8	34.6	46.2	
30-day rat							
Zn mass (mg)	2.27 ± 0.07		1.84 ± 0.10		2.18 ± 0.12		B
[Zn] (mg/kg)	31.4 ± 1.6		29.0 ± 2.0		27.6 ± 1.9		
[Zn] (μg/g protein)	171 ± 9		177 ± 11		190 ± 19		
60-day rat							
Zn mass (mg)	5.81 ± 0.12	6.93 ± 0.14	5.15 ± 0.12	5.69 ± 0.33	6.81 ± 0.15	12.11 ± 0.25	**B/B_R/B_C/D/D_W/D_L/D_O**
[Zn] (mg/kg)	30.3 ± 1.4	29.0 ± 2.2	35.3 ± 3.3	29.1 ± 3.0	25.3 ± 2.7	36.0 ± 7.8	
[Zn] (μg/g protein)	173 ± 11	188 ± 12	182 ± 18	169 ± 19	177 ± 20	323 ± 69	B/B_C/D_O
[Zn]/[Cu] ratio							
30-day rats	11		12		13		
60-day rats	12	10	12	15	13	17	

The data are the mean ± SEM of five to seven different animals. Statistical comparison between groups (ANOVA): B = significant overall effect of breed; B_R, B_C = significant effect of breed for reference and cafeteria diets; D = significant overall effect of diet; D_W, D_L, D_O = significant effect of diet for Wistar, Zucker lean and Zucker obese rats, respectively. The level of significance is $P < 0.05$; bold symbols (**B**, **B_R**, **B_C**, **D**, **D_W**, **D_L**, **D_O**) represent a higher significance: $P < 0.001$.

[a]Micrograms of zinc incorporated per day with respect to the mass of zinc in the rat in milligrams. This mass has been taken as the arithmetic mean of the mass at 30 and at 60 days.

Table 5. Chromium content and distribution in young Wistar and Zucker rats fed a reference and cafeteria diet

Parameter	Wistar control	Wistar cafeteria	Zucker Fa/? control	Zucker Fa/? cafeteria	Zucker fa/fa control	Zucker fa/fa cafeteria	
Cr ingested (mg/30 day)	0.31 ± 0.00	1.31 ± 0.06	0.28 ± 0.01	1.37 ± 0.04	0.45 ± 0.01	2.81 ± 0.13	**$B/B_C/D/D_W/D_L/D_O$**
Cr absorbed (% of ingested)	33.27 ± 16.51	26.64 ± 0.68	75.07 ± 3.55	32.09 ± 1.09	68.62 ± 1.17	18.05 ± 0.92	**$B/B_R/B_C/D/D_W/D_L/D_O$**
Cr accrued (mg/30 day)	0.10 ± 0.05	0.32 ± 0.01	0.21 ± 0.01	0.44 ± 0.03	0.31 ± 0.01	0.50 ± 0.01	**$B/B_R/B_C/D/D_W/D_I/D_O$**
(μg/mg per day)[a]	29.2	42.5	34.7	45.9	40.3	47.1	
30-day rat							
Cr mass (μg)	89.3 ± 3		99 ± 5		102 ± 5		
[Cr] (mg/kg)	0.88 ± 0.24		1.39 ± 0.64		2.65 ± 0.75		
[Cr] (μg/g protein)	5.7 ± 1.6		8.5 ± 3.1		7.8 ± 2.8		
60-day rat							
Cr mass (μg)	139 ± 3	413 ± 8	304 ± 7	540 ± 31	411 ± 9	605 ± 13	**$B/B_R/B_C/D/D_W/D_L/D_O$**
[Cr] (mg/kg)	0.57 ± 0.15	1.60 ± 0.47	1.76 ± 0.54	2.65 ± 0.75	1.38 ± 0.44	1.67 ± 0.38	
[Cr] (μg/g protein)	5.0 ± 2.6	10.5 ± 3.2	7.5 ± 2.8	17.0 ± 4.3	9.6 ± 3.1	14.4 ± 3.0	D/D_L

The data are the mean ± SEM of five to seven different animals. Statistical comparison between groups (ANOVA): B = significant overall effect of breed: B_R, B_C = significant effect of breed for reference and cafeteria diets; D = significant overall effect of diet; D_W, D_L, D_O = significant effect of diet for Wistar, Zucker lean and Zucker obese rats, respectively. The level of significance is $P < 0.05$; bold symbols (**B**, **B_R**, **B_C**, **D**, **D_W**, **D_L**, **D_O**) represent a higher significance: $P < 0.001$.

[a]Micrograms of chromium incorporated per day with respect to the mass of chromium in the rat in milligrams. This mass has been taken as the arithmetic mean of the mass at 30 and at 60 days.

Table 6. Manganese content and distribution in young Wistar and Zucker rats fed a reference and cafeteria diet

Parameter	Wistar control	Wistar cafeteria	Zucker Fa/? control	Zucker Fa/? cafeteria	Zucker fa/fa control	Zucker fa/fa cafeteria	
Mn ingested (mg/30 day)	33.3 ± 0.4	13.4 ± 0.8	30.5 ± 0.9	11.6 ± 0.6	50.9 ± 1.1	15.2 ± 1.7	**B/B_R/B_C/D/D_W/D_L/D_O**
Mn absorbed (% of ingested)	1.05 ± 0.06	1.11 ± 0.14	1.54 ± 0.07	1.80 ± 0.13	1.16 ± 0.05	0.64 ± 0.12	**B**/B_R/B_C/D_O
Urinary Mn (% of absorbed)	8.78 ± 2.07	16.34 ± 5.55	21.22 ± 2.23	6.49 ± 1.52	9.30 ± 1.26	3.17 ± 0.53	B/B_R/B_C/D/**D_L**
Mn accrued (mg/30 day)	0.32 ± 0.02	0.12 ± 0.01	0.37 ± 0.02	0.19 ± 0.03	0.54 ± 0.01	0.09 ± 0.02	**B/B_R/B_C/D/D_W/D_L/D_O**
(μg/mg per day)[a]	21.9	10.0	21.8	14.6	25.2	6.0	
30-day rat							
Mn mass (μg)	334 ± 11		383 ± 21		447 ± 25		B
[Mn] (mg/kg)	3.91 ± 0.21		5.96 ± 0.78		3.01 ± 0.60		B
[Mn] (μg/g protein)	25.4 ± 1.7		37.3 ± 5.0		39.4 ± 5.3		
60-day rat							
Mn mass (μg)	639 ± 14	464 + 9	746 ± 17	483 ± 34	980 ± 9	545 ± 11	**B/B_R/B_C/D/D_W/D_L/D_O**
[Mn] (mg/kg)	3.37 ± 0.31	1.96 ± 0.2	4.72 ± 0.39	3.01 ± 0.60	3.68 ± 0.32	1.63 ± 0.12	**B**/B_R/B_C/D/D_W/D_L/**D_O**
[Mn] (μg/g protein)	19.2 ± 1.9	12.8 ± 1.5	24.4 ± 2.3	18.1 ± 3.9	25.7 ± 2.3	14.5 ± 0.9	D/D_O

The data are the mean ± SEM of five to seven different animals. Statistical comparison between groups (ANOVA): B = significant overall effect of breed; B_R, B_C = significant effect of breed for reference and cafeteria diets; D = significant overall effect of diet; D_W, D_L, D_O = significant effect of diet for Wistar, Zucker lean and Zucker obese rats, respectively. The level of significance is $P < 0.05$; bold symbols (**B**, **B_R**, **B_C**, **D**, **D_W**, **D_L**, **D_O**) represent a higher significance: $P < 0.001$.

[a]Micrograms of manganese incorporated per day with respect to the mass of manganese in the rat in milligrams. This mass has been taken as the arithmetic mean of the mass at 30 and at 60 days.

very low in all groups, with little influence of diet or strain. Urinary losses showed a wide variation between groups, unrelated to the manganese ingested; maximal losses were found in reference-fed lean Zucker rats and cafeteria-fed Wistars, with lowest losses in obese rats fed the cafeteria diet.

The rats fed the cafeteria diet accrued less manganese than those on the reference diet, which showed much higher accrual rates. The manganese concentration of 30-day rats was highest in the lean Zucker group; 30 days later, the rats on the reference diet maintained similar manganese concentrations (lower when expressed per gram of protein), but the manganese content of the animals receiving the cafeteria diet decreased considerably.

Discussion

For most essential elements, the concentration in the diet is not a straightforward correlate of its absorption (Mertz 1991), since other factors, such as the chemical form or bioavailability (Johnson 1989) effectively modulate absorption. In addition, the internal availability/relative starvation of a given metal as well as overlapping competition by other abundant elements (L'Abbé & Fischer 1984) can result in widely changing rates of intestinal absorption (Sandström 1988) and retention by the kidney. The mammal is able to adapt its absorption/excretion/storage strategies to the needs and environmental availability for each and all essential metals it needs. The cafeteria diets have been charged as not providing enough essential micro-components of the diet (Moore 1987), despite being able to sustain growth (Rolls *et al.* 1980) and active metabolism. The data presented here partly support the assumption that one such self-selected high-energy diet can indeed provide some of the needed essential metals in adequate amounts despite low dietary content.

However, it must be taken into account that most standard diets recommended for the breeding and rearing of rats contain very high amounts of minerals (Rogers 1979), since it is often assumed that the ability of the rat to extract some minerals, such as calcium or iron, from the diet is very low (Allen 1982), and the needs of these elements rise considerably with pregnancy and lactation (Davies & Williams 1977, Munro 1981). The common presence of standard pelleted food as another constituent of the self-selected cafeteria diets, however, allows the rat to choose up to 30.5% (Wistar), 28.5% (lean Zucker) or 23.0% (obese Zucker) of the energy ingested in the form of chow pellets. This is a very significant part of the calcium and potassium ingested, as well as of iron and manganese.

Despite its low iron content, the cafeteria diet provided enough iron for the needs of even markedly obese animals. The intestinal absorptive mechanisms can compensate easily for the low iron content of the diet and the extra needs of growth and fat deposition. The $mg\,kg^{-1}$ concentration of iron is less affected by fat-mass dilution than by the distribution of other elements because of the high concentration of iron in blood hemoglobin, since a high mass of fat tissue produces a hypervolemia (Failla *et al.* 1988). The relative lack of changes in iron status observed here under dietary and genetic obesity in the rat agree with the unaltered plasma iron and transferrin found in genetically obese mice (Failla *et al.* 1988), despite the latter having lower tissue iron concentrations (Kennedy *et al.* 1986). The iron status of the fa/fa rats could not be considered as a situation of deficit or impaired iron metabolism or management, despite these animals having altered thyroid function (Beard *et al.* 1989), thermogenesis (Beard *et al.* 1984, Lukaski *et al.* 1990), thermoregulation and catecholamine turnover (Tobin & Beard 1990), a string of effects that can be induced by iron deficiency (Farrell *et al.* 1988), even at levels short of anemia. It can be assumed, then, that the altered energy regulation processes that afflict the genetically obese rat are not directly related to iron availability or altered metabolism.

Obesity alters copper tissue concentrations in mice, but does not lower the total copper budget of these animals (Kennedy *et al.* 1986). Similar lower tissue levels of copper have been found in the *corpulent* (Failla & Michaellis 1984) and Zucker fa/fa (Donaldson *et al.* 1987) rats. Our results agree with lower copper levels in the tissues of obese Zucker rats compared with their lean counterparts, but this condition is completely changed when these animals are subjected to a cafeteria diet, since then, the differences between Zucker rats disappear. This cannot be traced to large differences in diet copper content. More likely, the copper availability in the cafeteria diet—more fat content, the main copper provider being enriched milk—may induce higher rates of absorption. On the other hand, cafeteria feeding induced a significantly higher retention of copper in the rat, diminishing urinary losses in all strains. Thus, it can be assumed that the metabolic alterations induced by a high-energy high-fat diet increase the needs for copper and thus its retention is enhanced. This may have a direct bearing on the role of copper in oxidative metabolism (Prohaska

1988), strongly increased when the excess energy ingested has to be disposed of.

Copper and zinc absorption are closely intertwined (Hill *et al.* 1984), since they—in part—share a common transport mechanism (Oestreicher & Cousins 1985); the levels of zinc and copper in the diets studied did not result in cross-inhibition due to decompensation in their concentrations. In any case, copper and zinc were relatively poorly absorbed, as is common with these comparatively abundant dietary components (Scheibel & Mehta 1985). However, a low diet availability of zinc, such as that of cafeteria-fed fa/fa rats, is easily compensated by stepping up absorption. This behavior leaves no doubt on two accounts: genetic obesity did not impair zinc absorption and the rats had enough capacity to obtain the zinc they need even at levels of zinc lower than those found here. This can be done even when the zinc:copper ratio in the diet is one order of magnitude lower than that of the rat tissues, i.e. zinc is scarcer in the diet than copper.

Genetic obesity alters zinc metabolism in mice, with increased absorption and lower tissue levels (Kennedy *et al.* 1986), and increased urinary zinc excretion (Levine *et al.* 1983). In the Zucker fa/fa rat the pattern was different, since the absorption was affected by diet but not by strain (compared with lean Zucker rats), and the tissue concentrations were, likewise, much more influenced by diet than by obesity. Urinary zinc was higher in rats eating more zinc, despite them absorbing a lower proportion. The tissue zinc distribution is altered in fa/fa rats, which show higher levels in liver and higher in muscle than in lean controls (Donaldson *et al.* 1987); the low liver levels, however, are controversial (Serfass *et al.* 1988). In any case, as we have found, the overall zinc budget is not affected. The dilution effect of fat-mass is less apparent in obese rats probably, because of the high carbonic anhydrase content of fat tissue (Stanton *et al.* 1991). There was no deficit of zinc in any of the groups studied. Furthermore, the obese rats ate huge amounts of food (Bray & York 1979) and anorexia is a characteristic development of zinc deficiency (Kawamoto *et al.* 1986).

The need for chromium is enhanced in the animals receiving the carbohydrate-based reference diet compared with those with the lipid-based cafeteria diet, since additional chromium is required when large amounts of carbohydrate are to be processed (Glinsmann & Mertz 1966); in spite of this, the accumulation of chromium was higher in cafeteria-fed rats, probably due to higher availability. In humans, the dietary needs of chromium are hardly supplied by self-selected diets (Anderson & Kozlovsky 1985). This is not the case with the cafeteria diet used in our experiments, in which biscuits supplied cafeteria animals with about four times more chromium than that eaten with the reference diet, which is in line with the recommended chromium allowances in rats (Subcommittee on Laboratory Animal Nutrition 1978). The absorption of dietary chromium was inversely proportional to its concentration, as in humans (Anderson & Kozlowsky 1985). The ability to extract chromium from the diet and the very low urinary losses are a consequence of its scarcity in most dietary items, as can be seen from the data in Table 1. There is a parallel between chromium deficiency and glucose intolerance (Pilch & Senti 1984) and late-onset diabetes (Schroeder 1974), although a number of data seem to contest this interpretation (Offenbacher & Pi-Sunyer 1988). Obese rats showed chromium:protein concentrations similar to those of lean rats, and they maintained similar intestinal-absorbing and kidney-retaining abilities. Perhaps the relative abundance of chromium in both diets prevented the manifestation of the sensitivity to chromium deficiency attributed to the genetically obese mouse (Stoecker *et al.* 1987). In that same experimental model, supplementation of the diet with chromium resulted in normalization of insulin and liver lipid levels but with unchanged body weight (Li & Stoecker 1986).

Brown adipose tissue and muscle of ob/ob mice have low levels of manganese (Walsh *et al.* 1985), a condition that improves when dietary manganese supplements are provided; low mitochondrial manganese levels result in impaired oxidative capacity (Hurley *et al.* 1970). Thus, high oxidative rates require adequate supplies of manganese and unimpaired absorption/retention. Despite wide variations in the manganese of the diets, the absorption rates were practically unchanged except for obese rats fed the cafeteria diet. It seems that this low absorptive capacity is enough to supply the rat with the manganese it needs, since a sizeable—but highly variable—proportion of it is lost in the urine. This alone points towards a considerable excess of manganese in both diets studied, more than a limitation in the extractive capacity for this metal, since in all cases there is a significant loss through the urine, which is not found in severe manganese deficiency. Here again, the main differences between groups do not respond primarily to strain but to diet, the lower manganese content of the cafeteria diet may be the cause of the lower content of this metal in cafeteria-fed rats, but again, the dilution effect of fat-mass may have some effect,

since the mg kg^{-1} concentration differences between reference-fed lean and obese Zucker rats completely disappear when expressed on a protein-mass basis. Cafeteria-fed rats show an altered nitrogen metabolism (Esteve *et al.* 1992), with lower urinary losses and increased retention a condition parallel to situations of scarce dietary nitrogen, such as malnutrition. Manganese metabolism is altered in protein malnutrition, with increased toxicity (Shukla *et al.* 1988) and lowered oxidative capacity. Part of the alterations observed with the high-fat protein-sparing cafeteria diet may also be related to the nitrogen conservation configuration induced by the cafeteria diet.

The main conclusion that can be drawn from the data presented is that genetic obesity affects the management of essential metals in a way that is different from their handling in dietary-induced obesity. Genetic and diet-induced obesity do not significantly affect the ability to absorb and retain minerals, since these processes were much more correlated to their dietary availability than to the strain of rats studied. Most of the differences observed can be related to differences in diet concentration or—in some cases—to the excess fat content or different metabolic environment of obese animals.

Finally, but no less important, the data presented show that the cafeteria diet used, can supply the mineral needs of the rat, since the rat adapts its absorbing and retaining strategies to match the dietary availability of these minerals.

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References

Allen L. 1982 Calcium bioavailability and absorption. A review. *Am J Clin Nutr* **35**, 783–808.

Anderson RA. 1981 Nutritional role of chromium. *Sci Total Environm* **17**, 13–29.

Anderson RA, Kozlovsky AS. 1985 Chromium intake, absorption and excretion of subjects consuming self-selected diets. *Am J Clin Nutr* **41**, 1177–1183.

Anderson RA, Polansky MM, Bryden NA, Bhathena SJ, Canary J. 1987 Effects of supplemental chromium on patients with symptoms of reactive hypoglycemia. *Metabolism* **36**, 351–355.

Anderson RA, Mertz, W. 1977 Glucose tolerance factor: an essential dietary agent. *Trends Biochem Sci* **2**, 277–279.

Beard J, Green W, Miller L, Finch C. 1984 Effect of iron-deficiency on hormone levels and thermoregulation during cold exposure. *Am J Physiol* **247**, 114–119.

Beard J, Tobin B, Green W. 1989 Evidence for thyroid hormone deficiency in iron-deficient anemic rats. *J Nutr* **119**, 772–778.

Body DL, Curry DL, Keen CL, Hurley LS. 1968 Effect of manganese deficiency on insulin secretion and carbohydrate homeostasis in rats. *J Nutr* **94**, 89–94.

Bray GA, York DA. 1979 Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol Rev* **59**, 719–809.

Charlton RW, Bothwell TH. 1983 Iron absorption. *Annu Rev Med* **34**, 55–68.

Cousins RJ. 1985 Toward a molecular understanding of zinc metabolism. *Clin Physiol Biochem* **4**, 20–30.

Davies NT, Williams RB. 1977 Effects of pregnancy and lactation on the absorption of zinc and lysine by the rat duodenum *in situ*. *Br J Nutr* **38**, 417–423.

Donaldson DL, Smith CC, Koh E. 1987 Effect of obesity and diabetes on tissue zinc and copper concentrations in the Zucker rat. *Nutr Res* **7**, 393–399.

Esteve M, Rafecas I, Remesar X, Alemany M. 1992 Nitrogen balances of lean and obese Zucker rats subjected to a cafeteria diet. *Int J Obesity* **16**, 237–244.

Failla ML, Michaellis DM. 1984 Decreased tissue concentrations of essential trace metals in the obese diabetic rat. *Fed Proc* **43**, 677.

Failla ML, Kennedy ML, Chen ML. 1988 Iron metabolism in genetically obese (ob/ob) mice. *J Nutr* **118**, 46–51.

Farrell PA, Beard JL, Druckenmiller M. 1988 Increased insulin sensitivity in iron-deficient rats. *J Nutr* **118**, 1104–1109.

Folder J, Shih M-S, Levy J. 1992 Bone structure and calcium metabolism in obese Zucker rats. *Int J Obesity* **16**, 95–102.

Glinsmann WH, Mertz W. 1966 Effect of trivalent chromium on glucose tolerance. *Metabolism* **15**, 510–520.

Grodsky GM, Bennet LL. 1966 Cation requirements for insulin secretion in the isolated perfused pancreas. *Diabetes* **15**, 910–913.

Hill GM, Brewer GJ, Hogikyan ND, Stellini MA. 1984 The effect of depot parenteral zinc on copper metabolism in the rat. *J Nutr* **114**, 2283–2291.

Hopkins LL, Ransome-Kuti O, Majaj AS. 1968 Improvement of impaired carbohydrate metabolism by chromium(III) in malnourished infants. *Am J Clin Nutr* **21**, 203–211.

Hurley LS, Theriault L, Dreosti IE. 1970 Liver mitochondria from manganese-deficient and pallid mice, function and ultrastructure. *Science* **170**, 1316–1318.

Johnson PE. 1989 What can *in vitro* methods tell us about mineral availability? *Biol Trace Element Res* **19**, 3–10.

Kawamoto JC, Castonguay TW, Keen LL, Stern JS, Hurley LS. 1986 Age, sex and reproductive status alter the severity of anorexia in zinc deficient rats. *Physiol Behav* **38**, 485–493.

Kennedy ML, Failla ML, Smith JC. 1986 Influence of genetic obesity on tissue concentrations of zinc, copper, manganese and iron in mice. *J Nutr* **116**, 1432–1441.

Kochanowski BA, Sherman AR. 1983 Iron status of suckling rats in influenced by maternal diet during gestation and lactation. *Br J Nutr* **49**, 51–57.

L'Abbé MR, Fischer PWF. 1984 The effects of high dietary zinc and copper deficiency on the activity of copper-requiring metalloenzymes in the growing rat. *J Nutr* **114**, 813–822.

Levine AS, McClain CJ, Handwerger BS, Brown DM, Morley JE. 1983 Tissue zinc status of genetically diabetic and streptozotocin-induced diabetic mice. *Am J Clin Nutr* **37**, 382–386.

Li YC, Stoecker BJ. 1986 Chromium and yogurt effects on hepatic lipid and plasma glucose and insulin of obese mice. *Biol Trace Element Res* **9**, 233–242.

Lukaski HC, Hall CB, Nielsen FH. 1990 Thermogenesis and thermoregulatory function of iron-deficient women without anemia. *Aviat Space Environ Med* **61**, 913–920.

Mertz W. 1969 Chromium occurrence and function in biological systems. *Physiol Rev* **49**, 163–239.

Mertz W. 1991 General considerations regarding requirements and toxicity of trace elements. In: Chandra RK, ed. *Trace Elements in Nutrition of Children*, Vol. 2. New York: Raven Press: Nestlé Nutrition Series Workshop Series, Vol. 23; 1–13.

Moore B. 1987 The cafeteria diet—an inappropriate tool for studies of thermogenesis. *J Nutr* **117**, 227–231.

Morris ER. 1987 Iron. In: Mertz W, ed. *Trace Elements in Human and Animals Nutrition*, Vol. 1. San Diego: Academic Press; 79–142.

Munro HN. 1981 Nutrient requirements during pregnancy. *Am J Clin Nutr* **34**, 679–684.

Oestreicher P, Cousins RJ. 1985 Copper and zinc absorption in the rat: Mechanism of mutual antagonism. *J Nutr* **115**, 159–166.

Offenbacher EG, Pi-Sunyer FX. 1988 Chromium in human nutrition. *Annu Rev Nutr* **8**, 543–563.

Ogiso T, Moriyama K, Sasaki S, Ishimura Y, Minato A. 1974 Inhibitory effect of high dietary zinc on copper absorption in rats. *Chem Pharm Bull* **22**, 55–60.

Pilch SM, Senti FR, eds. 1984 *Assessment of the Iron Nutritional Status of the US Population Based on Data Collected in the Second National Health and Nutrition Examination Survey*. Bethesda: Federation of American Societies for Experimental Biology.

Prats E, Monfar M, Castellà J, Iglesias R, Alemany M. 1989 Energy intake of rats fed a cafeteria diet. *Physiol Behav* **45**, 263–272.

Prohaska JR. 1988 Biochemical functions of copper in animals. In: Prasad AS, ed. *Essential and Toxic Elements in Human Health and Disease*. New York: AR Liss; 105–124.

Roeser HP, Lee GR, Nacht S, Cartwright GE. 1970 The role of ceruloplasmin in iron metabolism. *J Clin Invest* **49**, 2408–2417.

Rogers AE. 1979 Nutrition. In: Baker HJ, Lindsey LR, Weisbroth SH, eds. *The Laboratory Rat. Vol. I, Biology and Diseases*. New York: Academic Press; 123–152.

Rolls BJ, Rowe EA, Turner RC. 1980 Persistent obesity in rats following a period of consumption of a mixed high energy diet. *J Physiol* **298**, 415–427.

Sandström B. 1988 Factors influencing the uptake of trace elements from the digestive tract. *Proc Nutr Soc* **47**, 161–167.

Scheibel MS, Mehta T. 1985 Effect of dietary fiber on bioavailability of zinc and copper and histology in rats. *Nutr Res* **5**, 81–93.

Schroeder HA. 1974 The role of trace elements in cardiovascular diseases. *Med Clin North Am* **58**, 381–396.

Sclafani A, Springer D. 1976 Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. *Physiol Behav* **17**, 461–471.

Serfass RE, Park KE, Kaplan ML. 1988 Developmental changes of selected minerals in Zucker rats. *Proc Soc Exp Biol Med* **189**, 229–239.

Shukla GS, Hussain T, Chandra SV. 1988 Protein-malnourishment alters organ distribution of ^{54}manganese in rat. *Biochem Arch* **4**, 151–157.

Stanton LW, Ponte PA, Coleman RT, Snyder MA. 1991 Expression of CA III in rodent models of obesity. *Mol Endocrinol* **5**, 860–866.

Stoecker BJ, Li YC, Webster DB, Chan SB. 1987 Effects of Torula and brewer's yeast diets in obese and lean mice. *Biol Trace Element Res* **14**, 249–254.

Subcommittee on Laboratory Animal Nutrition. 1978 Nutrient requirements of the laboratory rat. In: *Nutrient Requirements of Laboratory Animals 10*, 3rd edn. Washington: National Academy of Sciences; 7–37.

Tobin BW, Beard JL. 1990 Interactions of iron deficiency and exercise training relative to tissue norepinephrine turnover, triiodothyronine production and metabolic rate in rats. *J Nutr* **120**, 900–908.

Underwood EJ, Mertz W. 1987 Introduction. In: Mertz W, ed. *Trace Elements in Human and Animal Nutrition*, Vol. 1. San Diego: Academic Press; 1–19.

Walsh JJ, Narbaitz R, Bégin-Heick N. 1985 Metabolic effects of dietary manganese supplementation in ob/ob mice. *J Nutr* **115**, 919–928.