Dietary Macronutrient Composition Influences Tissue Trace Element Accumulation in Diabetic Sprague-Dawley Rats (43793)

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Abstract. **The purpose of the present investigation was to study the role of two potential contributory factors, hyperphagia and alterations in fuel metabolism, on the development of tissue trace element accumulation in the experimentally induced diabetic rat. The role of increased mineral intake associated with diabetic hyperphagia on tissue trace element accumulation was evaluated by feeding control and diabetic rats high-carbohydrate (HC) diets which varied in Zn, Cu, Mn, and Mg concentrations. Diabetic rats were hyperphagic and had lower plasma Mg, and higher liver Zn, Cu, and Mn concentrations than control rats, regardless of dietary mineral intake. In a second study, diabetic hyperphagia was reduced by feeding control and diabetic rats a HC, high-fat (HF), or high-protein (HP) diet; the effects of altering diabetic metabolism on trace element status was studied. Liver Mn and Zn concentrations of diabetic rats fed the HF diet were lower than diabetic rats fed the HC diet and HP diet, and were similar to control rats. Liver Cu concentrations of diabetic rats fed the HF and HP diets were lower than diabetic rats fed the HC diet and were similar to control rats. While diabetic rats, in general, had higher plasma glucagon concentrations and lower percent body fat than control rats, diabetic rats fed the HF diet had similar plasma glucagon and percent body fat to control rats. These data suggest that tissue-specific biochemical needs, such as the need for metals as cofactors for enzymes, rather than hyperphagia per se, may drive the accumulation of trace elements in the diabetic animal.**

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Diabetes in the human and the rodent is characterized by alterations in the metabolism of zinc (Zn), copper (Cu), manganese (Mn), and magnesium (Mg) (1–5). While high liver and kidney Zn, Cu, and Mn concentrations are observed in genetically and experimentally induced diabetic rats (2, 3), hypozincemia, hyperzincuria, hypercupremia, hypomagnesemia, and hypermagnesuria are observed in both diabetic humans and rodents (5). Alterations in the metabolism and concentrations of these elements, which have essential roles in metabolism, may pose a significant health risk and contribute to the development or progression of diabetic-induced complications. The purpose of the present investigation was to study the role of two potential contributory factors, hyperphagia and altered fuel metabolism, on the development of tissue trace element accumulation in the experimentally induced diabetic rat.

The characteristic hyperphagia observed in untreated diabetic rats fed high-carbohydrate diets is a complicating factor for determining the cause of dia-

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betic-induced trace element alterations since high trace element concentrations in diabetic tissues may reflect, in part, the increased mineral intake occurring with the diabetes-associated hyperphagia. Consistent with this idea, Craft and Failla (6) reported that the apparent absorption (μg/100 g body wt) of Zn by streptozotocin (STZ)-diabetic rats was 3-fold greater than control rats, and absorption of Zn positively correlated with food intake. However, Failla and Kiser (1), in an earlier study, demonstrated a persistent increase in tissue trace elements despite pair-feeding of Cu, Zn, and Mn to diabetic and control rats.

The first objective of the present project was to confirm previous reports (1, 7) that high tissue Zn, Cu, and Mn concentrations, characteristic of diabetic rats fed high-carbohydrate diets, are not a consequence of the increased mineral intake associated with hyperphagia. Rather than using pair-fed groups, we reduced the trace elements in the diabetic diet, so that despite hyperphagia, both controls and diabetics consumed the same amount of minerals. While the treatment in this study reducing dietary mineral concentrations was similar to that used in the previous studies (1, 7), it is important to note that the mineral concentrations used in this study, which range from more-than-adequate to marginally adequate, were broader than those used previously. Secondly, dietary Mg concentrations were also varied to study whether diabetes-induced alterations of Mg metabolism were sensitive to dietary Mg intake. This manipulation of dietary Mg content was considered important given reports that Mg supplementation reduces insulin resistance (8), platelet reactivity (9), and diabetic complications (10). Rude (11) has suggested that prophylactic Mg supplementation of diabetes may prevent or ameliorate complications such as arrhythmias, hypertension, and sudden cardiac death, thus improving the course of the diabetic condition.

Another means of reducing diabetic hyperphagia, and thereby limiting food intake as a variable, is to feed a diet with altered macronutrient composition. High-fat diets eliminate diabetic hyperphagia, while high-protein diets attenuate diabetic hyperphagia (12–15). The second objective was to determine if there was an increased accumulation of tissue trace elements when the metabolic fuel was changed and normophagia was achieved. Since key gluconeogenic and ureagenic enzymes are metalloenzymes, the demand for gluconeogenesis and ureagenesis in the insulin-deficient rodent is postulated to drive the accumulation of liver and kidney trace elements (16). High-protein and high-fat diets alter the activities of key gluconeogenic and ureagenic enzymes (17). We hypothesized that if the metabolic state of a diabetic rat is altered by macronutrient composition, the demand for the trace elements as cofactors and components of metalloenzymes would be altered, and this would be an underlying factor in diabetes-induced tissue trace element accumulation.

Materials and Methods

Materials. Lithium heparin syringes for blood collection were purchased from Sarstedt, Inc. (Newton, NC). Instra-analyzed quality HNO_3 used for trace element analysis was obtained from J. T. Baker Co. (Philipsburg, NJ). Certified reference solutions (1000 μg metal/ml) were obtained from Fisher Scientific (Pittsburgh, PA). Bovine liver standard (SRM 1577A) was purchased from the National Bureau of Standards (NBS) (Gaithersburg, MD). Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were from Fisher Scientific (Pittsburgh, PA) or Sigma Chemical Co. (St. Louis, MO).

Animals, Study 1. Virgin, female Sprague-Dawley rats (Charles River; Wilmington, MA) were housed individually in suspended stainless steel cages in a light- and temperature-controlled room. For 1 week prior to beginning the study, deionized water and a purified diet more than adequate in all minerals (high-carbohydrate/high-mineral = HCHM, Table I) were provided *ad libitum*. On Day 0 of the study, half

Table I. Diet Composition

Ingredient	Diet		
	HCHM or HC (g/kg diet)	HF (g/kg diet)	HP (g/kg diet)
Spray-dried egg white	210	270	490
Cerelose	595	250	315
Corn oil	80	333	80
Alphacel	40	50	40
Mineral mixture[a]	60	78	60
Vitamin mixture[b]	15	19	15

Note. HCHM: high-carbohydrate/high-mineral; HC: high-carbohydrate; HF: high-fat; HP: high-protein.
[a] Mineral mixture for diets contained (g/kg diet): $CaCO_3$, 20.33; $MgSO_4$, 3.96; $CaHPO_4$, 3.60; K_2HPO_4, 19.26; $FeSO_4 \cdot 7H_2O$, 0.60; NaCl, 10.08; $CrK(SO_4)_2 \cdot 12H_2O$, 0.024; $CuSO_4 \cdot 5H_2O$, 0.048; $MnSO_4 \cdot H_2O$, 0.092; KI, 0.012; $ZnCO_3$, 0.058.
[b] Vitamin mixture contained (g/kg diet): inositol, 0.375; ascorbic acid, 0.075; calcium pantothenate, 0.0375; thiamin hydrochloride, 0.0225; pyridoxine hydrochloride, 0.0225; nicotinic acid, 0.0225; menadione, 0.0188; riboflavin, 0.0075; para-aminobenzoic acid, 0.0075; folic acid, 0.00045; biotin, 0.0039; Rovimix E-50%, 0.117 (provided 5137 IU retinyl acetate/kg diet); Rovimix AD_3 A325/D325 (provided 1121 IU retinyl acetate and 1121 IU cholecalciferol/kg diet), 0.0034; Vitamin B_{12} + mannitol, 0.0225 (provided 22.5 μg vitamin B_{12}/kg diet; choline chloride (70% solution), 1.07 (ml/kg). (Sources: Rovimix, Hoffman-LaRoche, Nutley, NJ; Merck & Co., Rahway, NJ).

of the rats were anesthetized (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ) and injected via tail vein with streptozotocin (STZ; 40 mg/kg), in freshly prepared 0.1 *M* citrate buffer (pH 4.5), to induce diabetes. Control rats were anesthetized and injected with an equal volume of citrate buffer. Immediately after injection, rats were fed one of the following three diets: the HCHM diet containing 30, 12, 30, and 800 μg Zn, Cu, Mn, and Mg/g diet, respectively; a diet with 50% the mineral concentrations of the HCHM diet (HC/adequate mineral = HCAM) containing 15, 6, 15, and 400 μg Zn, Cu, Mn, and Mg/g diet, respectively; or a diet with 25% the mineral concentrations of the HCHM diet (HC/low mineral = HCLM) containing 7, 3, 7, and 200 μg Zn, Cu, Mn, and Mg/g diet, respectively. The Zn, Cu, Mn, and Mg concentrations of all diets were verified by flame atomic absorption spectrophotometry (AAS) (Model 551; Thermo-Jarrel Ash, Wilmington, MA) following wet-ashing of the diet with 16 *N* HNO_3 (18).

Food intake and body weights were measured daily throughout the study. Three days postinjection, following a 6-hr fast, heparinized tail vein blood samples were analyzed for glucose concentrations (Beckman Glucose Analyzer 2; Beckman Instrumentation, Fullerton, CA). Rats with plasma glucose concentrations >13.9 m*M* were considered diabetic. On Day 14, rats were killed via exsanguination under methoxyflurane anesthesia (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ).

Animals, Study 2. Male Sprague-Dawley rats (Charles River; Wilmington, MA) were housed individually in stainless steel cages in a light- and temperature-controlled room, with free access to deionized distilled water and the HCHM purified diet from Study 1 (HC, Table I) for one week prior to beginning the study. On both Day 0 and Day 3, 34 rats (diabetics) were injected subcutaneously with STZ (40 mg/kg), in freshly prepared 0.1 *M* citrate buffer (pH 4.5). Also, on Day 0 and 3, 30 rats (controls) were injected with an equal volume of citrate buffer. Based on our laboratory's experience, rates of diabetes development were found to be similar with intravenous and subcutaneous administration of STZ; therefore, in Study 2, subcutaneous injections were used due to ease of administration. Immediately after injection, rats were fed one of the following three diets: the purified HC diet (18.3% kcals as fat, 60.4% kcals as carbohydrate, 21.3% kcals as protein; Table I), a purified high-fat diet (HF; 60% fat, 19.7% carbohydrate, 20.3% protein), or a purified high-protein diet (HP; 18% fat, 32% carbohydrate, 50% protein). The Zn, Cu, and Mn concentrations of all diets were verified by AAS following wet ashing of the diet with HNO_3 (18). The trace element concentrations of the respective diets were as follows: (i) HC diet: 27 μg Zn, 6 μg Cu, and 43 μg Mn per g diet; (ii) HF diet: 36 μg Zn, 7 μg Cu, and 49 μg Mn per g diet; and (iii) HP diet: 26 μg Zn, 5 μg Cu, and 40 μg Mn per g diet.

Food intake and body weights were measured daily throughout the study. On Day 13, nonfasting heparinized tail vein blood samples were analyzed for plasma glucose concentrations (Beckman Glucose Analyzer 2; Beckman Instrumentation, Fullerton, CA). On Day 20 after a 4-hr fast, rats were killed via exsanguination under methoxyflurane anesthesia (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ). Rats were eviscerated and the remaining carcass was analyzed for body composition as previously described by Bell and Stern (19).

Since dietary macronutrient content has been shown to affect glycemia (20), plasma insulin concentrations were used as the diabetes-defining criteria. Rats with Day 20 plasma insulin concentrations <15.5 p*M* were considered diabetic. Six STZ-injected rats did not meet these criteria; data from these animals were excluded from the analyses.

Tissue Preparations. Cardiac blood was collected in heparinized tubes and placed on ice. Duplicate samples of whole blood were taken for the measurement of percent packed red blood cell volume or mixed with the enzyme inhibitor Trasylol and stored at −4°C until analyzed for plasma glucagon concentration (21; Study 2 only). The remaining blood was centrifuged (1700*g*, 20 min, 4°C) and the plasma fraction stored at −20°C until analyzed for glucose and insulin (22; Study 1 and 2) and trace element (Zn, Cu, and Mg; Study 1 only) concentrations.

Liver and plasma samples were wet-ashed with 16 *N* HNO_3, evaporated and diluted with 0.1% LaO_3/0.1 *N* HNO_3, as described by Clegg *et al.* (18). Concentrations of Zn, Cu, and Mn, and Mg (Study 1 only), were determined in the diluted ashed samples by flame AAS. Certified reference solutions (1000 μg metal/ml) were used to generate standard curves for each element. A sample of NBS bovine liver (SRM 1577A) was included with the samples to ensure accuracy and reproducibility.

Statistics. For Study 1, a 2 × 3-factor ANOVA was used to evaluate the influence of treatment (control and diabetic) and diet (HCHM, HCAM, and HCLM), on all dependent variables. For Study 2, a 2 × 3-factor ANOVA was used to evaluate the influence of treatment (control and diabetic) and diet (HC, HF, and HP) on all dependent variables. Data were also analyzed by 1-factor ANOVA. Post-hoc tests were performed using Duncan's Multiple Range test. Probability values of ≤0.05 were considered significant. All statistical analyses were performed using SAS software (23). Data in the text and tables are means ± SE.

Results

Study 1. Although Day 0 body weights were similar among the groups (controls, 200 ± 2 g; diabetics, 199 ± 2 g), body weights of the diabetic rats were lower than those of controls from Day 3 to Day 14 at sacrifice. There was no effect of dietary mineral concentration on body weight in either group. The daily food intake of control and diabetic rats was similar for the first 4 days after injection (controls, 15.0 ± 0.6 g/day; diabetics, 15.3 ± 1.2 g/day). On Day 5, diabetic rats had higher food intakes (25.6 ± 1.0 g) than controls (16.4 ± 0.6 g). This hyperphagia persisted throughout the study. By Day 14, diabetic rats were consuming approximately twice as much food (37.6 ± 1.8 g) as the control rats (15.7 ± 0.7 g). There was no effect of diet on food intake of control or diabetic rats. On Day 14, plasma insulin concentrations were characteristically lower and hematocrits were higher in the diabetic rats than in control rats. There was no effect of dietary mineral concentration on plasma glucose and insulin concentrations or hematocrit values in either diabetic or control rats (Table II).

Plasma Zn and Cu concentrations, which were within normal ranges, were similar between diabetic and control animals, and were not affected by dietary mineral intake (data not shown). Plasma Mg concentrations were lower in the diabetic groups compared with control groups (Table II). The significant effect of treatment on plasma Mg was due to lower plasma Mg concentrations observed in diabetic rats fed the HCAM and HCLM diets. Plasma Mg concentrations were not statistically affected by diet in either group; however, in the diabetic group plasma Mg tended to increase as dietary Mg concentration increased.

Liver Zn concentrations were higher in diabetic rats than in control rats (Table III). When animals with similar dietary Zn intakes were compared, diabetic rats fed the HCAM diet had higher liver Zn concentrations than control rats fed HCHM diet. Liver Cu and Mn concentrations were higher in the diabetic rats compared with the control rats. Diabetes-associated increases in hepatic trace element concentrations were independent of dietary mineral intake. Thus, despite comparable intakes of Cu and Mn between (i) the diabetic rats fed the HCLM diet and the control rats fed the HCAM diet, and (ii) the diabetic rats fed the HCAM diet and the control rats fed the HCHM diet, diabetic rats had higher liver Cu and Mn concentrations than controls. Liver Mg concentrations were similar among the groups.

Study 2. Diabetic rats were hyperglycemic compared with control rats on Day 13 (data not shown). On Day 20, there were significant effects of treatment and diet, as well as an interaction between treatment and diet with regard to plasma glucose concentrations (Table IV). Diabetic rats fed the HF diet had the lowest and diabetic rats fed the HC diets had the highest plasma glucose concentrations among the diabetic groups.

Plasma insulin concentrations were characteristically lower in the diabetic rats than in control rats. Due to alterations in plasma insulin concentrations only among control groups, there was a significant interaction between treatment and diet with regard to plasma insulin concentration. Plasma glucagon concentrations were significantly affected by both treatment and diet, and there was an interaction between diet and treatment (Table IV). Plasma glucagon concentrations

Table II. Study 1: Plasma Glucose, Insulin and Mg Concentrations and Hematocrit Values of Control and Diabetic Rats

	Control			Diabetic			ANOVA, *P*-value Treatment (T) Diet (D) T × D
	HCHM (n = 7)	HCAM (n = 7)	HCLM (n = 7)	HCHM (n = 9)	HCAM (n = 8)	HCLM (n = 8)	
Glucose Day 14 (m*M*)	8.5 ± 0.2^{a}	7.8 ± 0.2^{a}	8.0 ± 0.3^{a}	23.6 ± 1.1^{b}	24.7 ± 1.0^{b}	22.2 ± 2.9^{b}	0.0020 NS NS
Insulin Day 14 (p*M*)	317 ± 77^{a}	175 ± 16^{a}	276 ± 56^{a}	58 ± 16^{b}	36 ± 8^{b}	68 ± 17^{b}	0.0000 NS NS
Hematocrit (BErcs)	0.42 ± 0.03^{a}	0.41 ± 0.04^{a}	0.41 ± 0.05^{a}	0.44 ± 0.07^{b}	0.43 ± 0.05^{a,b}	0.44 ± 0.09^{b}	0.0000 NS NS
Mg (μmol/l)	686 ± 56^{b,c}	724 ± 63^{c}	694 ± 43^{b,c}	624 ± 51^{a,b,c}	554 ± 52^{a,b}	505 ± 45^{a}	0.0022 NS NS

Note. Control and diabetic rats were fed one of three high-carbohydrate purified diets either adequate in Zn, Cu, Mn, and Mg (HCHM) or containing 50% (HCAM) or 25% (HCLM) the Zn, Cu, Mn, and Mg of the HCHM diet. Superscripts denote differences between groups as determined by post-hoc analysis. Means not sharing the same superscripts are significantly different at $P \leqslant 0.05$. NS, not significant.

Table III. Study 1: Liver Trace Element Concentrations of Control and Diabetic Rats

	Control			Diabetic			ANOVA, *P*-value Treatment (T) Diet (D) T × D
	HCHM (n = 7)	HCAM (n = 7)	HCLM (n = 7)	HCHM (n = 9)	HCAM (n = 8)	HCLM (n = 8)	
Liver Zn (nmol/g)	467 ± 46[a,b]	419 ± 43[a,b]	395 ± 10[a]	531 ± 31[b,c]	597 ± 48[c]	506 ± 21[a,b,c]	0.0075 NS NS
Cu (nmol/g)	72.4 ± 2.8[a]	77.6 ± 3.3[a]	78.7 ± 1.4[a]	118.9 ± 10.1[b]	127.9 ± 19.8[b]	113.6 ± 10.5[b]	0.0000 NS NS
Mn (nmol/g)	31.3 ± 4.0[a]	34.0 ± 2.4[a]	35.1 ± 2.9[a]	54.4 ± 4.2[b]	53.9 ± 4.6[b]	52.1 ± 4.2[b]	0.0000 NS NS
Mg (μmol/g)	7.3 ± 0.4	7.4 ± 0.4	7.6 ± 0.4	7.0 ± 0.4	7.4 ± 0.4	6.9 ± 0.5	NS NS NS

Note. Control and diabetic rats were fed one of three high-carbohydrate purified diets either adequate in Zn, Cu, Mn, and Mg (HCHM) or containing 50% (HCAM) or 25% (HCLM) the Zn, Cu, Mn, and Mg of the HCHM diet. Superscripts denote differences between groups as determined by post-hoc analysis. Means not sharing the same superscripts are significantly different at $P \leq 0.05$. NS, not significant.

Table IV. Study 2: Plasma Glucose, Insulin, and Glucagon Concentrations and Hematocrit Values of Control and Diabetic Rats

	Control			Diabetic			ANOVA, *P*-value Treatment (T) Diet (D) T × D
	HC (n = 10)	HF (n = 10)	HP (n = 10)	HC (n = 6)	HF (n = 11)	HP (n = 11)	
Glucose Day 20 (m*M*)	8.4 ± 0.3[a]	8.7 ± 0.2[a]	8.9 ± 0.2[a]	26.0 ± 1.3[b]	21.4 ± 1.1[c]	23.8 ± 0.8[d]	0.0001 0.0241 0.0086
Insulin Day 20 (p*M*)	402 ± 52[a]	309 ± 41[b]	245 ± 21[b]	38 ± 10[c]	80 ± 10[c]	63 ± 13[c]	0.0001 NS 0.0167
Glucagon Day 20 (ng/l)	90.0 ± 7.8[a]	97.4 ± 13.0[a]	125.3 ± 13.6[a]	321.2 ± 90.7[b]	144.7 ± 11.4[a]	265.2 ± 40.5[b]	0.0001 0.0198 0.0314
Hematocrit (BErcs)	0.42 ± 0.01	0.42 ± 0.01	0.43 ± 0.02	0.43 ± 0.00	0.44 ± 0.01	0.44 ± 0.00	NS NS NS

Note. Control and diabetic rats were fed a high-carbohydrate (HC) diet, a high-fat (HF) diet, or a high-protein (HP) diet for 20 days. Values shown are means ± SE. Superscripts denote differences between groups as determined by post-hoc analysis. Means not sharing the same superscripts are significantly different at $P \leq 0.05$. NS, Not significant.

were lower in the control rats and diabetic rats fed the HF diet compared with diabetic rats fed the HC and HP diets. Hematocrits were similar between control and diabetic rats.

Diabetic rats had lower body weights than controls from Day 3 on (Fig. 1A). Diet had a significant effect on body weight, with the control rats fed the HF diet having higher body weight than the control rats fed the HC and HP diets.

With regard to food intake (g/day), diabetic rats fed the HP diet had higher food intake than control rats, but lower food intake than diabetic rats fed the HC diet (Fig. 1B). Food intake of the diabetic rats fed the HF diet was similar to control rats. Among the control groups, caloric intake was similar. Diabetic rats fed the HC diet had higher caloric intake than all other groups (data not shown).

Dietary macronutrient composition had significant effects on body composition in both control and diabetic rats (Table V). While the control rats fed the HF diet had higher percent body fat than all other groups, diabetic rats fed the HC and HP diets had lower percent body fat than all other groups. Diabetic rats fed the HC and HP diets had higher percent protein than control rats. Although diabetic rats had higher percent total body water than control rats, all percentages were within normal ranges indicating adequate hydration in the diabetic groups.

Overall, diabetic rats had higher liver Zn and Cu concentrations than control rats (Table VI) and these

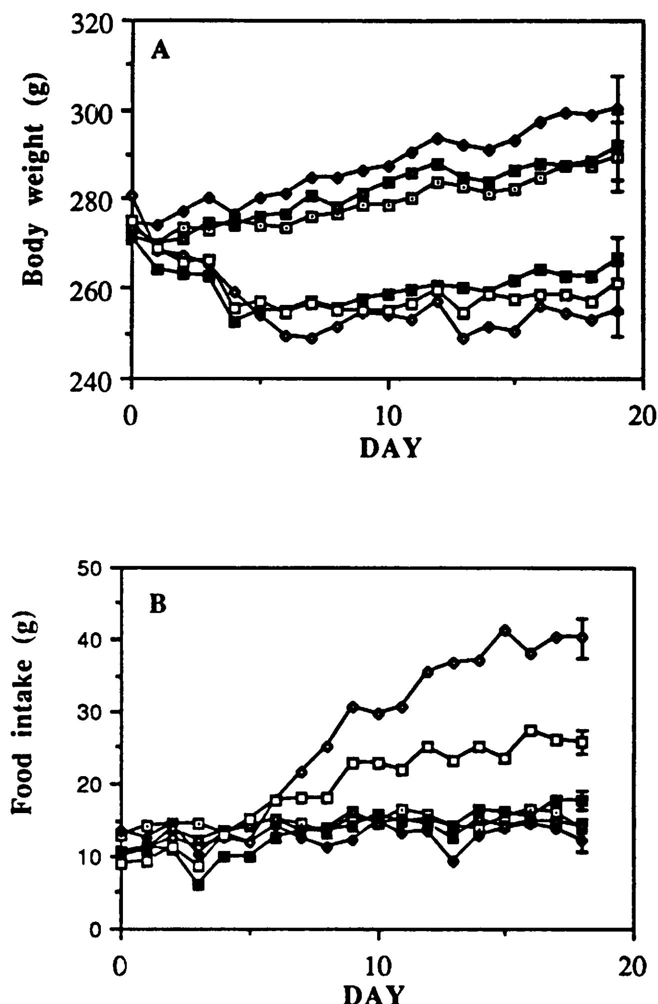

Figure 1. (A) Daily body weight (g) and (B) daily food intake (g) of control and diabetic rats fed diets of varying macronutrient composition. Values are mean ± SE. –⊡– Con High-CHO; –♦– Con High-Fat; –■– Con High-Pro; –♦– Diab High-CHO; –■– Diab High-Fat; –□– Diab High-Pro.

increased liver trace element concentrations were independent of gender (Study 1 versus Study 2). Diabetic rats fed the HC diet had higher liver Zn concentrations than control groups and the diabetic rats fed the HF diet. Diabetic rats fed the HC diet had higher liver Cu concentrations than all other groups. Diet significantly affected liver Mn concentrations. Liver Mn concentrations were higher in diabetic rats fed the HC and HP diets than in all other groups. Diabetic rats fed the HF diet had similar liver Mn concentrations to control rats.

Discussion

While alterations in dietary Mg concentrations did not significantly affect liver Mg concentrations in diabetic or control animals, diabetic animals fed the HCLM and HCAM diets had lower plasma Mg concentrations than controls. Hypomagnesemia has been reported in diabetic patients (5, 24) and experimentally induced diabetic animals (25), and hypermagnesuria has been reported in diabetic patients (5); thus, diabetics with low Mg intakes may be at risk for marginal Mg status. The significance of hypomagnesemia, hypermagnesuria, and Mg intake in diabetes has become a key concern (26) and deserves further study.

Johnson and Evans (7) reported that liver and kidney Zn and Cu concentrations were higher in diabetic rats compared with control rats with comparable Zn, Cu, and protein intakes. Similarly, when diabetic rats were fed a purified diet containing approximately 50% less Cu, Zn, Mn, and Fe than that of a purified diet adequate in all minerals, hyperphagic diabetic rats had significantly higher hepatic Zn, Cu, and Mn concentrations and higher kidney Cu concentrations than controls. This increase was independent of dietary trace element concentration (1). Restricting feeding of diabetic rats to the food intake level of nondiabetic controls resulted in higher tissue concentrations of Zn and Cu in the diabetic rats regardless of food restriction (6). These studies, Study 1 of this report, and previous reports that insulin-treated diabetic rats have normal levels of tissue trace elements (1, 4) support the concept that diabetic-induced alterations in trace element metabolism are not solely a function of increased mineral intake associated with hyperphagia. Instead, trace element metabolism in diabetes may be primarily affected by fuel metabolism. These results suggest that the tissue accumulation of trace elements in diabetic rats is independent of diabetic-associated hyperphagia.

With regard to liver Mn accumulation, Thompson *et al.* have recently reported that rats which were fed a Mn-deficient diet (1 μg Mn/g diet) for 8 weeks, made diabetic with STZ, and fed the Mn-deficient diet for 8 additional weeks had significantly lower liver Mn concentrations than diabetic rats fed a diet containing 45 μg Mn/g (27). It should be noted that the lowest dietary Mn concentration used in Study 1 of this report was 7 μg Mn/g diet. These data suggest that to reduce liver Mn accumulation, dietary intake must be severely restricted for a long period of time.

Dietary macronutrient modifications, such as high-protein and high-fat diets, have been previously shown to alter the metabolic profile of diabetic rats (14, 15, 17, 28). In addition, high-fat and high-protein diets alter the activities of key gluconeogenic/ureagenic enzymes (17) possibly in response to changes in the hormonal profile. Diabetic humans and rats have been shown to have alterations in glucocorticoid circadian rhythms (29–31), growth hormone (32, 33), thyroid status (34), glucagon secretion (35), and the capacity to oxidize and store exogenous carbohydrate (36). These metabolic aberrations can significantly affect trace element uptake and/or storage by stimulating enzymatic and/or storage protein synthesis (37). We

Table V. Study 2: Body Composition of Control and Diabetic Rats

	Control			Diabetic			ANOVA, *P*-value
	HC (*n* = 10)	HF (*n* = 10)	HP (*n* = 10)	HC (*n* = 6)	HF (*n* = 11)	HP (*n* = 11)	Treatment (T) Diet (D) T × D
Carcass (g)	232 ± 7[a]	243 ± 5[a]	232 ± 6[a]	185 ± 7[c]	209 ± 5[b]	189 ± 7[c]	0.0001 0.0115 NS
% Fat	12.2 ± 1.2[b]	16.4 ± 1.5[a]	11.5 ± 0.8[b]	4.3 ± 0.7[d]	11.5 ± 1.0[b]	7.7 ± 1.2[c]	0.0001 0.0001 NS
% Ash	4.3 ± 0.1	4.5 ± 0.2	4.9 ± 0.5	5.2 ± 0.1	4.5 ± 0.2	5.3 ± 0.2	NS 0.0519 NS
% Protein	21.7 ± 0.3[a]	21.8 ± 0.3[a]	21.6 ± 1.1[a]	24.6 ± 0.4[b]	22.7 ± 0.3[a,b]	24.7 ± 0.6[b]	0.0001 NS NS
% Body Water	61.6 ± 1.3[a]	57.4 ± 1.4[d]	62.0 ± 0.8[a]	66.2 ± 0.6[b]	61.2 ± 0.8[a]	62.4 ± 1.6[a]	0.0056 0.0019 NS

Note. Control and diabetic rats were fed a high-carbohydrate (HC) diet, a high-fat (HF) diet or a high-protein (HP) diet for 20 days. Values shown are means ± SE. Superscripts denote differences between groups as determined by post-hoc analysis. Means not sharing the same superscripts are significantly different at $P \leq 0.05$. Carcass, eviscerated body; NS, Not significant.

Table VI. Study 2: Liver Trace Element Concentrations of Control and Diabetic Rats

	Control			Diabetic			ANOVA, *P*-value
	HC (*n* = 10)	HF (*n* = 10)	HP (*n* = 10)	HC (*n* = 6)	HF (*n* = 11)	HP (*n* = 11)	Treatment (T) Diet (D) T × D
Liver Zn (nmol/g)	383 ± 24[a]	415 ± 23[a,c]	398 ± 26[a,c]	500 ± 30[b]	419 ± 32[a,c]	479 ± 16[b,c]	0.0034 NS NS
Cu (nmol/g)	73.6 ± 2.8[a]	76.8 ± 3.9[a]	72.8 ± 5.2[a]	104.5 ± 5.8[b]	79.2 ± 4.4[a]	87.3 ± 7.6[a]	0.0018 NS NS
Mn (nmol/g)	43.7 ± 3.4[a]	39.8 ± 2.8[a]	51.1 ± 2.8[a]	69.1 ± 8.4[b]	48.9 ± 4.1[a]	76.3 ± 4.6[b]	0.0001 NS NS

Note. Control and diabetic rats were fed a high-carbohydrate (HC) diet, a high-fat (HF) diet or a high-protein (HP) diet for 20 days. Values shown are means ± SE. Superscripts denote differences between groups as determined by post-hoc analysis. Means not sharing the same superscripts are significantly different at $P \leq 0.05$. NS, Not significant.

hypothesized that if the metabolic state of a diabetic rat is altered by macronutrient composition, the demand for trace elements as cofactors and components of metalloenzymes may be altered. For example, the need for Mn, a cofactor for arginase, pyruvate carboxylase, and phosphoenolpyruvate carboxylase, may be higher in high-carbohydrate- and high-protein-fed diabetic rats than in high-fat-fed diabetic rats. Thus, altered tissue mineral concentrations observed in diabetes may be due, in part, to the diabetic hormonal milieu secondary to changes in fuel metabolism.

In Study 2, the normalization of plasma glucagon concentrations and percent body fat of diabetic rats fed high-fat diets confirms that diabetic metabolism was altered. Schmidt *et al.* (28) reported that diabetic plasma glucose concentrations and urinary glucose concentrations were reduced and blood fatty acid and 3-hydroxybutyrate levels and urinary ketones were increased with high-fat feeding compared with high-carbohydrate feeding. High-carbohydrate-fed diabetic rats have increased activities of both arginase and PEPCK compared with diabetic rats fed high-fat diets (17). Bond and Failla have shown that diabetic and control rats have similar amounts of the arginase protein (38); however, the concentrations of Mn and arginase specific activity are higher in the livers of diabetic rats. Spolarics and Bond have shown that, while there were no marked changes in the physiochemical characteristics of diabetic rodent's arginase compared with nondiabetic controls, some changes were found in the interaction of arginase with Mn (39). Since liver Mn concentrations may regulate arginase activity and urea cycle flux may be dependent on the Mn concentrations, diabetic rats fed high-carbohydrate and high-protein diets may accumulate liver Mn due to high rates of gluconeogenesis and/or ureagenesis. In con-

trast, high-fat feeding may decrease diabetic-driven gluconeogenesis and ureagenesis, increase the oxidation of lipid fuels, and thus decrease Mn accumulation.

In summary, hyperphagic diabetic animals were characterized by elevated concentrations of liver Zn, Cu, and Mn when compared with control animals consuming equivalent amounts of Zn, Cu, and Mn. When diabetic fuel metabolism was altered by feeding high-fat and high-protein diets, liver Mn concentrations of the high-fat diabetic rats were similar to controls. Furthermore, liver Cu concentrations of diabetic rats fed high-fat and high-protein diets were similar to controls. Glucagon profiles were also normalized with high-fat feeding. Thus, the accumulation of metals in tissues of diabetic animals may be due to the metabolic state of the diabetic animal which is influenced by hormonal input. That these increased tissue concentrations of Zn, Cu, and Mn have been reported in spontaneously genetic diabetic rats (2) as well as experimentally induced diabetic animals (3) across a broad range of mineral intake suggests that the altered mineral metabolism may represent functionally important biochemical adaptations to the diabetic state and may be driven by the metabolic needs within specific tissues.

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