Effects of Selected Minerals on Leptin Secretion in Streptozotocin-Induced Hyperglycemic Mice

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The effects of lithium, magnesium, vanadate, and zinc on leptinemia and leptin secretion by adipose tissue were investigated in streptozotocin- (STZ) induced hyperglycemic mice. After the administration of studied minerals in drinking water for 4 weeks, fasting serum leptin concentrations were elevated, accompanied by normoglycemia in STZ-injected mice, regardless which mineral was provided ($P < 0.05$). However, the *in vitro* administration of lithium, magnesium, and vanadate did not significantly influence the leptin secretion of adipose tissue. A low zinc treatment (0.1 mM) augmented, whereas both a pharmacological treatment of zinc (1 mM) and zinc depletion (1 mM TPEN) attenuated, leptin secretion ($P < 0.05$). The present study shows that STZ-induced hyperglycemic mice have hypoleptinemia and reduced leptin secretion by adipose tissue. Moreover, these defects can be improved by a moderate zinc administration.

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Leptin is a polypeptide hormone mainly secreted by adipose tissue (1). It may act as a peripheral satiety factor to reduce appetite and adiposity (2). In addition, the hyperphagia in streptozotocin- (STZ) induced hyperglycemic animals has been shown attributed to hypoleptinemia (3). Exogenous leptin administration not only reduces hyperphagia, but also decreases hyperglycemia in these insulinopenic rats (4). Although leptin is secreted in proportion to the amount of body fat mass (5), a variety of hormonal factors (6, 7) such as insulin can also influence circulating leptin concentration and leptin secretion by adipose tissue.

Many trace elements such as lithium (8, 9), vanadate (10–12), and zinc (13–15) are known to have the insulinomimetic action of increasing peripheral glucose disposal. Magnesium, as a cofactor for enzymes that mediate carbohydrate metabolism, also takes part in glycemia homeostasis (16, 17). Furthermore, recent studies have shown that zinc-deficient subjects have hypoleptinemia (18, 19). Zinc supplementation can increase circulating leptin concentrations (19–21). It also augments leptin secretion by adipose tissue (22, 23).

To our knowledge, whether or not lithium, vanadate, and magnesium may also act like zinc to increase leptin secretion has not yet been examined. The present study was thus designed to investigate the *in vivo* and *in vitro* effects of these minerals on serum leptin concentrations and leptin secretion of adipose tissue in STZ-induced hyperglycemic mice. Moreover, the effects of zinc deficiency and zinc surplus on leptin secretion were assessed.

Materials and Methods

Animals. Male C57BL/6J mice were obtained from the National Laboratory of the Animal Breeding and Research Center (Taipei, Taiwan). Mice were kept in a temperature- and humidity-controlled room with a 12:12-hr light:dark cycle. Throughout the study, mice were provided standard laboratory chow *ad libitum*. Body weight and the amount of food intake and water intake (deionized water or mineral-added water) were recorded twice a week. This study was approved by the Animal Research Committee of the Taichung Veterans General Hospital. All chemicals and reagents, unless specifically indicated, were obtained from Sigma (St. Louis, MO).

***In Vivo* Study.** Mice at 6 weeks of age were made hyperglycemic by a single bolus intraperitoneal injection of STZ (100 mg/kg body wt) 2 weeks before the start of the experiments.

After establishing basal hyperglycemia (fasting blood sugar level greater than 250 mg/dl), STZ-induced hyperglycemic mice (8 weeks of age) were separated into five groups based on the supplementation of various studied minerals

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from drinking water (0.3 g LiCl/l, 0.3 g $MgSO_4$/l, 0.5 g Na_3VO_4/l, 0.3 g $ZnSO_4$/l, or none), respectively. Each group contained eight mice. The administered doses of minerals used in this study were consistent with the doses used previous studies (24, 25) that can significantly reduce insulinopenic hyperglycemia and have no effect on general health or taste acuity. After completing a 4-week period of experimentation with various mineral supplements, mice (12 weeks of age) were sacrificed by decapitation after a 12-hr overnight fast, and their trunk blood samples were collected.

***In Vitro* Study.** Thirty 8-week-old STZ-induced hyperglycemic mice (22.4 ± 0.5 g, as described above) and 20 age-matched normoglycemic control mice (26.4 ± 0.3) were used.

Excised epididymal adipose tissue (0.62 ± 0.21 and 0.36 ± 0.12 g/mouse for normal mice and STZ-diabetic mice, respectively) was immediately placed in ice-cold Krebs-Ringer-HEPES buffer. After removing the blood vessels and connective tissues, adipose tissue was washed and cut into small pieces. Pooled adipose tissue fragments (from three hyperglycemic mice or from two control mice) were equally separated into eight tubes (100–200 mg tissue per tube) and flushed with 1.0 ml of Dulbecco's Modified Eagle medium (DMEM, containing 25 mM glucose, into which 0.5% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate were added), respectively. Although DMEM contains no zinc, the culture medium in this study contained about 0.1 μM zinc contributed from fetal calf serum analyzed by an atomic absorption spectrophotometer. After preincubation (1 hr at 37°C), the medium in each tube was removed and re-flushed with 1.0 ml of fresh medium containing the studied chemicals. These studied chemicals included zinc (0.1 or 1.0 mM as $ZnSO_4$), TPEN (N,N,N′,N′-tetrakis-(2-pyridyl-methyl)-ethylenediamine, 1.0 mM), lithium (1.0 mM as LiCl), magnesium (5 mM as $MgSO_4$), and vanadate (0.1 or 1 mM as Na_3VO_4). For STZ-induced hyperglycemic mice or normoglycemic control mice, there were 10 observations for each studied chemical. The administered doses of zinc and vanadate were selected in agreement with doses in previous studies that can markedly enhance glucose uptake and lipogenesis of cultured adipocytes (10, 12, 15, 26). TPEN, a lipid-soluble cell-permeable zinc chelator (27), was used to induce a zinc-depleted condition (15). The prepared adipose tissue fragments showed greater than 96% viability at least 24 hr after, with or without the addition of studied chemicals. The medium collected at 24 hr after the initiation of incubation was taken for the measurements of lactate, leptin, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα). The amount of glucose uptake by the adipose tissue was determined by calculating the change in glucose concentration in the medium before and after incubation.

Biochemical Measurements. Glucose concentration was measured by the glucose oxidase method with an automated glucose analyzer (A&T, Tokyo, Japan). The determinations of triacylglycerol and lactate were performed spectrophotometrically. Free fatty acid concentration was measured by using a commercial kit obtained from Boehringer Mannheim (Mannheim, Germany). The measurements of insulin (Crystal Chem Inc., Chicago, IL), leptin, IL-1α, IL-1β, IL-6, and TNFα (R&D Systems, Minneapolis, MN) were performed by using the method of enzyme-linked immunoabsorbent assay with commercial kits according to the manufacturer's instructions, respectively.

Statistical Analysis. All data is expressed as mean ± SE. All analyses used $P < 0.05$ as the minimal criterion of statistical significance. Comparisons of the determined variables among groups were conducted by using analysis of variance (ANOVA). When ANOVA showed a significant difference, Tukey's multiple comparison test was used to define differences among the groups.

Results

***In Vivo* Study.** In agreement with pervious studies, STZ-hyperglycemic animals had increased food intake and water intake, but a decreased gain in body weight and body fat content than that of their normoglycemic controls (data not shown). As expected, STZ-injected mice had hyperglycemia (302 ± 14 vs. 104 ± 5 mg/dl, $P < 0.05$), hypertriglyceridemia (347 ± 42 vs. 170 ± 13 mg/dl, $P < 0.05$), and hypoinsulinemia (229 ± 59 vs. 433 ± 55 pg/ml, $P < 0.05$). STZ-hyperglycemic mice also had hypoleptinemia compared with normoglycemic control mice (0.26 ± 0.06 vs. 0.69 ± 0.15 ng/ml, $P < 0.05$). In addition, serum values of lactate and cytokines (IL-1α, IL-1β, IL-6, and TNFα) did not significantly differ between STZ-hyperglycemic mice and normoglycemic mice (data not shown).

Neither body weight gain nor body fat content of STZ-hyperglycemic mice was affected by any studied mineral (data not shown). Food intake (–0.3 vs. 0.1 g/day) and water intake (–0.9 vs. 0.9 ml/day) were significantly reduced only in vanadate-treated STZ-hyperglycemic mice. In accordance with previous studies, each studied mineral significantly alleviated STZ-induced hyperglycemia (Table I). Moreover, all of the studied minerals significantly increased the fasting serum leptin concentrations of STZ-hyperglycemic mice. The other determined serum variables (triacylglycerol, lactate, insulin, and cytokines) of STZ-hyperglycemic mice were not significantly affected by the studied minerals.

***In Vitro* Study.** Table II shows that STZ-hyperglycemic mice had reduced glucose uptake and leptin secretion by adipose tissues, and increased lactate and IL-6 production compared with that of normoglycemic mice.

An addition of small amount of zinc (0.1 mM) significantly increased glucose uptake (Table II) and leptin secretion (Fig. 1) by adipose tissue taken from either STZ-hyperglycemic mice or normoglycemic mice. Figure 1 also shows that leptin secretion of adipose tissue was significantly attenuated by a higher amount of zinc (1.0 mM) and by zinc depletion (1.0 mM TPEN). Table II also shows that

Table I. The Effects of Various Mineral Treatments on Determined Serum Variables in STZ-Induced Hyperglycemic Mice

	STZ	STZ + Li	STZ + Mg	STZ + V	STZ + Zn
Glu (mg/dl)	302 (14)	152 (13)[a]	157 (13)[a]	136 (11)[a]	137 (12)[a]
Ins (pg/ml)	229 (59)	268 (56)	196 (57)	124 (113)	247 (64)
Lep (ng/ml)	0.26 (0.06)	0.48 (0.14)[a]	0.56 (0.08)[a]	0.61 (0.18)[a]	0.71 (0.10)[a]

Note. Data is given as mean ± SE of eight mice. Glu, glucose; Ins, insulin; Lep, leptin.
[a] vs STZ, $P < 0.05$.

Table II. The Effects of Various Mineral Treatments on Glucose Uptake, Lactate, IL-6, and TNFα Productions by Adipose Tissues Taken from Normoglycemic Mice and STZ-Induced Hyperglycemic Mice

	Blank	0.1 mM Zn	1 mM Zn	1 mM TPEN	0.1 mM V	1 mM V
				Normoglycemic		
Glu (μg/g)	2628 (198)	4212 (450)[a]	2394 (378)	1692 (198)[a]	4554 (792)[a]	5076 (342)[a]
Lac (μg/g)	801 (239)	941 (122)	1706 (320)[a]	698 (275)	1724 (230)[a]	1859 (311)[a]
IL-6 (ng/g)	28.2 (7.3)	30.3 (3.3)	1.2 (0.7)[a]	3.1 (0.8)[a]	25.6 (4.1)	27.7 (9.0)
TNFα (ng/g)	0.91 (0.33)	0.90 (0.13)	0.74 (0.14)	0.90 (0.13)	0.99 (0.15)	0.79 (0.11)
				Hyperglycemic		
Glu (μg/g)	1620 (432)	2430 (432)[a]	1872 (738)	1224 (360)[a]	2718 (720)[a]	2790 (414)[a]
Lac (μg/g)	1121 (252)	1224 (387)	1593 (338)[a]	1013 (257)	1629 (450)[a]	2079 (680)[a]
IL-6 (ng/g)	37.8 (0.9)	37.2 (1.6)	2.9 (1.6)[a]	3.6 (1.6)[a]	42.7 (1.8)	36.3 (4.5)
TNFα (ng/g)	1.11 (0.27)	1.28 (0.34)	0.85 (0.16)	0.88 (0.34)	1.39 (0.80)	0.90 (0.35)

Note. Data are given as mean ± SE of 10 observations. Glu, glucose uptake; Lac, lactate production; IL-6, interleukin-6 production; TNFα, tumor necrosis factor-alpha production.
[a] The value is significantly different ($P < 0.05$) when compared with that of blank control, respectively.

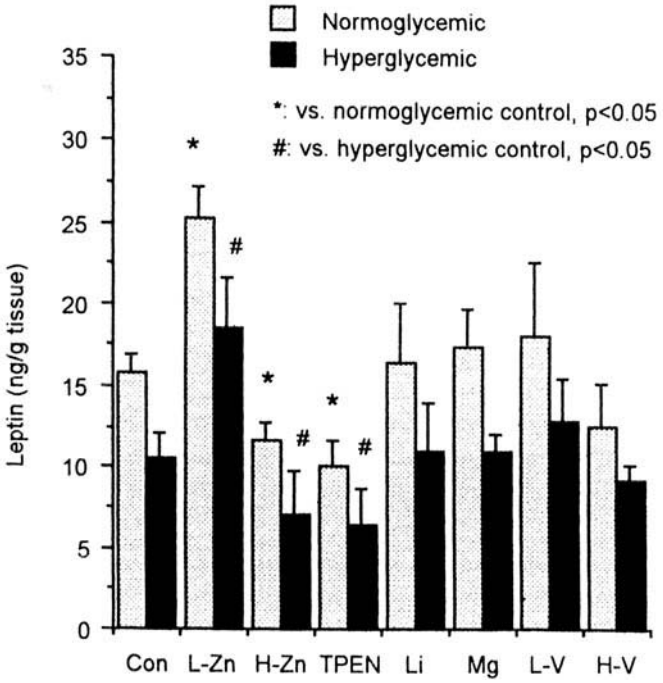

Figure 1. The effect of studied chemical on leptin secretion of adipose tissue into culture medium was measured. Con, blank control; L-Zn, 0.1 mM zinc; H-Zn, 1 mM zinc; TPEN, 1 mM TPEN; Li, 1 mM lithium; Mg, 5 mM magnesium; L-V, 0.1 mM vanadate; H-V, 1 mM vanadate.

a higher amount of zinc significantly increased lactate production. Moreover, zinc depletion markedly reduced glucose uptake by adipose tissues from all mice.

Lithium, magnesium, and vanadate did not significantly influence leptin secretion of adipose tissue (Fig. 1). In addition, lithium and magnesium did not affect the glucose uptake and lactate production of adipose tissues taken from all mice (data not shown). Table II shows that vanadate significantly increased the glucose uptake and lactate production of adipose tissues taken from all mice.

TNFα values did not significantly differ between hyperglycemic mice and normoglycemic mice, or among groups with or without the studied chemicals. Moreover, both high zinc administration and zinc depletion significantly reduced IL-6 production of adipose tissues derived from all mice (Table II).

After pooling all the data, leptin secretion of adipose tissue was positively correlated with the amount of glucose uptake ($r = 0.62$, $P = 0.01$). In addition, medium leptin concentrations were inversely correlated with the value of lactate production/glucose uptake (%, the conversion rate of lactate from glucose, $r = -0.77$, $P = 0.001$).

Discussion

In addition to hyperphagia, insulinopenic hyperglycemic rodents are also known to have a high central responsiveness to neuropeptide Y (28), a potent orexigenic agent whose activity can be suppressed by leptin (29). In this study, STZ-hyperglycemic mice had hypoleptinemia and reduced leptin secretion of adipose tissue. As suggested by previous studies (3, 4), a reduced leptin activity might explain why insulinopenic hyperglycemic rodents exhibit hyperphagia and have high central neuropeptide Y expression. Because insulin is known as a stimulant to increase circu-

lating leptin concentration (6, 7), the hypoleptinemia shown in STZ-hyperglycemic mice should be attributed to STZ-induced hypoinsulinemia. Moreover, the contribution of increased glucose uptake into adipose tissue in augmenting leptin secretion is well noted (7, 30). Because the amount of glucose uptake of adipose tissues from STZ-hyperglycemic mice was markedly reduced as opposed to that in normoglycemic mice, it seems that the defective glucose utilization of adipose tissue may also contribute to the manifestation of hypoleptinemia in STZ-hyperglycemic mice. Although less epididymal fat content in the STZ-hyperglycemic mice might also be a factor contributing to a lower leptin level, a decreased leptin secretion from a given weight of fat indicated that the hypoleptinemia in our STZ mice was more of a function of the glucose uptake rather than the amount of the adipose tissue.

Some cytokines like TNFα and IL-1 have been shown to increase circulating leptin concentrations (7, 31). In this study, the serum values of cytokines (TNFα, IL-1α, IL-1β, and IL-6) did not significantly differ between STZ-hyperglycemic mice and normoglycemic mice. These results also indicate that hypoleptinemia of STZ-hyperglycemic mice is not due to changes in cytokinemia.

As expected, STZ-induced hyperglycemia was significantly decreased after the administration of the studied minerals. The antihyperglycemic effect of these minerals (24, 25) has been supposed to be attributed to the increment in hepatic glycogen synthesis, peripheral tissues' glycolysis, and dephosphorylation of postinsulin receptor signal substrates. However, the detailed mechanisms of these minerals on glycemia homeostasis are still largely unknown. Nevertheless, there is no doubt that the supplementation of these minerals can effectively alleviate hyperglycemia (9, 11, 12, 15, 17). Moreover, exogenous leptin administration has been shown to significantly decrease STZ-induced hyperglycemia (3, 4). Because serum leptin concentrations were elevated in STZ-hyperglycemic mice after the administration of the studied minerals, it seems reasonable to suppose that the antihyperglycemic effect of the studied minerals might be also attributable to the hyperleptinemia induced by studied minerals.

In this study, only moderate zinc administration significantly increased glucose uptake and leptin secretion by adipose tissue. Moreover, as is consistent with a previous study (22), zinc deficiency attenuated leptin secretion of adipose tissue. This effect might be due to the decreased glucose uptake induced by zinc deficiency. A high dose of zinc has been shown to increase cellular lactate production (32). In this study, 1 mM zinc treatment also significantly reduced leptin secretion and increased lactate production. Both zinc deficiency (decreased glucose uptake) and a high zinc treatment (increased lactate production) decreased leptin secretion. These results suggest that changes in glucose uptake and lactate production by adipose tissue may influence leptin secretion.

Surprisingly, lithium and magnesium did not significantly influence glucose uptake or lactate and leptin secretion by adipose tissues in this study, though they have been previously shown to augment glucose uptake of adipose tissue (8, 16). Because glucose uptake by adipose tissue can be stimulated only by the addition of higher amount of lithium (5 mM, five times that used in this study) (33), the discrepancy might be due to the lower dose used in this study. Moreover, vanadate did not significantly influence leptin secretion in this study. Although it can be expected that vanadate can increase glucose uptake of adipose tissue (10, 12), in this study vanadate also markedly augmented lactate production by adipose tissues. Thus, the increased lactate/glucose conversion rate may be used to explain why vanadate cannot significantly increase leptin secretion by adipose tissues. Although lithium, magnesium, and vanadate did not directly augment leptin secretion of adipose tissue, an elevated leptinemia was observed in STZ-hyperglycemic mice after the supplementation of these minerals. It seems that this discrepancy may be due to a long-term improvement of mineral-induced changes in peripheral glucose disposal and fuel utilization.

Adipose tissue has been shown to secrete TNFα and IL-6 (34, 35). In this study, these cytokines were also measured. Adipose tissue TNFα production did not significantly differ among groups with or without the administration of the studied chemicals. The administrations of 1 mM zinc (high zinc treatment) and 1 mM TPEN (zinc depletion) significantly reduced IL-6 production by adipose tissues. There is no correlation between IL-6 production and other determined variables, such as glucose uptake and the levels of lactate, TNFα, and leptin. The reason for the decrement in IL-6 production by a high zinc administration and by a zinc-depleted treatment is not clear. The *in vitro* data also indicates that the changes in leptin secretion are not associated with the paracrine effect of these cytokines.

In summary, the present study shows that STZ-induced hyperglycemic mice had hypoleptinemia and reduced leptin secretion of adipose tissue. Among these minerals that have an insulin-like action, only zinc significantly increases both leptinemia and leptin secretion of adipose tissue. The antihyperglycemic effect of zinc on STZ-hyperglycemic mice may also be partly attributed to the increased leptinemia induced by the mineral.

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