

Glucagon and insulin have opposite effects on tissue chromium distribution in an obese mouse model

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ABSTRACT

Aims/Introduction: Previous studies have suggested that chromium (Cr) is an essential cofactor for normal carbohydrate metabolism and affects insulin sensitivity, especially in rodent models. Several factors, such as insulin challenge, high carbohydrate intake, and response to stress (e.g., in obesity), alter Cr excretion or distribution. Glucagon is known to regulate carbohydrate metabolism and hyperglucagonemia plays a role in the development of hyperglycemia in diabetic subjects.

Materials and Methods: In the present study we investigated possible modulation of Cr levels by glucagon using an obese mouse model. Mice were kept on a high-fat diet and then used as an obesity model. These obese mice were injected with one dose of glucagon or insulin and Cr levels in their tissues were determined.

Results: In obese mice, glucagon challenge significantly increased Cr levels in bone but decreased them in the fat and liver. In contrast, insulin challenge significantly decreased Cr levels in bone but increased them in the fat, liver and muscle.

Conclusions: The results show that glucagon and insulin have opposite effects on Cr levels in bone, fat, liver, and muscle. (*J Diabetes Invest*, doi: 10.1111/jdi.12097, 2013)

KEY WORDS: Chromium, Glucagon, Insulin

INTRODUCTION

Trivalent chromium (Cr) is considered an essential cofactor for normal carbohydrate, lipid, and protein metabolism¹. For example, previous studies have suggested the importance of Cr as a cofactor for effective insulin responses, and have demonstrated that Cr is transported to tissues to bind chromodulin and form a biologically active compound^{2,3}. Thus, it is important to understand tissue Cr distribution.

Some studies have demonstrated that hyperglucagonemia, or an elevated glucagon-to-insulin ratio, plays an important role in the development of hyperglycemia in diabetic subjects^{4,5}. Treatment to reduce glucagon levels has been shown to reduce blood glucose levels and alleviate symptoms of hyperglycemia^{6,7}, thus confirming the importance of glucagon in diabetes. However, the exact mechanisms of action of glucagon and its possible effects on Cr levels in diabetic or obese subjects remain unclear. Conversely, high carbohydrate intake and some stress factors have been reported to promote Cr excretion in the urine^{8,9}. Therefore, in the present study we evaluated the role of glucagon in modulation of Cr levels in obese mice. Our results

should improve the current understanding of the role of glucagon in modulation of Cr levels.

MATERIALS AND METHODS

Cr-containing milk powder

Powder containing 325 p.p.m. trivalent Cr, Cr chloride hexahydrate, lactoferrin, whey protein concentrate, and powdered milk (1:6:200:393) was provided by Maxluck Biotechnology Corporation (Taipei, Taiwan)¹⁰. This milk powder has been examined and shown to be biologically active in a previous study¹⁰.

Animals, diet, and tissue preparation

Fifty-two male C57BL/6JNarl mice were maintained at a constant temperature of $22 \pm 2^\circ\text{C}$ and under a 12-h light–dark cycle, with free access to food and water. Mice were divided into the following four experimental groups on the basis of the type of chow fed: (i) normal chow (5008 Rodent LabDiet; PMI Nutrition International, St Louis, MO, USA); (ii) high-fat chow (high-fat Rodent TestDiet; PMI Nutrition International; 67% of calories provided by fat); (iii) high-fat chow supplemented with placebo milk; and (iv) high-fat chow supplemented with Cr-containing milk (40 $\mu\text{g}/\text{kg}$ bodyweight per day). Mice were fed high-fat chow for 4 weeks to obtain a diet-induced obesity model¹¹. The obese mice were further divided into two groups: one group was fed placebo milk and the other was fed Cr-containing milk daily by oral gavage for another 4 weeks. Mice were anesthetized and then killed by cervical dislocation,

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and bone, epididymal fat, liver, and gastrocnemius muscle were harvested and stored at -20°C until analysis. In all animal experiments, the authors adhered to the guidelines for the Care and Use of Laboratory Animals, as recommended by the Taiwanese Government. Note, all animal tests described below were performed at the end of the feeding protocol just before mice were killed.

Insulin or glucagon challenge

After 8 weeks, mice were injected with glucagon (200 $\mu\text{g}/\text{kg}$, i.p.; Sigma, St Louis, MO, USA), insulin (2 IU/kg, i.p.; Lilly, Indianapolis, IN, USA), or saline (control group). Blood samples were collected 1 h after injection and blood glucose levels were determined.

Biochemical analyses

After 8 weeks feeding, blood samples were collected from overnight-fasted mice. Blood glucose was measured using a glucometer (One Touch II; LifeScan, Milpitas, GA, USA). Serum insulin and glucagon concentrations were measured using commercially available ELISA kits, namely the Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Chicago, IL, USA) and the Glucagon Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA), according to the manufacturers' instructions.

Cr analysis

Tissue concentrations of Cr were determined as reported previously¹⁰. Briefly, tissue samples were digested in 65% nitric acid and heated at 65°C for 1 h. Cr levels were determined using graphite furnace atomic absorption spectrophotometry (Hitachi Z-2000 series polarized Zeeman atomic absorption spectrophotometer; Hitachi Co. Ltd, Tokyo, Japan).

Glucose tolerance test

Mice were subjected to a glucose tolerance test as described previously¹². Briefly, mice were fasted overnight and then injected with glucose (0.5 g/kg, i.p.). Blood samples were collected from the tail vein at 0, 30, 90 and 180 min after injection, and blood glucose concentrations determined by the glucose oxidase reaction.

Statistical analysis

Data are expressed as mean \pm SD. The significance of differences was evaluated using Student's *t*-test in Microsoft EXCEL (Microsoft, Richmond, WA, USA). $P < 0.05$ was considered significant.

RESULTS

Effects of a high-fat diet on Cr distribution in mice

As indicated in Figure 1a, all mice became obese after feeding of the high-fat chow, and the blood glucose levels in these obese mice tended to be slightly higher than those in mice fed normal chow (Figure 1b). In addition, Cr levels in the blood of

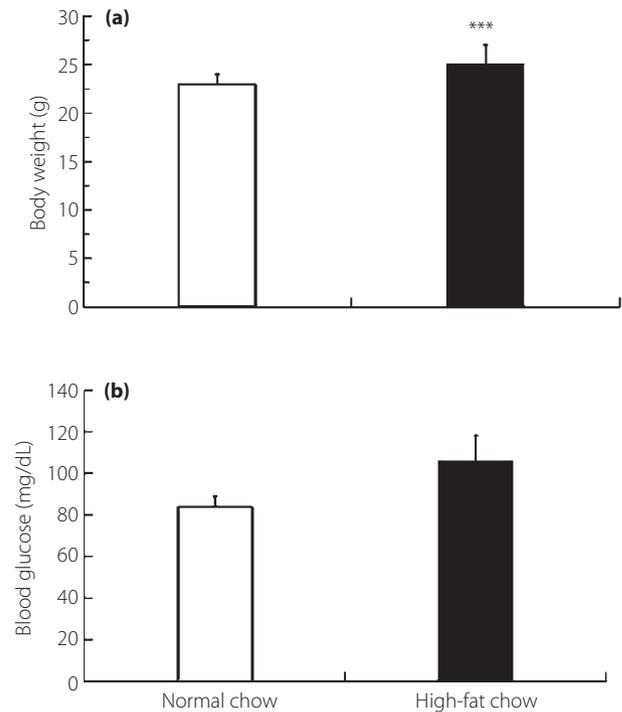


Figure 1 | High-fat diet induced obesity. (a) Body weight and (b) blood glucose were measured in mice fed normal chow ($n = 10$) or high-fat chow ($n = 42$) for 8 weeks. Data are the mean \pm SD. * $P < 0.05$, *** $P < 0.001$ compared with normal chow.

Table 1 | Chromium levels in different tissues in mice fed either normal or high-fat chow for 8 weeks

	Chromium (p.p.b.)	
	Normal chow	High-fat chow
Blood	165 \pm 11	243 \pm 60*
Bone	139 \pm 25	94 \pm 32*
Fat	32 \pm 2	27 \pm 4*
Liver	35 \pm 3	30 \pm 4*
Muscle	35 \pm 2	28 \pm 6*

Data are the mean \pm SD. * $P < 0.05$ compared with mice fed normal chow.

obese mice were significantly higher than those in mice fed normal chow (Table 1). In contrast, Cr levels in the bone, fat, liver, and muscle of obese mice were significantly lower than those in mice fed normal chow (Table 1). These findings confirm that a high-fat diet has a negative effect on Cr accumulation in selected tissues.

Effects of glucagon or insulin on blood glucose, serum glucagon, and insulin in obese mice

In obese mice, 4 weeks of placebo or chromium supplementation had a negligible effect on blood glucose levels (Table 2). One hour after bolus injection of glucagon, blood glucose levels

Table 2 | Blood glucose levels after saline, glucagon or insulin challenge in obese mice supplemented with placebo or chromium-containing milk

	Saline challenge		Glucagon challenge		Insulin challenge	
	Placebo	Chromium	Placebo	Chromium	Placebo	Chromium
Blood glucose (mg/dL)						
Baseline	104.7 ± 14.4	100.1 ± 5.1	102.3 ± 9.4	99.3 ± 10.4	110.9 ± 11.1	102.0 ± 15.6
1 h	106.0 ± 12.4	105.7 ± 9.4	140.1 ± 16.6*	134.3 ± 26.4*	53.1 ± 5.6*	54.7 ± 6.1*

Data are the mean ± SD. * $P < 0.05$ compared with baseline. Mice were fed high-fat chow for 4 weeks and then fed with the same diets supplemented with chromium-containing or placebo milk for an additional 4 weeks. These mice were then injected intraperitoneally with saline, glucagon or insulin ($n = 7$ in each group) and blood glucose levels were determined 1 h later.

increased; in contrast, insulin injections decreased blood glucose levels (Table 2). These observations were seen regardless of chromium supplementation. Bolus glucagon injection increased circulating glucagon levels, but had a little effect on insulin. However, bolus injection of insulin increased circulating levels of both glucagon and insulin. The effects of glucagon and insulin injections did not change after chromium supplementation (Table 3).

Effects of glucagon or insulin on Cr distribution in obese mice

To investigate the role of glucagon in modulation of tissue Cr levels in obese mice, the effects of glucagon challenge on tissue Cr distribution were analyzed. For comparison, one experimental group was challenged with insulin. As shown in Figure 2, glucagon challenge significantly increased Cr levels in bone, but decreased Cr levels in fat and liver. In contrast, insulin challenge significantly decreased Cr levels in bone, but increased them in fat, liver, and muscle. These findings show that glucagon and insulin have opposite effects on Cr levels in these selected tissues. Similar results have been found using other obese (B6.V-*Lep^{ob/fj}*) and diabetic (BKS.Cg-*Lep^{db/+Lep^{db}}*) mouse models (C-C Sun, unpubl. obs., 2006).

The above data demonstrate that glucagon has opposite effects on Cr accumulation to insulin. Previously, we showed that Cr supplementation effectively increased Cr levels in serum, muscle, and fat¹⁰. Then, we were interested to investigate whether dietary Cr supplementation was capable of reversing tissue Cr levels in obese animals. In preliminary studies, the change in tissue chromium was most obvious in the 1 h after glucagon and

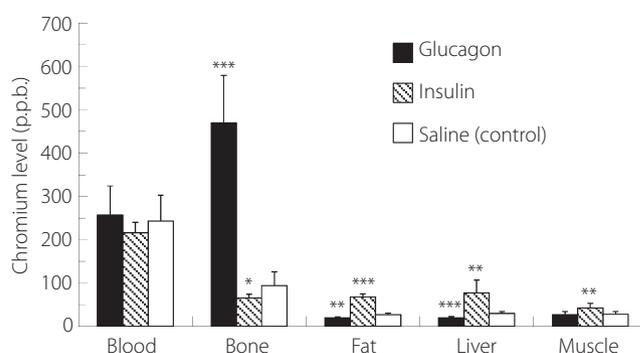


Figure 2 | Glucagon and insulin have opposite effects on tissue chromium (Cr) distribution in obese mice. Mice were fed high-fat chow for 8 weeks and then injected intraperitoneally with saline (control; $n = 7$), glucagon ($n = 7$), or insulin ($n = 7$). Tissues samples were harvested and Cr levels measured 1 h after injection. Values are the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the saline control.

insulin challenge. Moreover, tissue chromium levels were evaluated in obese mice 4 h after challenge with saline, glucagon, and insulin. Chromium supplementation significantly increased chromium levels in fat 4 h after saline, glucagon, and insulin challenge (data not shown). Thus, we investigated chromium tissue levels 1 h after glucagon and insulin challenge. The Cr supplementation protocol used in the present study has been reported previously to effectively increase Cr levels in serum, muscle, and fat¹⁰. In saline-challenged mice, chromium

Table 3 | Serum levels of glucagon and insulin after saline, glucagon or insulin challenge in obese mice supplemented with placebo or chromium-containing milk

	Saline challenge		Glucagon challenge		Insulin challenge	
	Placebo	Chromium	Placebo	Chromium	Placebo	Chromium
Glucagon (pg/mL)	194.3 ± 58.8	138.4 ± 35.5	333.8 ± 26*	195.5 ± 49.2	259.4 ± 3.5	182.5 ± 62.7
Insulin (pg/mL)	303 ± 42.8	275.7 ± 24.7	332.5 ± 53.5	250.0 ± 42.8	3871.5 ± 44.5*	2416 ± 592*

Data are the mean ± SD. * $P < 0.05$ compared with saline challenge. Mice were fed high-fat chow for 4 weeks and then fed with the same diets supplemented with chromium-containing or placebo milk for an additional 4 weeks. These mice were then injected intraperitoneally with saline, glucagon or insulin ($n = 7$ in each group) and serum levels of glucagon and insulin were determined 1 h later.

supplementation significantly increased Cr levels in fat, liver, and muscle compared with the placebo group (Table 4). As indicated in Table 4, Cr levels in fat and liver of glucagon-challenged mice increased significantly after administration of additional dietary Cr. Moreover, Cr levels in insulin-challenged mice increased with the administration of Cr supplements. Relative variations in Cr levels in the tissues listed in Table 4 were also calculated; Cr levels in a given tissue from the control group (supplemented with placebo milk and challenged with either glucagon or insulin) were subtracted from Cr levels in the same tissue from Cr-supplemented groups (challenged with glucagon or insulin), and the result was divided by Cr levels in the same tissue from the control group. Administration of a Cr supplement to glucagon-challenged mice increased Cr levels in fat, liver, and muscle by >30%, and slightly increased Cr levels in fat, liver, and muscle by 20–30% in insulin-challenged mice.

DISCUSSION

The results of the present study show that a high-fat diet has a negative effect on Cr accumulation in tissues. In a recent report¹³, Cr was shown to be a non-essential trace element in terms of body composition, glucose metabolism, and insulin sensitivity in a rodent model. The authors also concluded that previously reported results of Cr supplementation should be treated as pharmacological effects¹³. However, it is believed that Cr should be considered a cofactor, especially in individuals with certain disorders. In support of this, Cr has been shown to activate glucose transporter four trafficking and enhance insulin-stimulated glucose transport in 3T3-L1 adipocytes¹⁴. Moreover, in our rodent models, Cr supplementation has attenuated hepatic damage in a rat model of chronic cholestasis¹⁵ and has helped attenuate high-fat diet-induced non-alcoholic fatty liver disease in KK/HIJ mice¹⁶. Furthermore, Cr supplementation enhances insulin signaling in skeletal muscle in an obese KK/HIJ diabetic mouse model¹⁰. Collectively, the data suggest that a high-fat diet could contribute to the detrimental

effects caused by changes in Cr accumulation within insulin-sensitive tissues. Of note, the present study has demonstrated that mice fed a high-fat diet have decreased Cr levels in bone compared with normal healthy mice. The role of Cr in bone is unclear and needs to be investigated further. In the present study, plasma insulin levels in mice fed the normal diet and high-fat diet were 420 ± 153 and 303 ± 43 pg/mL, respectively. Insulin measurements revealed that feeding of the high-fat diet did not cause hyperinsulinemia. The homeostatic Cr levels in tissues were produced by the interplay between the high-fat diet and insulin, and favored the former. After injection of a bolus of insulin, plasma insulin levels increased to 3875 ± 45 pg/mL. We believe that these high insulin levels override the effects of the high-fat diet on chromium mobilization and reversed tissue Cr levels in obese animals.

Our results demonstrate that glucagon and insulin challenge have opposite effects on tissue Cr distribution in obese mice. It has been shown that physiological insulin indirectly promotes Cr mobilization to insulin-sensitive tissues, possibly in association with the effect of insulin on glucose transport^{17–20}. In agreement with these findings, the present study shows that insulin injection promotes Cr accumulation in fat, liver, and muscle. Intriguingly, glucagon is a counterregulatory hormone to insulin, and the present study provides interesting data on modulation of Cr levels by glucagon and insulin. As mentioned above, chromium uptake is glucose dependent in insulin-sensitive tissues. Therefore, the effects of both insulin and glucagon on tissue Cr distribution may all be secondary to the effects of these hormones on glucose uptake in liver, muscle, and fat.

Previous studies have reported that hyperglucagonemia, or an elevated glucagon-to-insulin ratio, plays an important role in the development of hyperglycemia in diabetes^{4,5}. Hyperglucagonemia destabilizes normal blood glucose control mostly because of glucagon-mediated increases in blood glucose levels. In the present study, we have shown that the effect of glucagon on Cr levels in tissues may also contribute, in part,

Table 4 | Tissue chromium levels after saline, glucagon or insulin challenge in obese mice supplemented with placebo or chromium-containing milk

	Chromium (p.p.b.)					
	Saline challenge		Glucagon challenge		Insulin challenge	
	Placebo	Chromium	Placebo	Chromium	Placebo	Chromium
Blood	243 ± 60	256 ± 58	257 ± 67	267 ± 53	216 ± 24	234 ± 19†
Bone	94 ± 32	245 ± 144*	469 ± 110	306 ± 98*	65 ± 9	56 ± 12
Fat	27 ± 4	34 ± 10	20 ± 2	28 ± 4***	68 ± 7	91 ± 31†
Liver	30 ± 4	44 ± 12*	20 ± 3	26 ± 6*	77 ± 30	96 ± 34†
Muscle	28 ± 6	38 ± 9*	27 ± 7	36 ± 10†	40 ± 11	42 ± 11

Data are the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with placebo; † $P < 0.1$ compared with placebo. Mice were fed high-fat chow for 4 weeks and then fed with the same diets supplemented with chromium-containing or placebo milk for an additional 4 weeks. These mice were then injected intraperitoneally with saline, glucagon or insulin ($n = 7$ in each group) and tissue chromium levels were determined 1 h later.

to impairment of blood glucose control in terms of Cr modulation by glucagon.

We also examined and confirmed the role of glucagon in tissue Cr distribution in other obese and diabetic animal models (data not shown), and found that glucagon challenge significantly decreased Cr levels in epididymal fat, liver, and muscle, and increased Cr levels in bone. Using three types of animal models, our studies clearly demonstrate that glucagon challenge has a negative effect on Cr accumulation in insulin-sensitive tissues.

In conclusion, the present study demonstrates that both a high-fat diet and glucagon challenge can decrease Cr levels in tissues. Of note, as discussed above, the role of glucagon in tissue Cr distribution may be secondary to the effects of the hormone on glucose uptake in tissues. However, our findings support the assumption that the detrimental effects of a high fat intake and hyperglucagonemia in diabetics could be associated with changes in tissue Cr levels. Further studies are needed to elucidate the mechanisms involved.

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