# Dietary Chromium Decreases Insulin Resistance in Rats Fed a High-Fat, Mineral-Imbalanced Diet

John S. Striffler, Marilyn M. Polansky, and Richard A. Anderson

**The effects of chromium (Cr) supplementation on diet-induced insulin resistance produced by feeding a high-fat, low-Cr diet were studied in rats to ascertain the role of Cr in insulin resistance. Wistar male rats were maintained for 16 weeks after weaning on a basal diet containing 40% lard, 30% sucrose, and 25% casein by weight and adequate vitamins and minerals without added Cr (−Cr). Fasting levels of insulin, glucose, and triglycerides and the responses during an intravenous glucose tolerance test (IVGTT) were compared as indices of insulin resistance and the effectiveness of dietary Cr. IVGTTs and blood sampling for data analyses were performed over a 40-minute period after IV glucose injection (1.25 g/kg body weight) in overnight-fasted animals under pentobarbital anesthesia (40 mg/kg body weight). All animals were normoglycemic (−Cr, 109 ± 3 mg/dL; +Cr, 119 ± 5), with fasting insulin levels elevated in the −Cr group (65 ± 10 μU/mL) versus the +Cr group (31 ± 4 μU/mL). Increases in plasma triglycerides in the −Cr group were not significant. Following glucose injection, the rate of glucose clearance was lower in the −Cr group (1.74 ± 0.22 *v* 2.39 ± 0.11%/min), and 40-minute glucose areas in the −Cr group tended to be higher than in the +Cr group. The insulin response to glucose injection was 20% higher in the −Cr group. Forty-minute plasma triglyceride areas were lower in +Cr rats (875 ± 62 *v* 1,143 ± 97 mg/dL · min in −Cr rats). These data demonstrate that the insulin resistance induced by feeding a high-fat, nutrient-stressed diet is improved by Cr.**


IT IS GENERALLY ACCEPTED that high dietary fat intake causes glucose intolerance and insulin resistance in man and in laboratory rodents. Impaired glucose tolerance associated with a high fat intake is the result of fat-induced changes in membrane-associated proteins in various tissues. Studies[1,2] suggest that functioning of both the insulin receptor and the glucose transporter can be modulated by such diet-induced alterations in plasma membranes. Glucose intolerance and abnormal pancreatic functioning comparable to non–insulin-dependent diabetes in humans are also observed in animals with a diet-induced deficiency in various minerals including copper,[3-5] zinc,[6] and chromium (Cr).[7] In addition, significant interactions between these dietary micronutrients are known to occur in the development of mineral deficiency states that are also influenced by the level and type of macronutrients (carbohydrate, fat, and protein) present in the diet.[7-9]

In contrast to other mineral deficiency states, Cr deficiency is difficult to produce in animals.[7] Signs of Cr deficiency in rats are extremely marginal if Cr is simply omitted from the diet. Cr exerts larger effects under dietary or physical stress.[10] Studies reporting borderline or no effects of Cr on glucose tolerance in Cr-supplemented compared with unsupplemented animals have usually used specially prepared low-Cr diets high in sucrose containing added cholesterol,[11] torula yeast as the protein source,[12] or commercially available rat chow.[13,14] The presence of Cr in the basal diet,[13,14] inadequate amounts of Cr in supplemented groups, and use of starch-containing diets[15] may explain the absence of response to Cr supplementation in some studies.

Studies reporting an absence of any effects of Cr deficiency on glucose tolerance and related metabolic parameters[12-15] have given little attention to the early experimental approaches used and conclusions derived.[16] Flatt et al[15] observed no effects on 30- or 60-minute glucose levels after intraperitoneal administration of glucose in rats maintained on a low-Cr diet. Other investigators using oral glucose tolerance tests and finding either no effects or borderline effects of Cr supplementation or deficiency have similarly used only the 60-minute time point or even longer intervals after glucose administration.[11,13] In the early experiments using intravenous glucose tolerance tests (IVGTTs), rats were fed a low-Cr diet for 2 to 3 months, and only "responders" with a low $K_G$ (measured 2 weeks before Cr administration) were used in the bioassay for glucose tolerance factor (GTF) activity in Cr-containing compounds. Glucose tolerance was significantly impaired in the Cr-deficient responders with glucose disappearance rates less than 2.0%/min, as compared with 4.0%/min typically measured in normal rats fed regular diets.[17] Positive effects of Cr-containing GTF compounds on the restoration of the $K_G$ were routinely observed in these animals. Assuming that insulin secretion is not increased by administration of Cr, the twofold increase in the rate of glucose disappearance was attributed to enhanced insulin effectiveness on peripheral glucose uptake.[16]

The objective of these studies was to assess the influence of dietary Cr on both the tissue sensitivity to endogenous insulin and the insulin secretory responsiveness to intravenous administration of glucose in rats. The effects of dietary Cr were measured by comparing glucose and insulin responses to intravenous glucose administration in rats maintained on the basal diet versus rats fed the same diet supplemented with Cr as $CrCl_3$ added to the drinking water. As compared with our previous study,[18] the effects of Cr supplementation on glucose tolerance were not associated with any significant effects on β-cell glucose responsiveness, although insulin levels were consistently lower during the IVGTT in supplemented rats. These relative differences in β-cell responsiveness to intravenous glucose in the Cr-deficient rats used here may be the result of the high fat content of the low-Cr diet. In these studies,

*From the Nutrient Requirements and Functions Laboratory, Beltsville Human Nutrition Research Center, Beltsville, MD.*

*Submitted March 20, 1997; accepted October 15, 1997.*

*Current address for J.S.S.: Department of Diabetes, City of Hope National Medical Center, 1300 E Duarte Rd, Duarte, CA 91010.*

*Address reprint requests to Richard A. Anderson, PhD, USDA, ARS, NRFL, Bldg 307, Beltsville, MD 20705-2350.*



*0026-0495/98/4704-0006$03.00/0*

besides an enhanced glucose removal efficiency, we observed that Cr may also have a role in the maintenance of normal triglyceride metabolism in the rat.

## MATERIALS AND METHODS

The study protocol and animal care procedures were approved by the US Department of Agriculture Beltsville Area Research Animal Care Committee. Weanling male Wistar rats (Charles River, Wilmington, MA) were housed on a 12-hour light/dark cycle with constant ambient temperature (25°C) and environmental conditions that minimize external Cr contamination from air, dust, cages, etc.[19] Fourteen weanling animals were randomly assigned to either a Cr-deficient (−Cr) or Cr-supplemented (+Cr) diet. Technical problems were encountered with two +Cr rats and one −Cr rat, resulting in five +Cr and six −Cr animals. +Cr rats were provided with 5 ppm Cr as $CrCl_3$ added to the drinking water.[18] Cr was added to the water rather than to the diet to minimize cross-contamination. Any water spillage was readily contained on absorbent paper. The basal low-Cr diet (33 ± 14 ng Cr/g diet) was 30% sucrose, 40% lard, and 25% casein by weight. The standard reference materials and methods of Cr analysis have been described.[20] Recommended levels of vitamins were present in the diet,[21] and to minimize Cr contamination, the macroelement content of the diet was decreased marginally. The diet contained the following (in mg/kg diet): 1,400 sodium, 5,035 potassium as chloride, 5,000 calcium as carbonate, 4,000 phosphorus as potassium phosphate, and 400 magnesium as sulfate. The following trace elements (in mg/kg diet) were also added: 10 zinc as carbonate, 3 iron as sulfate, and 0.2 iodine as potassium iodate. To compromise the functioning of the endocrine pancreas,[3-5] copper as carbonate was added at 1 mg/kg during the initial 6 weeks of rapid growth. Copper was present in the diet at recommended levels (6 mg/kg) after the sixth week. Stock animals were fed a commercial stock diet (Rat Mouse Hamster 3200; Agway, Waverly, NY).

### *Experimental Protocol*

Fasting plasma insulin, glucose, and triglyceride levels were measured as indicators of the dietary effect on insulin resistance. Blood samples for baseline measurements were drawn by heart puncture under pentobarbital anesthesia (40 mg/kg body weight). Following collection, blood samples were transferred to polypropylene tubes containing EDTA (12 mg/mL). Plasma samples were harvested and stored frozen until assay.

### *Insulin, Glucose, and Triglyceride Responses During an IVGTT*

The effects of Cr deficiency and supplementation on the insulin responsiveness of the pancreas and sensitivity to endogenous insulin during an IVGTT were assessed after 16 weeks on the low-Cr diet. Tolerance tests were performed on overnight-fasted rats anesthetized with pentobarbital (40 mg/kg). The procedure for blood sampling via the cannulated jugular vein has been described in detail.[18] In addition to the −Cr and +Cr groups, tolerance tests were also performed on five chow-fed rats for comparison to animals fed a regular stock diet.

### *Assay Procedures*

Plasma glucose levels were measured with a Centrifichem System 600 using the hexokinase method (Baker Instruments, Allentown, PA). Plasma triglycerides were also assayed enzymatically using the Centrifichem 600. Insulin concentrations were measured using the radioimmunoassay procedure of Albano et al.[22] Samples were assayed in triplicate using standards prepared from rat insulin (kindly provided by Eli Lilly, Indianapolis, IN). Counting and data reduction were performed with an LKB gamma-counting system (Model 1282; Wallac, Gaithersburg, MD).

### *Data Analyses*

Glucose clearance ($K_G$) was determined using computer-generated linear regression lines. Glucose, insulin, and triglyceride response areas were determined from plasma levels using procedures outlined previously.[18] The plasma values shown are the mean ± SEM. The significance of differences between −Cr and +Cr groups was determined using Student's *t* test.

## RESULTS

Both groups of rats fed the low-Cr, high-fat diet showed an essentially normal growth rate and body weight gain over the 16-week period of study. Growth patterns in the two groups of rats fed the basal low-Cr diet were similar to those of chow-fed animals, and the mean body weights of both groups were similar (data not shown).

After 16 weeks on the low-Cr diet, unsupplemented rats showed pronounced fasting hyperinsulinemia with normoglycemia, indicating the presence of insulin resistance induced by the experimental diet (Table 1). In contrast, plasma insulin and glucose levels were essentially normal in +Cr animals and were similar to the levels measured in chow-fed rats (data not shown). Plasma triglyceride levels tended to be lower in +Cr versus −Cr animals.

The insulin response was lower in both groups of rats fed the high-fat diet compared with chow-fed animals (Fig 1). In addition, modulatory effects of Cr supplementation on insulin responsiveness were also apparent, with insulin levels consistently lower in +Cr versus −Cr rats.

More efficient glucose utilization due to Cr is illustrated by comparing the glucose decay constant ($K_G$) as represented by the slope of the lines shown in Fig 2. In contrast to the $K_G$ in chow-fed rats (5.18%/min), the $K_G$ in −Cr animals was decreased more than 50% (1.74%/min). Glucose removal efficiency was also lower than normal in +Cr rats, although a significant restorative effect of Cr was observed, with the $K_G$ (2.39%/min) being increased by almost 40% relative to that of −Cr animals. It should be noted that at all time points before minute 40, insulin levels were approximately 100 μU/mL, indicating that hepatic glucose release was fully suppressed.[23] Thus, differences in the plasma glucose response measured in these animals represent differences in peripheral glucose utilization and not hepatic glucose production.

Table 2 provides a comparison of insulin, glucose, and plasma triglyceride response areas during the 40-minute tolerance test in −Cr and +Cr rats fed the basal high-fat diet. As compared with the insulin areas near 7,500 μU/mL · min in chow-fed animals (data not shown), the areas in rats fed the purified diet were almost 50% lower, illustrating the marked

**Table 1. Effects of Cr Supplementation on Fasting Plasma Insulin, Glucose, and Triglyceride Levels in Rats Fed a High-Fat Diet Low in Cr**

| Parameter | −Cr (n = 7) | +Cr (n = 6) |
|---|---|---|
| Insulin (μU/mL) | 65 ± 10* | 31 ± 4† |
| Glucose (mg/dL) | 109 ± 3 | 119 ± 5 |
| Triglycerides (mg/dL) | 82 ± 5 | 71 ± 5 |

NOTE. Values are the mean ± SEM. Values with different superscripts are significantly different at $P < .05$ (Student's *t* test).

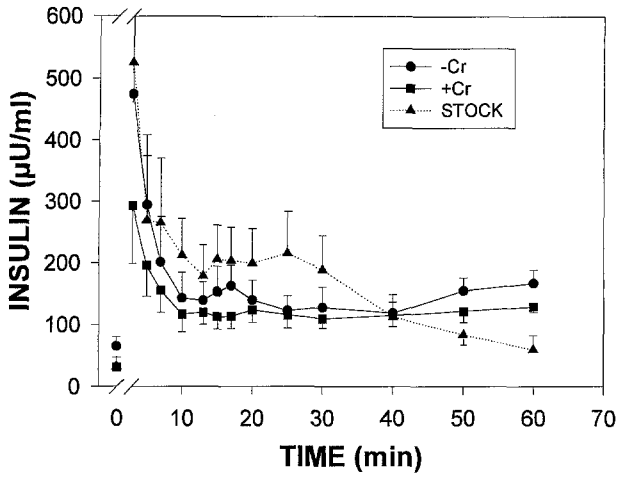


**Fig 1. Plasma insulin response during an IVGTT in −Cr and +Cr rats fed a low-Cr, high-fat diet and in stock diet–fed rats. Values before the axis break denote fasting levels. The dashed line denotes values for 5 stock-fed rats.**

depressive effect of the high-fat diet on the responsiveness of the endocrine pancreas to glucose (Fig 1). In relation to this, it should be noted that 40-minute insulin areas in animals fed a diet lower in fat (15% lard) and containing identical levels of minerals as used herein are nearly fourfold greater (~15,000 μU/mL · min; unpublished results, June 1994) than in the high-fat–fed rats (~3,800 μU/mL · min; Table 2). In accordance with the higher rates of glucose clearance measured in +Cr rats, 40-minute glucose areas tended to be smaller in the supplemented animals.

In addition to insulin and glucose responses, there was a pronounced decrease in plasma triglyceride levels during the 40-minute tolerance test. This triglyceride-lowering effect was observed in all rats, with 40-minute steady-state levels lower in +Cr compared with −Cr animals. These differences resulted in smaller 40-minute triglyceride areas in +Cr animals, indicating an enhanced effectiveness of endogenous insulin in +Cr

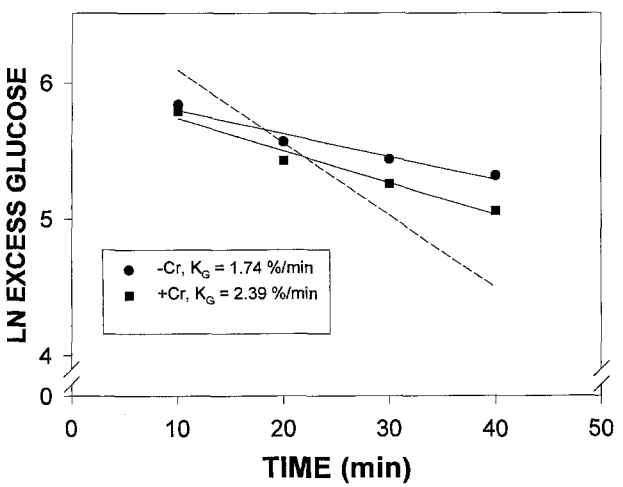


**Fig 2. Glucose clearance ($K_G$) during an IVGTT in −Cr and +Cr rats fed a low-Cr, high-fat diet. Excess glucose represents glucose levels corrected for basal glucose concentrations. The dashed line denotes values for 5 stock rats and corresponds to a $K_G$ of 5.18%/min.**

**Table 2. IVGTT Plasma Insulin, Glucose, and Triglyceride Responses in −Cr and +Cr Rats Fed a High-Fat Diet Low in Cr**

| | −Cr (n = 6) | +Cr (n = 5) |
|---|---|---|
| Insulin area above basal‡ | 3,838 ± 1,493 | 3,791 ± 1,153 |
| Glucose area above basal§ | 11,238 ± 568 | 9,907 ± 300 |
| Triglyceride area‖ | 1,143 ± 97* | 875 ± 62† |
| $K_G$ (%/min) | 1.74 ± 0.22† | 2.39 ± 0.11* |

NOTE. Values in the same row with different superscripts are significantly different at $P < .05$.

‡40-minute insulin area (μU/mL · min).

§40-minute glucose area (mg/dL · min).

‖40-minute triglyceride area under the curve (mg/dL · min).

animals (Table 2). Triglyceride areas in +Cr animals were similar to those in chow-fed animals (data not shown).

## DISCUSSION

The observations reported here and in previous studies from this laboratory examining intravenous glucose tolerance in Cr-deficient rats[18] support the proposal that impaired glucose tolerance in Cr deficiency reflects defects in peripheral tissue sensitivity to insulin.[16] Glucose removal rates were nearly 40% higher and occurred at circulating insulin levels that were consistently lower in the Cr supplemented rats. In previous studies, we observed even larger modulatory effects of Cr supplementation on the insulin response to intravenous glucose.[18] In those experiments using rats fed a low-Cr sucrose diet high in iron, β-cell hypersecretion of insulin was prevented by Cr and insulin responses in supplemented animals were 50% lower during the 40-minute glucose tolerance test. Except for studies performed in severely glucose-intolerant human subjects,[24] the modulatory effects of Cr supplementation on the insulin response to oral glucose in humans have been relatively small. A greater insulin response after oral compared with intravenous glucose administration is well documented and may, in part, underlie the response differences.[25] Comparisons between rat and human responses are complicated by differences in dietary fat content, which is typically high in the average human diet (>40% by calories in Western countries) in contrast to commercial rat diet, which often contains no added animal fat.[21]

In these experiments, the basal diet was high in fat to enhance insulin resistance in the experimental animals. The use of a high-fat diet was motivated by studies from many investigators demonstrating that the tissues of rats fed a high-fat diet are less sensitive to insulin versus those of animals fed a regular diet.[26,27] The presence of insulin resistance was indicated by the hyperinsulinemia in the fasting state and marked decrease of the $K_G$ in the fat-fed Cr-deficient rats used here. In accordance with studies from other groups examining interactions between dietary sucrose and various factors such as exercise, obesity, and cold acclimatization on glucose tolerance,[28-30] we have shown previously that the expression of Cr deficiency and supplementation effects is also significantly dependent on the sucrose content of the experimental diet. Animals fed low-Cr, high-carbohydrate diets show no impairment of glucose tolerance and glucose clearance are unaffected by Cr supplementation.[31] Beneficial effects of Cr supplementation on glucose tolerance are not observed in rats fed a low-Cr diet excessively

high (>70%) in sucrose.[31] Thus, an apparent discordance exists between this lack of any Cr effects on the $K_G$ and the amply documented effects of high-sucrose diets in producing a depletion of Cr stores in rats fed low-Cr, high-carbohydrate diets. Since high-carbohydrate diets are usually low in fat, it is clear that diet-induced changes in metabolism are also the result of an altered (decreased) fat content of the diet. The need for the use of low-Cr diets containing adequate amounts of saturated fat in Cr supplementation studies is underscored by the findings from several investigators using diets containing insufficient levels of saturated fat and showing no or borderline effects of Cr supplementation on glucose tolerance.[11,13-15] In addition to compensatory changes in β-cell secretion of insulin,[16] direct effects of high-carbohydrate diets on glucose metabolism in tissues may also underlie the borderline effects of Cr supplementation on glucose tolerance in rats fed high-carbohydrate diets low in fat. In support of this conclusion are studies using rats fed a diet lower in fat (15% lard) containing the same levels of trace minerals used here. These animals have a clearance of injected glucose two to three times faster than that of chow-fed rats, and no effects of Cr supplementation on glucose clearance are detectable.[31] In addition, insulin secretion is significantly enhanced, with 40-minute insulin areas being more than twofold greater than those measured in chow-fed rats.

Exaggerated insulin responses also occur in rats fed diets containing corn oil,[32] supporting the speculation that corn oil diets may directly affect β-cell insulin release by changing the membrane fatty acid composition.[32] In contrast, as seen in this study, high levels of animal fat (lard) in the diet have opposite effects on the pancreas, resulting in a marked decrease in β-cell responsiveness to glucose. The in vivo observations shown in this study are in accordance with studies by other investigators reporting that high-fat feeding is associated with relative postprandial hypoinsulinemia in rats,[26] as well as in vitro studies suggesting that insulin secretion is negatively influenced by high-fat feeding.[33-35] Thus, in contrast to the effects of feeding diets high in sucrose (low animal fat) or containing corn oil, the decreased glucose clearance and insulin resistance observed in the high-fat–fed rats used in this study were more likely the result of direct effects of chronically elevated circulating free fatty acids on peripheral tissues caused by consumption of the high-fat diet. As seen here, Cr supplementation had restorative effects on insulin resistance, with the $K_G$ being increased approximately 40% in the supplemented animals. The observation that fasting insulin levels were higher in Cr-deficient rats is indicative of more severe insulin resistance in response to the absence of Cr in the experimental diet. The development of fasting hyperinsulinemia was also prevented in response to Cr supplementation, with normalization of fasting insulin levels.

In this study, no significant beneficial effects of Cr on fasting triglyceride levels were seen, although triglyceride levels tended to be lower in Cr-adequate animals. In contrast, large differences between Cr-adequate and Cr-deficient rats were observed in response to intravenous glucose administration. Steady-state plasma triglyceride levels during the 40-minute tolerance tests were decreased in all animals. However, the incremental decrease in the triglyceride concentration was larger in Cr-adequate rats, resulting in lower steady-state levels and smaller 40-minute triglyceride areas as compared with the unsupplemented Cr-deficient rats. This acute triglyceride-lowering effect is likely the result of two separate actions of insulin: activation of lipoprotein lipase, leading to an enhanced degradation of triglycerides,[36] and concurrent suppression of lipolysis, resulting in a decreased supply of free fatty acids required for triglyceride biosynthesis.[37] It is well documented that even small increases in insulin can cause marked inhibition of lipolysis, with this effect being complemented by elevated blood glucose levels[38] as occur following intravenous glucose administration. This conclusion is also supported by the well-established correlation between elevated plasma free fatty acids and hypertriglyceridemia.[37] Experimental support for the involvement of Cr in this acute triglyceride-lowering response also derives from studies by Mirsky[39] reporting a 50% decrease of plasma free fatty acids in streptozotocin-diabetic rats 30 minutes after administration of a Cr complex. Concerning these Cr effects on blood lipids, Mirsky cited early studies by Tuman et al[40] showing a decrease in plasma triglyceride within hours of intraperitoneal administration of organic forms of Cr to genetically diabetic mice. The conclusion that Cr is required for maintenance of the sensitivity of these processes to insulin is also supported by in vitro studies showing significantly decreased free fatty acid release in adipose tissue from Cr-supplemented rats compared with rats with marginal Cr deficiency.[13]

In conclusion, Cr supplementation improves the glucose removal efficiency in rats with dietary insulin resistance induced by feeding a low-Cr diet high in saturated fat. This action of Cr to enhance glucose removal in response to endogenous insulin also includes a significant effect on blood triglyceride metabolism. The more pronounced decreases of triglycerides in Cr-adequate rats suggest that Cr may also be required for maintenance of the normal antilipolytic action of insulin, a well-documented effect of the hormone.

## REFERENCES

1. Kahn BB, Cushman SW: Subcellular translocation of glucose transporters: Role in insulin action and its perturbation in altered metabolic states. Diabetes Metab Rev 1:203-227, 1985

2. Clandinin MT, Cheema S, Field CJ, et al: Dietary fat: Exogenous determination of membrane structure and cell function. FASEB J 5:2761-2769, 1991

3. Cohen AM, Teitelbaum A, Miller E, et al: Effect of copper on carbohydrate metabolism in rats. Isr J Med Sci 18:840-844, 1982

4. Cohen AM, Miller E: Effect of copper on insulin release by the intact rat pancreas and the perfused rat pancreas. Pancreas 1:309-314, 1986

5. Hassel CA, Marchello JA, Lei KY: Impaired glucose tolerance in copper-deficient rats. J Nutr 113:1081-1083, 1983

6. Mooradian AD, Morley JE: Micronutrient status in diabetes mellitus. Am J Clin Nutr 45:877-895, 1987

7. Anderson RA: Effects of exercise, physical trauma, and high sugar intake on chromium, copper, and zinc metabolism, in Tomita H (ed): Trace Elements in Clinical Medicine. Tokyo, Japan, Springer-Verlag, 1990, p 185

8. Seaborn CD, Stoecker BJ: Effects of starch, sucrose, fructose, and glucose on chromium absorption and tissue concentrations in obese and lean mice. J Nutr 119:1444-1451, 1989

9. Fields M, Ferretti RJ, Reiser S, et al: The severity of copper deficiency in rats is determined by the type of dietary carbohydrate. Proc Soc Exp Biol Med 175:530-537, 1984

10. Anderson RA: Stress effects on chromium nutrition of humans and farm animals, in Lyons TP, Jacques KA (eds): Biotechnology in the Feed Industry, Proceedings of Alltech's Tenth Annual Symposium. Nottingham, England, University Press, 1994, pp 267-274

11. Donaldson DL, Lee DM, Smith CC, et al: Glucose tolerance and plasma lipid distributions in rats fed high-sucrose, high-cholesterol, low-chromium diet. Metabolism 34:1086-1093, 1985

12. Davies DM, Holdsworth ES, Sherriff JL: The isolation of glucose tolerance factors from brewer's yeast and their relationship to chromium. Biochem Med 33:297-311, 1985

13. O'Flaherty EJ, McCarty CP: Alterations of rat adipose tissue metabolism associated with dietary chromium supplementation. J Nutr 108:321-328, 1978

14. Hubner G, Noack K, Zuhlke H, et al: Influence of trivalent chromium on the beta-cell function. Exp Clin Endocrinol 93:293-298, 1989

15. Flatt PR, Juntti-Berggren L, Berggren P-O, et al: Effects of dietary inorganic trivalent chromium ($Cr^{+3}$) on the development of glucose homeostasis in rats. Diabete Metab 15:93-97, 1989

16. Mertz W: Chromium—An overview, in Shapcott D, Hubert J (eds): Chromium in Nutrition and Metabolism. New York, NY, Elsevier, 1979, pp 1-14

17. Mertz W, Schwarz K: Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. Am J Physiol 196:614-618, 1959

18. Striffler JS, Law JS, Polansky MM, et al: Chromium improves insulin response to glucose in rats. Metabolism 44:1314-1320, 1995

19. Polansky MM, Anderson RA: Metal-free housing units for trace element studies in rats. Lab Anim Med 29:357-359, 1979

20. Anderson RA, Bryden NA, Polansky MM: Dietary chromium intake. Biol Trace Elem Res 32:117-121, 1992

21. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 177:1340-1348, 1977

22. Albano JDM, Ekins RP, Maritz G, et al: A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol (Copenh) 70:487-509, 1972

23. DeFronzo RA: The triumvirate: B-Cell, muscle, liver—A collusion responsible for NIDDM. Diabetes 37:667-687, 1988

24. Doisy RJ, Streeten HP, Freiberg JM, et al: Chromium metabolism in man and biochemical effects, in Prasad AS, Oberleas D (eds): Trace Elements in Human Health and Disease, vol 2. New York, NY, Academic, 1976, pp 79-104

25. Creutzfeldt W, Nauck M: Gut hormones and diabetes mellitus. Diabetes Metab Rev 8:149-177, 1992

26. Pederson O, Kahn CR, Flier JS, et al: High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (Glut 4) in fat cells of rats. Endocrinology 129:771-777, 1991

27. Susini C, Lavau M: In-vitro and in-vivo responsiveness of muscle and adipose tissue to insulin in rats rendered obese by a high-fat diet. Diabetes 27:114-120, 1978

28. Vallerand AL, Lupien J, Bukowiecki LJ: Synergistic improvement of glucose tolerance by sucrose feeding and exercise. Am J Physiol 250:E607-E614, 1986

29. Vallerand AL, Lupien J, Bukowiecki LJ: Cold exposure reverses the diabetogenic effects of high-fat feeding. Diabetes 35: 329-334, 1986

30. Pfeiffer EF: Obesity, islet function and diabetes mellitus, in Gretin H, Levine R, Pfeiffer EF, Renold AE (eds): Lipid Metabolism, Obesity, and Diabetes Mellitus: Impact Upon Atherosclerosis. Stuttgart, Germany, Thieme, 1974, p 143

31. Striffler JS, Polansky MM, Anderson RA: Expression of chromium deficiency in rats may be related to dietary fat content. FASEB J 9:A449, 1995 (abstr)

32. Wiersma MML, Bakker IM, Radder JK: Effect of high fat feeding on glucose tolerance in neonatally streptozotocin-treated rats at 3 and 6 months of age. Ann NY Acad Sci 683:228-236, 1993

33. Seino Y, Usami M, Okamoto Y, et al: Effect of dietary environment on the development of impaired glucose tolerance and pancreatic hormone secretion in neonatal streptozotocin-treated (N6Z) rats. Acta Diabetol Lat 27:95-104, 1990

34. Takahashi RF, Curi R, Capinelli AR: Insulin secretion to glucose stimulus in pancreatic islets isolated from rats fed unbalanced diets. Physiol Behav 50:787-791, 1991

35. Linn TH, Schmitz P, Kloer HU, et al: Experimental islet isolation from donors with different nutrition regimens. Influence of the fatty acid content and composition of the chow. Horm Metab Res 25:17-20, 1990 (suppl)

36. Sadur CN, Eckel RR: Insulin stimulation of adipose tissue lipoprotein lipase. Use of euglycemic clamp technique. J Clin Invest 69:1119-1124, 1982

37. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1596-1607, 1988

38. Miles JM: Does glucagon regulate adipose tissue lipolysis? J Clin Endocrinol Metab 77:5A-5B, 1993 (editorial)

39. Mirsky N: Glucose tolerance factor reduces blood glucose and free fatty acids levels in diabetic rats. J Inorg Biochem 49:123-128, 1993

40. Tuman RW, Bilbo JT, Doisy RJ: Comparison of the effects of natural and synthetic glucose tolerance factor in normal and genetically diabetic mice. Diabetes 27:49-56, 1978