Maternal and Fetal Insulin-Like Growth Factor System and Embryonic Survival During Pregnancy in Rats: Interaction between Dietary Chromium and Diabetes

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ABSTRACT Chromium (Cr) depletion may exacerbate hyperglycemia and negative outcomes of pregnancy in the streptozotocin (STZ) diabetic pregnant rat model through the regulation of the insulin-like growth factor (IGF) system. To test this hypothesis, 40 female rats, all fed a low Cr diet (i.e., 70 μg Cr/kg diet) from 21 d of age, were randomly assigned one of four treatments, applied on Day 1 of pregnancy, in a 2 × 2 factorial design: 1) very low Cr diet (40 μg Cr/kg diet) + citrate buffer injection, 2) very low Cr diet + STZ injection (30 mg STZ/kg body wt in citrate buffer), 3) adequate Cr diet (2 mg Cr [from added CrK(SO₄)₂/kg diet] + citrate buffer injection) and 4) adequate Cr diet + STZ injection. Blood and tissues were collected on Day 20 of pregnancy. Chromium depletion increased (P < 0.05) maternal insulin concentrations, 32-kDa IGFBP, glucose, or placental and fetal hydroxyproline concentrations. Diabetes decreased (P < 0.05) maternal wt gain, embryonic survival, litter size, mean pup wt and maternal insulin concentrations, increased (P < 0.05) maternal blood glucose, IGF-I concentrations and maternal hydroxyproline excretion but did not affect fetal concentrations of hormones, IGFBP, glucose or hydroxyproline. Interaction between chromium and diabetes tended (P < 0.10) to affect maternal IGF-II concentrations, but had no effect on other maternal or fetal variables. In conclusion, maternal chromium depletion did not exacerbate hyperglycemia or pregnancy outcome in STZ-induced diabetic rats, but may negatively affect fetal protein content by decreasing fetal IGFBP-II concentrations. Diabetes may negatively affect fetal growth through its effect on maternal glucose, insulin and IGF-I. J. Nutr. 128: 2341–2347, 1998.

KEY WORDS: • Chromium • diabetes • insulin-like growth factors • pregnancy • rats

Chromium deficiency in humans and animals is associated with impaired glucose tolerance and a diabetic-like state (Anderson 1992 and 1997, Mertz 1993). In humans, chromium supplementation improves glucose tolerance, lowers fasting blood glucose concentrations, potentiates the action of insulin and increases HDL cholesterol (Anderson 1997, Anderson et al. 1997b, Mertz 1993), but has no effect on body composition (Lukaski et al. 1996, Pasman et al. 1997). Chromium deficiency in rats leads to retarded growth, insulin hyperresponsiveness to glucose, decreased glycogen reserves, increased incidence of aortic lesions and disturbances in amino acid utilization for protein synthesis (Mertz 1969, Striffler et al. 1995). However, the effect of chromium deficiency or its interaction with diabetes on the growth regulating insulin-like growth factor (IGF) system is unknown.

Chromium is best known for its insulin potentiating effect (Anderson 1992 and 1997, Mertz 1993). Chromium supplementation decreased the insulin-secretory response to glucose in rats (Striffler et al. 1995) and reduced the growth hormone-induced insulin resistance and resultant rise in blood glucose in pigs (Evock-Clover et al. 1993). Pigs fed chromium from chromium picolinate had faster glucose disappearance rates in response to i.v. glucose tolerance tests and shorter glucose half-life in response to i.v. insulin challenge tests when compared to controls not supplemented with chromium (Amonoikin et al. 1995). The effects of supplemental chromium, from chromium picolinate, on average daily body weight gain in pigs has been inconsistent (Boleman et al. 1995, Lindemann et al. 1995a and b, Mooney and Cromwell 1995 and 1997, Myers et al. 1995, Page et al. 1993, Ward et al. 1997), as has the effect on litter size in pigs (Lindemann et al. 1995a and 1995b). Furthermore, during gestation, insulin response to feeding in sows supplemented with chromium was significantly less compared to the response in un-supplemented sows (Lindemann et al. 1995b). However, the effect of supplemental chromium on pregnancy outcome or its interaction with diabetes in rats or humans is unknown.

Although streptozotocin (STZ)-induced diabetic pregna-
cies result in impaired fetal growth (Aerts et al. 1990, Mooradian and Morley 1987, Pitkin and Orden 1974, Uriu-Hare et al. 1985), little is known about the effect of STZ on the IGF system in pregnancy. The IGFs and their binding proteins (IGFBP) are necessary for murine embryogenesis and fetal growth (D’Ercole 1991, Heyner et al. 1993, Murphy and Barron 1993, Owens 1991). For example, both IGF-I and -II increased incorporation of [3H]thymidine in fetal rat islets (Hogg et al. 1993) and [3H]proline in fetal rat type I collagen (McCarthy et al. 1989) as well as induced neuronal and muscular differentiation in cocultures of embryonic rat brainstem slices and skeletal muscle fibers (Eustache et al. 1994). Furthermore, marked retardation of fetal growth occurred in mice with a hemizygous disruption of the IGF-II gene (DeChirara et al. 1990). In normal rat pregnancies, maternal plasma IGF-I concentrations decrease in conjunction with the disappearance of IGFBP-3 (Gargosky et al. 1990). This is associated with an increase in IGFBP-3 protease activity, which promotes the availability of IGF-I to act upon the retrogut tissues (Davenport et al. 1992). Growth-retarded fetal rats have decreased IGF-I and -II gene expression, decreased fetal serum IGF-I and increased IGFBP-I gene expression (Davenport et al. 1990, Unterman et al. 1993). In addition, decreased serum IGF-I, differential gene expression of IGF-I and IGFBP-1 as well as discordant organ specific regulation of IGF-I mRNA were observed in diabetic male and nonpregnant female rats (Binz et al. 1989, Catanese et al. 1993, Luo and Murphy 1991). Diminished IGF-II expression and retarded development were evident in 6-d-old conceptuses of diabetic mice (Chernicky et al. 1994). Whether chromium supplementation could interact with diabetes to alter the maternal or fetal IGF system is unknown. Changes in IGF-I, IGF-II and their binding proteins during normal and diabetic pregnancy might be related to an altered metabolic state and the supply of glucose and insulin in both the mother and fetus, which contributes to changes in fetal growth; supplemental dietary chromium may improve metabolism during gestational diabetes. Thus, the objective of this study was to determine the effect of interaction between dietary chromium depletion and STZ-induced diabetes on maternal and fetal insulin, glucose, IGFBP, IGF-I and IGF-II concentrations, pregnancy outcome, and fetal and placental protein and hydroxyproline content.

MATERIALS AND METHODS

Experimental Design. At Day 1 of pregnancy, 40 female rats were assigned to one of four treatments in a 2 × 2 factorial design: 1) very low chromium diet (40 μg chromium/kg diet) with citrate buffer injection, 2) very low chromium diet with STZ (a potent pancreatic β-cell toxin) injection (30 mg STZ/kg body weight in citrate buffer), 3) adequate chromium diet (2 mg chromium from CrK(SO4)2/kg diet) with citrate buffer injection and 4) adequate chromium diet with STZ injection. The STZ was obtained from Sigma Chemical Co. (St. Louis, MO), solubilized in 1 mL of 0.1 M citrate buffer (pH 4.5) and injected into the tail vein. Treatments were applied on Day 1 of pregnancy, which was confirmed by the presence of a vaginal plug. The rats were bred at a mean (±SEM) weight of 267 ± 8 g and age of 126 ± 3 d; these measures did not significantly differ among treatment groups.

Animals and diets. Weanling female Sprague Dawley rats were obtained from Sasco Inc. (Omaha, NE) and raised in a light (12 h light: 12 h dark cycle) and temperature controlled (22 ± 1°C) environment in Plexiglass cages with plastic grate flooring. From 21 d of age until breeding, all rats were fed a low chromium diet similar to the AIN-93G formulation (Reeves et al. 1993) without added chromium, resulting in 70 μg chromium/kg diet (adaptation diet). Distilled, deionized water was provided for drinking water throughout

<table>
<thead>
<tr>
<th>Components</th>
<th>Adaptation diet</th>
<th>Treatment diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch 1</td>
<td>430</td>
<td>150</td>
</tr>
<tr>
<td>Casein 1</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Dextrose 1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>Celulfit 1</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>AIN-76 mineral mix 2</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN-76 vitamin mix 2</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-Glycine</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Diet ingredients were obtained from Harlan Teklad (Madison, WI). Sucrose and soybean oil were obtained from a local retail store. All ingredients utilized were those determined to contain the lowest chromium concentrations by atomic absorption spectrophotometry. 2 Mineral mix contained ingredients with minimal amounts of chromium and was composed of (g/kg mix): CaHPO4, 500; CaH2O2Cr2O7, H2O, 220; NaCl, 74; K2SO4, 502; MgO, 24; FeCl3, 3.56; ZnSO4, 1.6; MnO2, 0.65; CH2Cu2O5, 0.3; KIO3, 0.01; Na2SeO3, 0.01. Mineral mix used for the adequate chromium diet included 0.55 g CrK(SO4)2/kg mix (AIN, 1977). 3 Vitamin mix (AIN-76) was obtained from U.S. Biochemical Corp. (Cleveland, OH).
for IGF-I in the IGF-II RIA and for IGF-II in the IGF-I RIA were 5.0% and 0.2%, respectively. Cross-reactivities for insulin in the IGF-I and IGF-II RIA were <0.001% and <0.006%, respectively. Concentrations of IGFBP were analyzed by Western ligand blot method described by Echternkamp et al. (1994), except that a 4 μL sample of plasma was electrophoresed and the duration of exposure to X-ray film was 12 d. Fetal samples were analyzed following the method described by Bergman and Loxley (1969). Moisture content of fetal samples were analyzed using the general linear models procedure of SAS (1988). Maternal IGF-I and maternal 22 kDa IGFBP exhibited heterogenous variance (determined by SAS UNIVARIATE procedure) and thus, values were transformed to natural log (x + 1) before analysis. Values reported herein are expressed as mean ± SEM.

**RESULTS**

Dietary chromium depletion resulted in decreased placental weight relative to maternal body wt (P < 0.05) and increased the number of pups per litter (litter size) (P < 0.05, Table 2). There was a tendency for dietary chromium depletion to increase maternal wt gain (P < 0.10, Table 2) but chromium depletion did not affect mean pup wt. Diabetes had negative effects on maternal wt gain (P < 0.05), embryonic survival (P < 0.01), litter size (P < 0.01) and average pup wt (P < 0.05). No significant interaction between dietary chromium and diabetes was observed on maternal wt gain, embryo loss, litter size or mean pup wt (Table 2).

For 17 d of treatment, dietary chromium depletion increased (P < 0.01) urinary hydroxyproline excretion from 0.38 to 0.46 ± 0.07 μg/(12 h · g body wt) in control rats and from 0.88 to 1.22 ± 0.07 μg/(12 h · g body wt) in diabetic rats. Diabetes increased (P < 0.001) urinary hydroxyproline excretion from 0.46 to 1.22 ± 0.07 μg/(12 h · g body wt) in chromium-deficient rats and from 0.38 to 0.87 ± 0.07 μg/(12 h · g body wt) in rats fed adequate dietary chromium. No significant interaction between dietary chromium and diabetes existed for maternal urinary hydroxyproline excretion.

Dietary chromium had no significant effect on maternal insulin or glucose concentrations (Table 3). In contrast, diabetes significantly decreased (P < 0.001) maternal plasma insulin such that mean plasma insulin concentration for the diabetic group was 2.2 ± 0.6 nmol/L compared with 8.7 ± 2.4 nmol/L in the nondiabetic group. As a result, there was a significant elevation of plasma glucose (P < 0.01); mean plasma glucose for the diabetic group was 6.8 ± 0.7 mmol/L compared with 4.4 ± 0.2 mmol/L in the nondiabetic group. Dietary chromium had no effect (P < 0.10) on maternal plasma IGF-I concentrations (Table 3). Diabetes affected (P < 0.05) maternal plasma IGF-I concentration such that mean IGF-I concentration for the diabetic dams was 167 ± 21 μg/L compared with a mean of 129 ± 17 μg/L in the nondiabetic group. There was a tendency (P < 0.08) for a chromium × diabetes interaction to affect maternal plasma concentrations of IGF-I.

### Table 2

Comparison of pregnancy outcomes of normal (−STZ, no streptozotocin in citrate buffer injection) and streptozotocin (STZ)-induced diabetic (+STZ, streptozotocin in citrate buffer injection) dams fed diets with (+Cr) or without (−Cr) added chromium (Cr)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal wt gain, g</td>
<td>−Cr−STZ</td>
<td>−Cr+STZ</td>
</tr>
<tr>
<td>Embryo loss, n</td>
<td>3 ± 0.8</td>
<td>6 ± 2.3</td>
</tr>
<tr>
<td>Pups per litter, n</td>
<td>10 ± 2.0</td>
<td>10 ± 1.7</td>
</tr>
<tr>
<td>Mean pup wt, g</td>
<td>4.2 ± 0.2</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Placental wt, g</td>
<td>0.45 ± 0.01</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>g/100 g body wt</td>
<td>0.12 ± 0.01</td>
<td>0.15 ± 0.01</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10.

### Table 3

Comparison of plasma concentrations of insulin, glucose, insulin-like growth factor (IGF)-I and IGF-binding protein (IGFBP) on Day 20 of gestation in normal (−STZ, no streptozotocin in citrate buffer injection) and streptozotocin (STZ)-induced diabetic (+STZ, streptozotocin in citrate buffer injection) dams fed diets with (+Cr) or without (−Cr) added chromium (Cr)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, nmol/L</td>
<td>−Cr−STZ</td>
<td>−Cr+STZ</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.7 ± 0.3</td>
<td>6.5 ± 0.8</td>
</tr>
<tr>
<td>IGF-I, μg/L</td>
<td>129.0 ± 28.2</td>
<td>170.6 ± 26.8</td>
</tr>
<tr>
<td>IGFBP, ADU³</td>
<td>1.21 ± 0.46</td>
<td>1.02 ± 0.46</td>
</tr>
<tr>
<td>32 kDa IGFBP, ADU³</td>
<td>0.76 ± 0.36</td>
<td>0.37 ± 0.36</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10.
2 Results from ANOVA of values transformed to natural log (x + 1).
3 ADU = arbitrary densitometric units.
IGF-II (Fig. 1). Within the chromium-depleted group, diabetics had a tendency to decrease (P < 0.08) maternal plasma IGF-II concentrations, whereas in the adequate chromium group, diabetes had no effect (P > 0.10) on maternal plasma IGF-II (Fig. 1).

Figure 2 is a representative autoradiograph from Western ligand blotting of maternal and fetal IGFBP. In maternal plasma, concentration of the 32-kDa IGFBP was very low compared to that of fetal plasma and was not significantly affected by dietary chromium, diabetes or their interaction (Table 3). However, concentration of the 22-kDa IGFBP in maternal plasma was increased (P < 0.08) by chromium depletion but not affected by diabetes or its interaction with chromium (Table 3). The most prominent IGFBP detected in fetal plasma was a 32-kDa species (Fig. 2); this IGFBP was not affected (P > 0.10) by treatments (Table 4). The level of the 22-kDa IGFBP in fetal plasma was less than 2% of the 32-kDa IGFBP, with no significant difference observed among the treatment groups (Table 4).

Fetal insulin, glucose, IGF-I and fetal protein content are listed in Table 4. Fetal insulin and glucose concentrations were not affected (P > 0.10) by dietary chromium, diabetes or their interaction. There was a tendency for plasma IGF-I concentrations to be lower (P < 0.10) in the fetuses from the chromium-depleted dams versus those fed adequate/normal chromium. Diabetes and its interaction with chromium had no significant effect on fetal plasma IGF-I or IGF-II. Dietary chromium depletion also decreased fetal plasma on IGF-II concentrations (P < 0.05; Fig. 1) and fetal protein content (P < 0.05; Table 4). Fetal moisture content was not significantly different among the treatment groups (data not shown). Mean plasma IGF-II concentration for the adequate chromium pups was 779 ± 38 μg/L compared to 697 ± 34 μg/L in the chromium-depleted pups (P < 0.05); this difference was more pronounced in fetuses from nondiabetic dams (Fig. 1). Mean protein content for the adequate chromium pups was 9.2 ± 0.7% compared to 8.7 ± 0.5% in the chromium-depleted pups. Fetal and placental hydroxyproline concentrations were not significantly affected by chromium, STZ or their interaction (data not shown).

DISCUSSION

We observed that feeding the recommended AIN-76 level of dietary chromium compared to a very low level of dietary chromium to pregnant rats had no effect on the hyperglycemia of STZ-induced diabetes and had no effect on plasma glucose, insulin, IGF-I, or IGF-II in late pregnancy. Dietary chromium depletion increased litter size and maternal weight gain without a change in mean fetal weights. Because adequate dietary chromium increased placental weights relative to maternal body weight, intrauterine competition per pup may have been greater thereby reducing litter size. This is in contrast to previous studies (Lindemann et al. 1995b) where a positive effect of chromium was observed on maternal weight gain and litter size in sows supplemented with 200 μg chromium/kg feed from chromium picolinate. Total chromium concentration of the feed was not specified in these previous experiments, but based on other swine studies (Anderson et al. 1997b), unsupplemented corn-soybean meal diets contain about 2.5 mg chromium/kg diet. In the present experiment, we utilized the AIN-76 recommendation of 0.55 g chromium potassium sulfate/kg mineral mix resulting in a diet containing 2 mg chromium/kg diet. Feeding our pregnant rats this adequate chromium diet resulted in a significantly greater concentration of chromium in the kidneys compared with those rats fed the very low chromium diet (Spicer & Stoecker 1997). In view of the preliminary studies by Ward et al. (1995), it is unlikely that the dietary source of chromium may have influenced the animals’ reproductive performance. Further studies are needed to determine the precise amount of total dietary chromium needed to optimize reproductive performance. In addition, a duration of a dietary chromium deficiency may influence the outcome on reproductive performance because female rats bred to male rats fed a chromium deficient diet for 6 mo, but not 3 mo, had lower pregnancy rates compared with those bred to male rats fed an adequate chromium diet (Anderson and Polansky, 1981).

Fetal plasma IGF-II concentrations as well as fetal protein content were greater in the group with adequate dietary chromium in spite of the fact that the number of pups per litter was reduced in the present study. Furthermore, we observed an increase in urinary hydroxyproline excretion, a measure of collagen breakdown, in dams fed the low chromium diets compared with dams fed the adequate chromium diet. Whether increased dietary chromium directly or indirectly influenced urinary hydroxyproline excretion remains to be determined. However, it is likely that chromium affected fetal protein through its effect on IGF-II as it has been observed that the hemizygous disruption of the IGF-II gene in fetal mice results in marked growth retardation (Unterman et al. 1993). In addition, IGF-II has been shown to stimulate DNA and protein synthesis in numerous fetal rat tissues in vitro (Binz et al. 1981, Davenport et al. 1990, Luo and Murphy 1991). The increase in placental weight in our study may have resulted in the subsequent increase in maternal nutrient transfer and in turn may have contributed to the increase in fetal IGF-II and protein concentration of the fetuses from the dams fed adequate dietary chromium. Also, adequate dietary chromium increased maternal IGF-II concentrations and decreased the 22 kDa IGFBP concentrations in plasma of diabetic dams compared to dams fed the low chromium diet. This may have contributed to the increase in fetal IGF-II. Because fetal plasma IGFBP concentrations were not influenced by dietary...
chromium, changes in IGFBP levels cannot account for the observed changes in fetal IGF-II levels. However, the precise mechanism by which chromium alters fetal IGF-II secretion will require further elucidation.

In agreement with the literature, the toxic effect of STZ on the pancreas and the subsequent development of diabetes was demonstrated by the reduced production of insulin and the resultant elevated maternal glucose concentrations. Diabetes also had a negative effect on reproductive performance and fetal growth in the present study. As has been observed by other researchers (Hogg et al. 1993; Lea et al. 1996; McCarthy et al. 1989), diabetic dams gained less during pregnancy, had smaller fetuses, had a greater number of embryo resorptions (i.e., decreased embryonic survival) and a smaller number of viable fetuses per litter. In addition to increased maternal glucose concentrations, in the present study maternal plasma IGF-I concentrations were greater in the diabetic group when compared to the nondiabetic group in spite of the fact that IGF-I concentrations were greater in the diabetic group. As has been observed by previous studies support the hypothesis that increased fetal IGF-I mRNA, but does not affect uterine IGF-I mRNA in nonpregnant female rats (Catanese et al. 1993, Luo and Murphy 1991, Murphy 1988). Because kidney IGF-I content (Bach and Jerums 1990, Phillip et al. 1995) and IGF-I mRNA (Catanese et al. 1993) can be increased by STZ, and we observed a significant increase in kidney weight (data not shown); in the present study, it is possible that increased production of kidney IGF-I contributed to the increase in maternal plasma IGF-I concentrations.

Diabetic dams had no significant effect on fetal plasma concentrations of insulin, glucose, IGF-I, IGF-II, or the 22- and 32-kDa IGFBP. Maternal IGFBP levels were almost undetectable and were not affected by treatment. Previously, hepatic IGFBP mRNA levels were reported to increase in adult diabetic rats (Boni-Schnetzler et al. 1989, Rechler and Brown 1992) and in diabetic castrated pigs (White et al. 1993). Also, based on increased embryonic survival, lower birth weight fetuses of diabetic dams have greater hepatic IGFBP-I mRNA expression than fetuses of nondiabetic dams, although this difference does not exist on Days 15 and 16 of gestation (Rajaratnam et al. 1997, Streck et al. 1995). Collectively, results from the present and previous studies suggest that increased fetal}

### Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group</th>
<th>P value</th>
<th>Cr</th>
<th>STZ</th>
<th>CxSTZ</th>
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<tbody>
<tr>
<td>Insulin, nmol/L</td>
<td>−Cr−STZ</td>
<td>18.8 ± 3.1</td>
<td>15.8 ± 3.0</td>
<td>21.8 ± 2.9</td>
<td>15.9 ± 2.4</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>−Cr−STZ</td>
<td>7.7 ± 0.9</td>
<td>6.8 ± 1.9</td>
<td>4.7 ± 0.4</td>
<td>8.4 ± 2.2</td>
</tr>
<tr>
<td>IGF-I, μg/L</td>
<td>−Cr−STZ</td>
<td>48.8 ± 4.8</td>
<td>50.1 ± 3.5</td>
<td>59.6 ± 3.0</td>
<td>55.2 ± 5.0</td>
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<tr>
<td>32 kDa IGFBP, ADU²</td>
<td>−Cr−STZ</td>
<td>26.9 ± 9.1</td>
<td>21.9 ± 9.1</td>
<td>21.6 ± 9.1</td>
<td>24.1 ± 9.1</td>
</tr>
<tr>
<td>22 kDa IGFBP, ADU²</td>
<td>−Cr−STZ</td>
<td>0.40 ± 0.15</td>
<td>0.24 ± 0.05</td>
<td>0.37 ± 0.15</td>
<td>0.32 ± 0.15</td>
</tr>
<tr>
<td>Protein content, %</td>
<td>−Cr−STZ</td>
<td>8.8 ± 0.5</td>
<td>8.6 ± 0.4</td>
<td>9.2 ± 0.7</td>
<td>9.3 ± 0.7</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 10.
2 ADU = arbitrary densitometric units.
IGFBP secretion may contribute to the growth retardation observed in fetuses of diabetic dams. Hydroxyproline is a good indicator of collagen breakdown and is thereby considered to reflect the connective tissue alterations in the diabetic state. In the present study, 12-h urinary hydroxyproline excretion per unit body weight was two- to three-fold greater in pregnant rats after STZ treatment compared to placebo treatment, regardless of the level of dietary chromium. This is probably because of increased collagen breakdown in STZ-induced pregnant rats. In diabetes, collagen tends to be less cross-linked and more susceptible to collagenase digestion because a high glucose environment can inhibit fibril formation and subsequent collagen cross-linking. Thus, urinary hydroxyproline excretion was increased as a product of collagen breakdown. Mavrakis et al. (1993) reported 24-h urinary hydroxyproline concentration in rats increased 3.4-fold 57 d after the induction of diabetes by STZ. Our findings are in agreement with their report. However, we found that STZ or chromium treatment had no significant effect on placental or fetal hydroxyproline concentrations on Day 20 of pregnancy. The latter observation suggests that placental or fetal collagen breakdown is not very susceptible to maternal influences.

In conclusion, the present study demonstrates that despite its negative effect on litter size, dietary chromium at a level of 2 mg/kg diet increased placental weight and fetal protein concentrations, which was associated with an increase in fetal IGF-II concentrations. The present study also demonstrated that STZ-induced diabetes during pregnancy has profound effects on reproductive performance and fetal growth and that these effects may be mediated by changes in maternal plasma insulin, glucagon and IGF-I, but not by changes in plasma IGFBP concentrations. Thus, maternal chromium depletion did not exacerbate hyperglycemia and the negative outcomes of pregnancy in STZ-induced diabetic rats.

ACKNOWLEDGMENTS

We thank the National Hormone and Pituatory Program (Rockville, MD) for the IGF-I antiserum, Monsanto Company (St. Louis, MO) for the generous donation of recombinant bovine IGF-II, the OSU Recombinant DNA/Protein Resource Facility for use of its molecular imager and scanning densitometer, Beth Keefer for technical assistance, and Paula Cinnamon for secretarial assistance.

LITERATURE CITED


