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Original Article

# Chromium restriction in the intrauterine environment and development of metabolic diseases in mice offspring

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**الملخص**

**أهداف البحث:** ثبت أن البيئة الرحمية الضارة الناتجة عن نظام غذائي مقيد بالكروم مرتبطة بتطور الأمراض الأيضية في مرحلة البلوغ، إلا أن مسبباتها لا تزال مجهولة.

**طريقة البحث:** فصلت عينة من 24 فأرة بالغة من نوع "بالب/س" إلى مجموعتين، حيث أُطعمت فئران المجموعة ٠١ بحرية تامة، بينما أُطعمت فئران المجموعة ٠٢ نظاما غذائيا مقيدا بالكروم (٠،١٢٥ ملغ كروم/كغ من النظام الغذائي) لمدة ١٢ أسبوعا. بعد التأكد من وجود كمية كافية من الكروم، سمح للحيوانات بالتلبد، وتم حساب فترة الحمل مع ظهور سدادة مهبلية. استمر اتباع نفس النظام الغذائي المقيد بالكروم طوال فترة الحمل. بعد الولادة، بقيت الجراء على نظام الأم الغذائي حتى عمر ١٥ شهرا، وتمت مراقبة سلوكها وحركاتها. بعد خمسة عشر شهرا من الولادة، قمنا بقياس مستوى الجلوكوز في الدم، والأنسولين في المصل، والكوليسترول، وكوليسترول البروتين الدهني عالي الكثافة. وعندما بلغ النسل ١٥ شهرا، أُزيلت أنسجة البنكرياس والكلى والأنسجة الدهنية والرحم بعد التضحية بها، ودرست بنيتها الخلوية.

**النتائج:** أظهرت حيوانات المجموعة "جي٢" سلوكا وأنماط حركة شاذة. وبالمقارنة مع المجموعة الضابطة، أظهرت ذرية المجموعة "جي٢" ارتفاعا في مستوى الجلوكوز في الدم، والأنسولين في المصل، والكوليسترول، وكوليسترول البروتين الدهني عالي الكثافة. كشف تلطيخ الهيماتوكريت والإستروجين عن تغيرات في أنسجة البنكرياس والكلى والرحم، والتي تم التحقق من صحتها من خلال تلطيخ الأجسام المضادة لـ "الجزيرة-١"، والكادهيرين الخاص بالكلى، والأجسام المضادة لـ "ام ال اتش"، مما يشير إلى آثار تقييد الكروم على الأعضاء الرئيسية للنسل، مما يؤدي في النهاية إلى تغيرات أيضية وأمراض في وقت لاحق من الحياة.

**الاستنتاجات:** يؤدي نقص الكروم في الرحم إلى الإصابة بمرض السكري وتصلب الشرايين في البنكرياس والكلى والرحم مع قلة الأنسجة الحشوية.

**الكلمات المفتاحية:** تقييد الكروم؛ ذرية الفئران؛ أمراض البالغين؛ برمجة النمو

**Abstract**

**Objective:** A detrimental intrauterine environment generated by a chromium-restricted diet has been implicated in the development of metabolic diseases in adulthood, but the etiology remains unknown.

**Methodology:** A sample of 24 BALB/c female adult mice were separated into two groups, where mice in group 1 were fed ad libitum with normal animal chow, whereas mice in group 2 received a chromium-restricted diet (0.125 mg Cr/kg diet) for 12 weeks. After determining their chromium levels, animals were allowed to mate and pregnancy were counted based on vaginal plug appearance. The same chromium-restricted diet regime was continued throughout pregnancy. After birth, pups were kept on the mother's diet until the age of 15 months, and

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their behavior and movements were monitored. At five and 15 months postnatal, the blood glucose, serum insulin, cholesterol, and high-density lipoprotein cholesterol (HDLc) levels were measured. After reaching the age of 15 months, the offspring were sacrificed and the pancreas, kidney, adipose tissue, and uterine tissue were removed to assess the cytostructure.

**Results:** The animals in group 2 exhibited aberrant behavior, and mobility patterns. Compared with the controls in group 1, the offspring in group 2 had elevated blood glucose, serum insulin, cholesterol, and HDLc levels. Hematoxylin and eosin staining indicated alterations in the pancreas, kidneys, and uterus parenchyma, which were validated by anti-islet-1, kidney-specific (Ksp) cadherin, and anti-MLH antibody staining, thereby demonstrating the impacts of chromium restriction on key organs to ultimately lead to metabolic alterations and diseases later in life.

**Conclusion:** In utero chromium restriction induced diabetes and atherosclerosis in the endocrine pancreas, kidney, and uterus, with less parenchymal tissue.

**Keywords:** Adult diseases; Chromium restriction; Development programming; Mice offspring



## Introduction

Diet significantly influences health in all life stages and nutrition during prenatal and early postnatal development is particularly crucial for long-term health outcomes. Studies are increasingly indicating that adult disorders, such as diabetes, are associated with the intrauterine nutritional status.[1–4] Malnutrition in this critical period may result in the development of metabolic disorders later in life.[5,6] Alterations in genes during this critical period of development also have long-lasting effects since they persist in adulthood even after dietary recovery.[7] In addition to proteins, lipids, and carbohydrates, micronutrients have crucial roles in the human body. It has been demonstrated that in-utero exposure to environmental factors can have profound effects on the susceptibility to disease in adult life.[8,9] The pathogenesis of illness and its consequences are linked to the time of exposure to high susceptibility environmental-related factors and the developmental stage when the fetus is exposed during intrauterine life. When the fetus passes through the susceptible developmental stage of genomic activity, cells undergo programmed growth and differentiation, which may be affected by environmental factors. Any alteration in the gene expression process will trigger changes during the embryonic developmental period.[10,11]

Evidence suggests that micronutrients are essential for mediating glucose and fat metabolism.[12–14] Chromium has been shown to lower fasting blood glucose levels in diabetic people and diabetic rodent models, as well as increasing insulin action by activating insulin receptor sites.[15,16] Previous research indicates that supplemental Cr reduces body fat and body weight, and regulates hunger in humans and animal models.[12] The minimum recommended daily intake of chromium is 30 μg in adults. However, in many countries, the average dietary intake of chromium by adults is far below this recommendation.[17,18] Due to increased metabolic stress and low chromium absorption rates,[19,20] pregnant women and elderly individuals are more prone to chromium deficiency.[17,21,22] Cr can be estimated by measuring its plasma concentration. However, clinical studies indicate that blood levels of chromium are inadequate indicators of actual deficiency because they do not correlate with chromium resources in the liver, spleen, bone, and soft tissues. Research also indicates that the chromium level may decrease during acute illness, despite adequate total body chromium stores.[23]

Many studies have examined the impacts of chromium deficiency during intrauterine development on insulin resistance and glucose intolerance,[24–26] which ultimately result in metabolic disturbances. In the present study, we investigated whether these disturbances may also influence the functions of other organs, such as the kidney and uterus, as well as insulin resistance, potentially leading to adult disorders related to these organs in addition to insulin resistance and glucose intolerance. We aimed to determine the impacts of chromium deficiency on the onset of adult illnesses resulting from in utero exposure, such as diabetes mellitus, kidney disease, and uterine disorders, by examining the pancreas, kidneys, and uterus.

## Materials and Methods

All experiments were conducted in the Animal House, Sindh Agricultural University Tando Jam and Diagnostic and Research Laboratory, Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan.

### *Preparation of diet*

The control animal chow was prepared according to the laboratory protocol by mixing plain flour (500 g), soya bean oil (40 mL), chickpeas (60 g), dried meat powder (60 g), dried milk powder (120 g), poultry feed (60 g), and mineral water, and baking into cakes for consumption by mice.[27] The chromium-restricted diet (only chromium restricted) was prepared in a similar manner to the control diet but with some modifications, where it contained plain flour (500 g), dried meat powder (60 g), soya bean oil (40 mL), chickpeas (60 g), skimmed milk powder (chromium restricted) (120 g), poultry feed (60 g), and mineral water. All of the ingredients were purchased from a local market. The method used for restricting chromium in the diet was as described previously by Zhang et al.[28]

*Experimental protocol*

All experimental protocols were conducted as described previously by Bano et al.[29] In total, 24 adult female mice were segregated into two groups (12 mice per group) and housed in conventional polypropylene cages under a light:dark cycle of 12 h:12 h, temperature of 22 ± 2 °C, and relative humidity of 55 ± 10 %. The baseline weights were measured in both groups before the experiment and they did not differ significantly (Table 1). Control mice in group 1 were provided ad libitum access to a diet containing 0.190 mg Cr/kg, whereas mice in group 2 had ad libitum access to a chromium-restricted diet containing 0.125 mg Cr/kg for 12 weeks. After completing this dietary regimen, the plasma chromium levels were assessed using samples obtained from the retro-orbital vein and analyzed with an atomic absorption spectrophotometer (Norwalk, CT, USA). After determining the chromium levels (Table 2), animals were allowed to mate with control males (two females with one male), and pregnancy day 1 was recorded based on observation of the vaginal plug. The diet of the pregnant mice was modified to chromium-restricted during gestation. Postnatally, pups were allocated to groups of ten and they received the same food regimen as the mother until 15 months of age. A double-blind experimental design was employed where the researcher responsible for administering treatments, evaluating effects, and analyzing results was unaware of the animal groupings and dosage allocations, which were managed through coding by a separate researcher.

*Gross examination of pups*

Pups were examined to assess their behavior according to their behavior grading system[30] shown in Table 3. Observations of pup movements were performed based on the 10 grades shown in Table 4.[31]

*Biochemical analyses*

Blood samples were obtained after five and 15 months to assess the blood glucose and serum insulin levels by using the enzyme-linked immunoassay (ELISA) assay kit method and spectrophotometry (Hitachi 902, Roche Diagnostics, USA). Blood cholesterol and high-density lipoprotein cholesterol (HDLc) levels were measured using an enzymatic colorimetric approach with commercially available kits and the Microlab Clinical Chemistry Analyzer (Microlab 300 Spectrophotometer, Roche, USA).

*Hematoxylin & eosin (H&E) staining*

Routine H&E staining was conducted using 3–5 μm thick slices of tissue sample as described in a previous study.[32]

*Immunohistochemistry (IHC)*

Slides were dried in an oven at 800 °C for 25–30 min, before dewaxing with xylene, alcohol, and distilled water. To suppress nonspecific antibodies, slides were treated with 3 % hydrogen peroxide for 10 min, before extensive rinsing with distilled water. Following antigen retrieval, slides were placed in a pressure cooker for 8 min at 120 °C with EDTA buffer, and then permitted to cool for an additional 10 min within the cooker. The residual EDTA buffer was removed from the slides by rinsing with phosphate-buffered saline (PBS). Primary antibodies targeting pancreatic tissue (anti-islet 1 antibody) [1B1], kidney-specific (Ksp) cadherin (anti-Ksp-cadherin), and uterine tissue (anti-MLH-1 antibody) were obtained from Sigma–Aldrich UK and applied to slides, which were maintained at room temperature for 1 h. The antibody was eliminated by washing the slide three times with PBS. After washing the slides, the amplifier (link) was administered for 15 min and subsequently removed using PBS. A secondary antibody was applied, and the slides were kept in the dark at ambient temperature for 10–15 min. The slides were rinsed with PBS, followed by distilled water to remove any residual secondary antibody. Following washing, a single drop of 3,3′-diaminobenzidine (DAB) chromogen (5:1) was applied for 5 min. Surplus DAB was removed by comprehensive washing. Hematoxylin counter-staining was conducted before rinsing the slides with distilled water. Slides were affixed post-drying with dibutylphthalate polystyrene xylene.

*Sudan Black B staining*

Adipose tissue was stained with Sudan Black B as described by Chiffelle and Putt.[32] Rinsed cryostat sections were immersed in 100 % propylene glycol for 15 min and subsequently differentiated in 85 % propylene glycol twice before staining with Sudan Black B for 10 min. Excess stain was removed by washing thoroughly in distilled water. Sections were affixed using aqueous mounting medium and examined under a light microscope.

*Statistical analysis*

Statistical analyses were performed using SPSS 26.0 (IBM, Incorporation, USA). Continuous variables were compared by using the Student's *t*-test and results were

**Table 1: Body weights (g) of female mice before experiment at baseline.**

| | Mean body weight (g) ± SD | *p*-value |
|---|---|---|
| Group 1. Control mice | 31.03 ± 3.63 | 0.083 |
| Group 2. Chromium-restricted mice (0.125 mg/kg) | 31.79 ± 4.78 | |

**Table 2: Serum chromium (μg) levels in female mice before the experiment at baseline.**

| | Serum chromium levels (μg) mean ± SD | *p*-value |
|---|---|---|
| Group 1. Control mice | 0.24 ± 0.04 | 0.021 |
| Group 2. Chromium-restricted mice (0.125 mg/kg) | 0.08 ± 0.09 | |

**Table 3: Behavior grading system for mice (n = 20).**

| Grade | Criteria |
|---|---|
| Grade 0 | No observable deficit |
| Grade 1 | Slightly abnormal gait |
| Grade 2 | Markedly abnormal gait |
| Grade 3 | Significant mobility problems |
| Grade 4 | Immobility >24 h |
| Grade 5 | Tense and nervous about handling |
| Grade 6 | Marked distress on handling (shaking, vocalizing, aggressive) |

**Table 4: Movement grading system for mice (n = 20).**

| Grade | Criteria |
|---|---|
| Grade 1 | Moving quickly around the cage |
| Grade 2 | Frequently standing at the sides of the cage |
| Grade 3 | Active investigation of surroundings |
| Grade 4 | Reduced movement around the cage |
| Grade 5 | Little or no investigation of the cage |
| Grade 6 | Seeks shelter |
| Grade 7 | Moves around the cage when stimulated |
| Grade 8 | No movement around the cage |
| Grade 9 | Maybe moribund |
| Grade 10 | Typically isolated from cage mates |

expressed as the mean ± standard deviation (SD). Categorical variables were compared using the Chi-square test and results were expressed as frequencies and percentages. In all cases, $p \leq 0.05$ was considered to indicate a significant difference.

## Results

### *Body weight (g) of offspring mice on chromium-restricted diet*

The mean (±SD) body weights of offspring in the chromium-restricted group differed significantly compared with those in the control group from the second month (Figure 1). The weight reductions in both groups were correlated with the serum chromium levels, and the mean (±SD) serum chromium concentration was significantly lower in the chromium-restricted group (0.10 ± 0.1 µg and 0.13 ± 0.02 µg after five and 15 months, respectively) compared with the control group ($p = 0.0001$). Table 5 compares the serum chromium levels and body weights in both groups.

### *Behavior of offspring mice on chromium-restricted diet*

The observed behaviors of mice in the control group exhibited no observable deficits (grade 0) but they differed

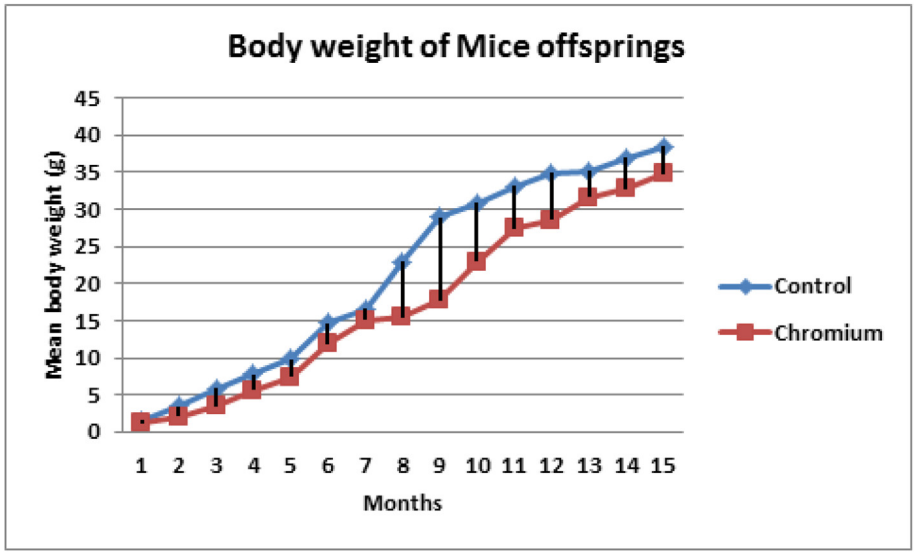


**Figure 1:** Body weights of mice offspring under control and chromium-restricted diets.

**Table 5: Comparisons of body weights and serum chromium levels in mice offspring (n = 20).**

| | Serum chromium (µg) mean ± SD | *p*-value | Body weight (g) mean ± SD | *p*-value |
|---|---|---|---|---|
| **05 Months** | | | | |
| Group 1. Control mice | 0.18 ± 0.0 | 0.0001 | 9.78 ± 0.98 | 0.003 |
| Group 2. Chromium-restricted diet mice (0.125 mg/kg) | 0.10 ± 0.10 | | 7.42 ± 0.52 | |
| **15 Months** | | | | |
| Group 1. Control mice | 0.20 ± 0.05 | 0.0001 | 38.53 ± 0.97 | 0.001 |
| Group 2. Chromium-restricted diet mice (0.125 mg/kg) | 0.13 ± 0.02 | | 34.83 ± 0.94 | |

**Table 6: Behaviors of mice offspring according to the scoring criteria (n = 20).**

| | G1: Control | G2: Chromium-restricted (0.125 mg/kg) | $\chi^2$-value | *p*-value |
|---|---|---|---|---|
| 1st month | 0 | 6 | 14.4 | 0.0001 |
| 2nd month | 0 | 6 | 14.4 | 0.0001 |
| 3rd month | 0 | 6 | 15.71 | 0.0001 |
| 4th month | 0 | 6 | 15.6 | 0.0001 |
| 5th month | 0 | 6 | 15.72 | 0.0001 |
| 6th month | 0 | 6 | 14.61 | 0.0001 |
| 7th month | 0 | 5 | 13.51 | 0.0001 |
| 8th month | 0 | 5 | 12.50 | 0.0001 |
| 9th month | 0 | 5 | 13.51 | 0.0001 |
| 10th month | 0 | 4 | 14.60 | 0.0001 |
| 11th month | 0 | 4 | 14.61 | 0.0001 |
| 12th month | 0 | 4 | 14.65 | 0.0001 |
| 13th month | 0 | 4 | 13.62 | 0.0001 |
| 14th month | 0 | 4 | 14.63 | 0.0001 |
| 15th month | 0 | 4 | 15.52 | 0.0001 |

**Table 7: Movements of mice offspring (n = 20).**

| | G1: Control | G2: Chromium-restricted (0.125 mg/kg) | $\chi^2$-value | *p*-value |
|---|---|---|---|---|
| 1st month | 5 | 8 | 16.71 | 0.0001 |
| 2nd month | 4 | 8 | 17.61 | 0.0001 |
| 3rd month | 3 | 8 | 16.63 | 0.0001 |
| 4th month | 2 | 7 | 17.51 | 0.0001 |
| 5th month | 2 | 7 | 16.65 | 0.0001 |
| 6th month | 1 | 6 | 15.61 | 0.0001 |
| 7th month | 1 | 6 | 14.71 | 0.0001 |
| 8th month | 1 | 6 | 13.75 | 0.0001 |
| 9th month | 1 | 5 | 15.62 | 0.0001 |
| 10th month | 1 | 5 | 14.63 | 0.0001 |
| 11th month | 1 | 4 | 15.72 | 0.0001 |
| 12th month | 1 | 4 | 13.65 | 0.0001 |
| 13th month | 1 | 3 | 15.70 | 0.0001 |
| 14th month | 1 | 3 | 12.65 | 0.0001 |
| 15th month | 1 | 3 | 13.59 | 0.0001 |

significantly compared with those of the chromium-restricted mice, which exhibited significant gait and mobility abnormalities as well as tense, nervous, and aggressive behavior ($p < 0.0001$), as shown in Table 6.

*Movement of offspring mice on chromium-restricted diet*

The chromium-restricted mice exhibited significantly reduced movements, with little or no investigation around the cage and shelter-seeking movements, and no movement around the cage, where they were typically isolated and nearly moribund compared with the control offspring (Table 7).

*Biochemical analyses*

The mean blood glucose level was elevated in the offspring of chromium-restricted mice compared with the control. The mean serum insulin level was also raised in offspring of chromium-restricted female mice compared with the control. After five and 15 months, the blood cholesterol levels were elevated in the chromium-restricted mice compared with the control. The mean blood HDL-cholesterol (HDLc) was lower in the offspring of chromium-restricted female mice compared with the control offspring ($p = 0.0001$), as shown in Table 8.

*Histological and immunohistochemical examinations*

Histological examination detected atrophy of the islets of Langerhans with mononuclear lymphocytic infiltration in the pancreas (Figure 2b) of chromium-restricted mice compared with the control (Figure 2a). Immunostaining with anti-islet 1 antibody indicated a normal pancreatic architecture (Figure 3a), but the chromium-restricted mice had smaller islets of Langerhans (Figure 3b).

Histological analysis of the kidneys of chromium-restricted mice detected dilated hypervascular tubules, hypercellularity of glomeruli, and a few atrophic glomeruli (Figure 4b). Normal appearing glomeruli and renal tubules stained by Ksp-cadherin antibody were observed

**Table 8: Biochemical parameters at the five months of age for all groups (n = 20).**

| Parameters | Groups | | *p*-value |
|---|---|---|---|
| | G1: Control | G2: Chromium-restricted (0.125 mg/kg) | |
| Blood glucose (mg/dL) 5 months | 117.54 ± 28.10 | 165.07 ± 3.94 | 0.0001 |
| Blood glucose (mg/dL) 15 months | 119.54 ± 31.92 | 168.08 ± 0.98 | 0.0001 |
| Serum insulin (mIU/L) 5 months | 8.92 ± 0.73 | 10.33 ± 0.84 | 0.003 |
| Serum insulin (mIU/L) 15 months | 9.36 ± 1.27 | 10.20 ± 1.50 | 0.0001 |
| Blood cholesterol (mg/dL) 5 months | 115.02 ± 31.01 | 164.79 ± 6.51 | 0.0001 |
| Blood cholesterol (mg/dL) 15 months | 118.76 ± 32.25 | 169.52 ± 3.87 | 0.0001 |
| Blood HDLc level (mg/dL) 5 months | 35.17 ± 6.63 | 24.32 ± 1.67 | 0.0001 |
| Blood HDLc level (mg/dL) 15 months | 36.63 ± 6.82 | 26.00 ± 0.68 | 0.0001 |

Results represent the mean ± standard deviation.

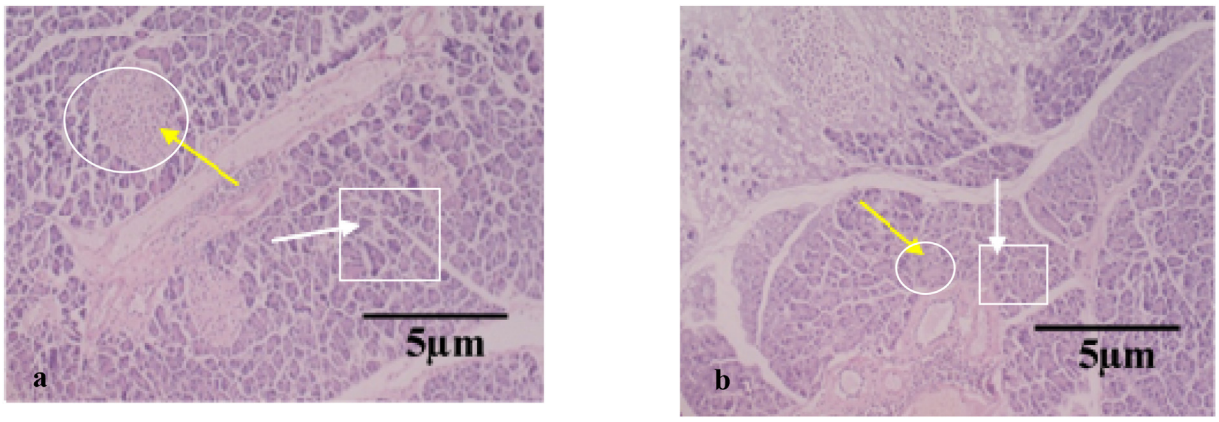


**Figure 2:** (a) Histological examination of pancreatic tissue stained with H&E showing the normal pancreatic architecture in the control, where the white arrow indicates the exocrine part and the yellow arrow indicates the islets of Langerhans. (b) Distorted pancreatic architecture in chromium-restricted mouse, where the white arrow indicates the exocrine part and the yellow arrow indicates the small islets of Langerhans, suggesting a reduction in size or potential damage. Images at 10 × magnification.

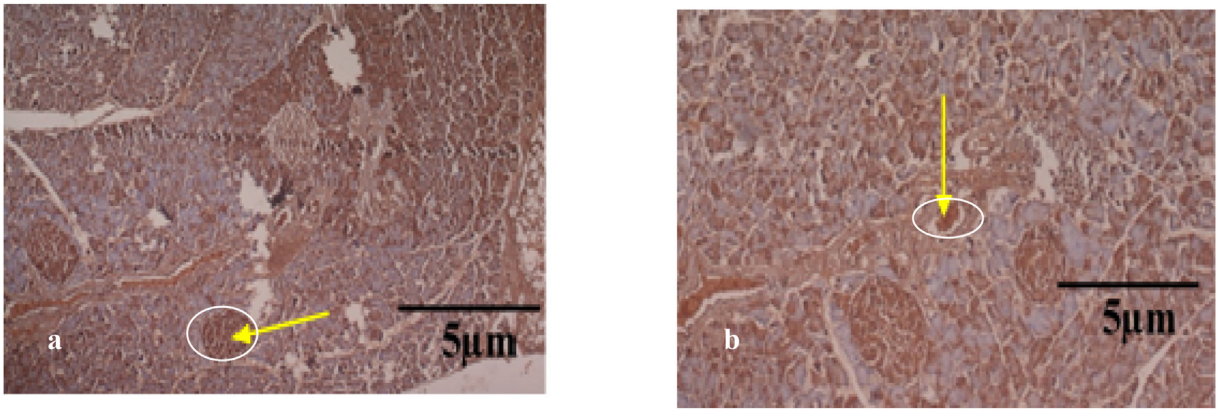


**Figure 3:** IHC results obtained by staining with anti-islet 1 antibody. (a) Normal pancreatic tissue section at 10 × magnification showing islets of Langerhans (yellow arrow) in control stained with anti-islet 1 antibody. Pancreatic tissue from chromium-restricted mouse stained with anti-islet 1 antibody at 10 × magnification showing three visible islets (yellow arrow) and one shrunken islet (white arrow), indicating possible structural alterations.

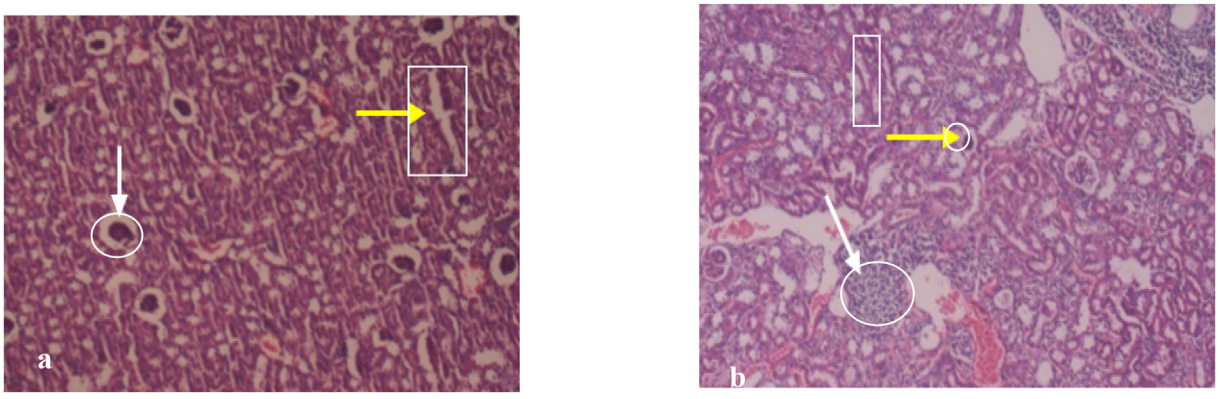


**Figure 4:** (a) H&E stained histological examination of renal tissue showing normal renal glomeruli (white arrow) and renal tubules (yellow arrow). (b) Section of kidney from chromium-restricted mouse showing interstitial mononuclear lymphocytic infiltration (white arrow) and two to three areas with glomerular fibrosis (yellow arrow), suggesting structural damage. Images at 10 × magnification.

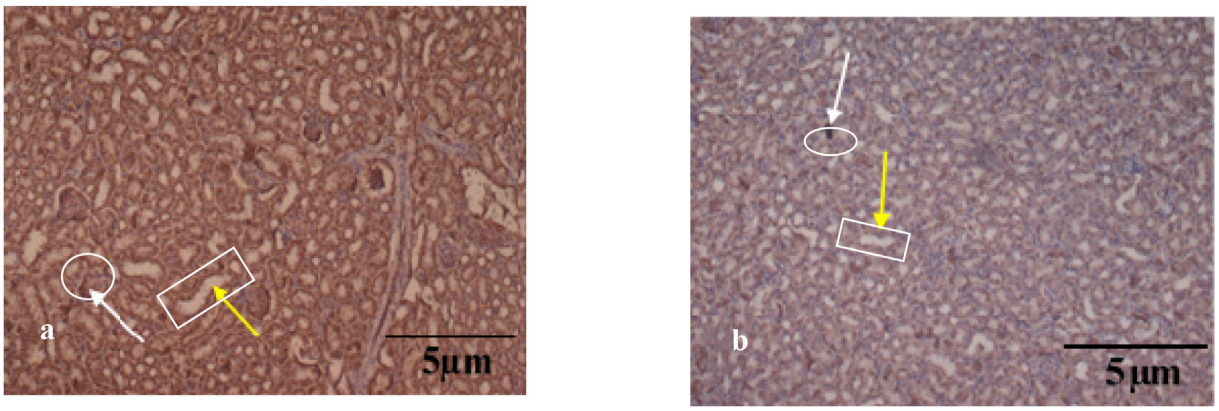


**Figure 5:** (a) IHC results at 10 × magnification for the control showing normal appearance of the kidney with glomeruli (yellow arrow) and tubules (White arrow) after staining with anti-Ksp-cadherin. (b) IHC results at 10 × magnification for the kidney of a chromium-restricted mouse stained with anti-Ksp-cadherin antibody showing glomerular fibrosis (white arrow) and reduced staining by the marker in the tubules (yellow arrow) due to infiltration of a chronic inflammatory cell (fibroblast).

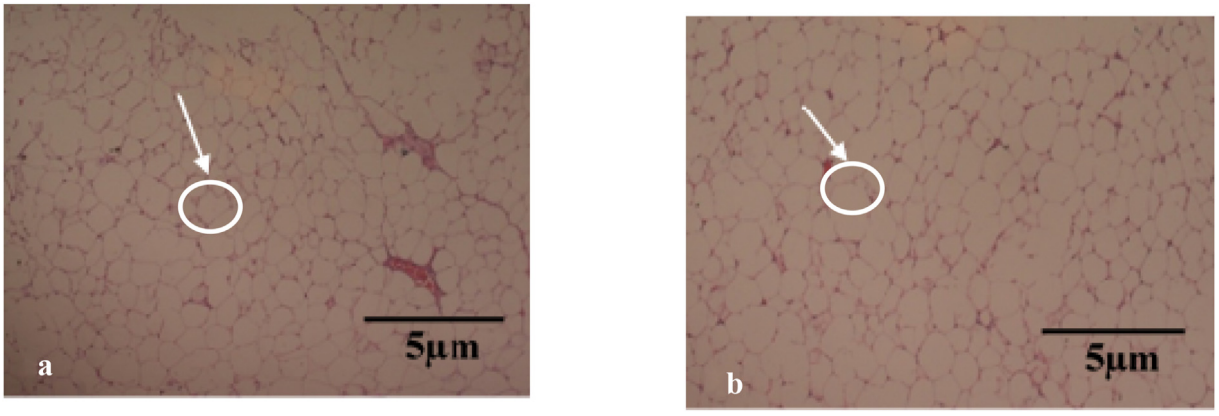


**Figure 6:** (a) Section of adipose tissue from the control with H&E staining, showing normal adipocytes at 10 × magnification. The white arrow indicates adipocytes. (b) Section of adipose tissue from a chromium-restricted mouse with H&E staining showing normal adipocytes (white arrow) at 10 × magnification.

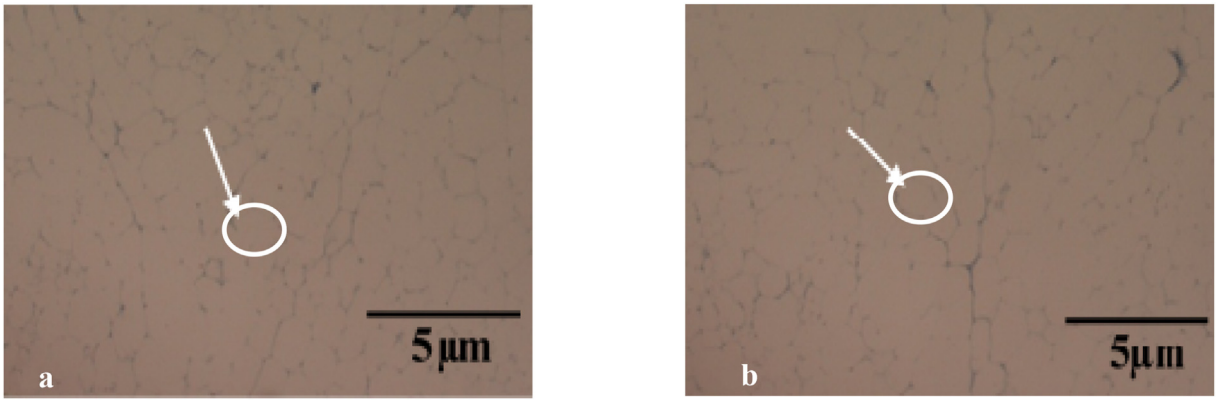


**Figure 7:** (a) Section of adipose tissue from control stained with Sudan Black B showing normal adipocytes at 10 × magnification. The white arrow indicates adipocytes. (b) Section of adipose tissue from a chromium-restricted mouse stained with Sudan Black B showing normal adipocytes at 10 × magnification. The white arrow indicates adipocytes.

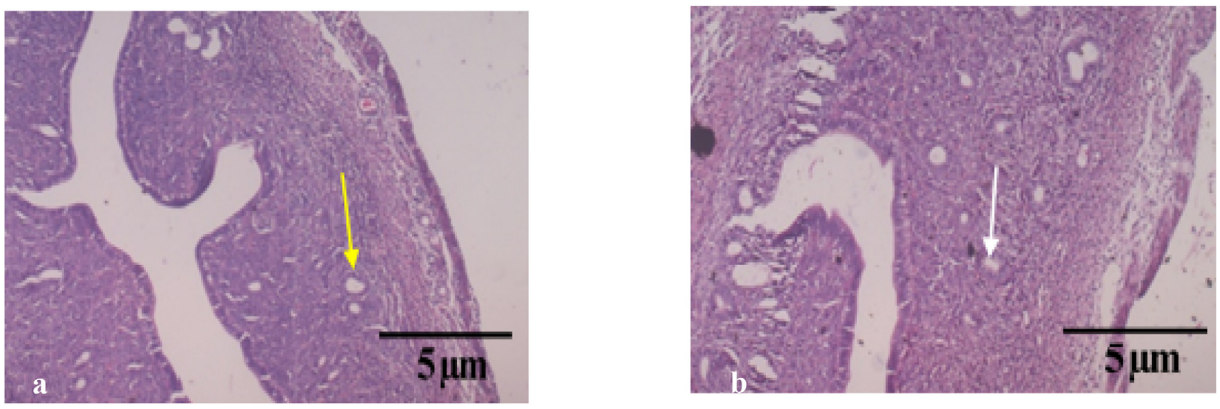


**Figure 8:** 8(a) Section of uterus from the control stained with H&E showing normal endometrium with endometrial glands (yellow arrow) at 10 × magnification. (b) Section of uterus from a chromium-restricted mouse stained with H&E showing normal endometrial glands (white arrow) at 10 × magnification.

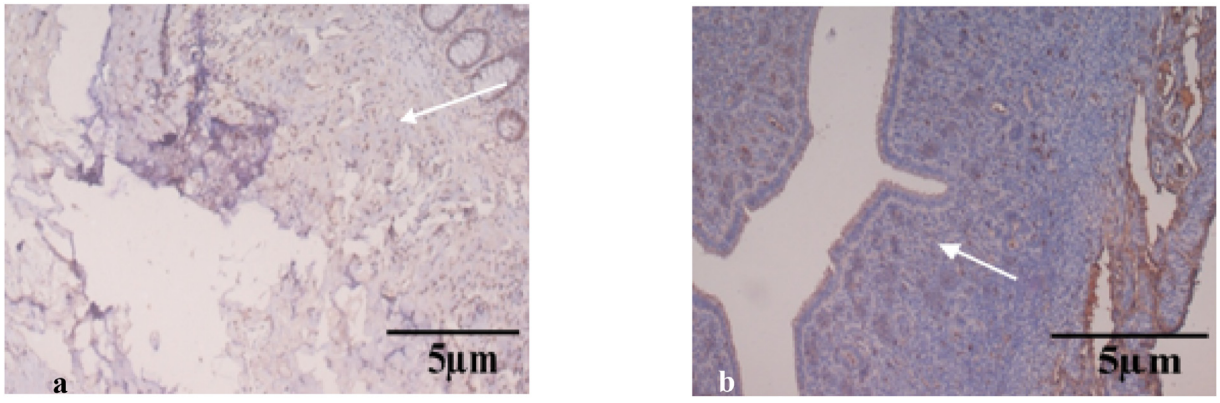


**Figure 9:** (a) IHC analysis of uterus tissue from the control group showing nuclear staining (yellow arrow) with anti-MLH. (b) IHC analysis of uterus tissue from a chromium-restricted mouse stained with anti-MLH 1 showing cytoplasmic staining of endometrial tissue (white arrow) at 20 × magnification.

in the control (Figure 5a). Chromium-restricted mice (Ksp-cadherin staining) had atrophic renal tubules and glomerular fibrosis (Figure 5b).

Analysis of adipose tissue (Figures 6a, 6b) and uterine tissue (Figures 8a, 8b) samples found no histological abnormalities in the chromium-restricted mice after H&E staining. Sections stained with Sudan Black B dye also contained no cytostructural abnormalities in the adipose tissues of chromium-restricted pups compared with the control (Figures 7a, 7b). However, abnormal cytoplasmic staining of endometrial cells with anti-MLH 1 antibody was observed in chromium-restricted mice, and nuclear damage was evident compared with the control (Figures 9a, 9b).

## Discussion

This study investigated the potential effects of a low chromium diet on adult-onset illnesses when exposure occurred in pregnancy. The experimental strategy in the current study involved depriving pregnant female mice of chromium by restricting its availability in their diet. The experiment was successful as the serum chromium levels were lower in the offspring of female mice who were fed a chromium-deficient diet during pregnancy. The body weights did not differ significantly during the first month ($p > 0.05$), but in the subsequent months up to the 15th postnatal month, the body weights of chromium-restricted mice offspring were significantly lower ($p = 0.0001$), probably due to the requirement for chromium for the biological activities of important enzyme systems for somatic growth. Blood glucose metabolism was also impaired according to the higher mean (±SD) blood glucose levels in the chromium-restricted offspring, and this is a common manifestation of the epigenetic impact of chromium shortage during fetal development, resulting in hyperglycemia in offspring during postnatal life.

The behavioral and movement grades were significantly different in chromium-restricted mice compared with the control, probably because the biochemical markers of their glucose, insulin, and HDL levels deteriorated. The results demonstrated that the behavior and mobility of chromium-restricted offspring were abnormal in postnatal life. A previous study[33] also showed that chromium deficiency is uncommon in animals and results in peripheral

neuropathy, which manifests as gait and mobility impairments similar to those observed in the present study. A placebo-controlled, double-blind study reported in 2010 investigated the effects of chromium supplementation on elderly individuals with mild cognitive impairment and showed that chromium supplementation reduced semantic interference in learning, improved recognition in memory tasks, and aided recall. According to this study, insulin resistance is associated with the histological alterations seen in Alzheimer's disease, and thus managing glucose intolerance in people with mild cognitive impairment may mitigate the risk of dementia development.[34] An evaluation of the effects of vitamin and mineral supplementation on individuals with mild cognitive impairment concluded that the quality of studies of chromium supplementation was insufficient to make definitive conclusions.[35]

The findings obtained in the present study support the involvement of chromium in blood glucose regulation. The chromium-restricted diet resulted in hyperglycemia and hyperinsulinemia in offspring, and these two changes indicate the metabolic defect known as insulin resistance in postnatal life, predisposing to adult diseases. Studies have demonstrated the beneficial effects of trace elements such as chromium on glucose homeostasis and insulin secretion. At the molecular level, chromium participates in insulin signaling, thereby regulating blood glucose levels at target cell membranes.[36,37] Our findings regarding hyperglycemia and hyperinsulinemia support those obtained in previous studies,[38] which indicated that chromium supplementation enhanced glucose metabolism. However, another previous study[39] indicated that chromium had no effect on blood glucose improvement, which contradicts our findings. This difference may be due to inadequate research control, researcher prejudice, statistical errors, and material collection fallacies, as well as the use of outmoded blood glucose analytical techniques. We detected glucose using the blood glucose oxidase method, which is a highly sensitive approach, and insulin using the highly sensitive and specific ELISA detection technique. Previously, Padmawati et al. observed a decrease in HbA1c and an increase in HDLc, but no change in fasting blood glucose.[38] The first findings are consistent with our results but the detection of no change in fasting blood is inconsistent our results. This difference could be due to the study design, experimental duration, research bias, blood sample processing, and laboratory biochemical estimation procedures. The findings obtained in a systematic study indicated that chromium supplementation in animals may reduce the levels of malondialdehyde, thiobarbituric acid reactive substances (TBARS), 4-hydroxynonenal, protein carbonyls, and other indicators of lipid peroxidation. Furthermore, the outcomes of clinical trials in a similar study indicated that chromium supplementation decreased the levels of TBARS. Furthermore, the results obtained in animal studies indicate that chromium supplementation enhances the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferases, and may elevate the levels of glutathione and the total antioxidant capacity. However, although chromium supplementation enhanced the levels of antioxidant biomarkers in clinical studies, these changes were not statistically significant, except in one study. These discrepancies may be attributed to variations in the dosage and duration of therapies, as well as factors such as sex, age, genetics, physical activity, food intake, and other confounding variables, including a family history of diabetes mellitus.[40]

We also investigated the effects of the chromium-restricted intrauterine diet on blood lipids and detected dyslipidemia according to the elevated blood cholesterol level and reduced HDLc level, which both lead to an atherogenic tendency similar to insulin resistance. Our detection of dyslipidemia agrees with the results obtained in previous research.[41,42] Another study showed that chromium supplements reduced the serum cholesterol level and enhanced the HDLc level but without changing the blood triglycerides.[41] A chromium-restricted diet obtained comparable results in the present study, thereby validating these findings, possibly because chromium deficiency affects the enzyme systems involved in lipid metabolism during fetal development, resulting in epigenetic effects in postnatal life.

Histological examinations of the control mice demonstrated that the pancreas, adipose tissue, kidneys, and uterus were normal, whereas histological abnormalities were found in the chromium-restricted mice, such as small islets of Langerhans in the pancreas and dilated renal tubules with mononuclear lymphocytic infiltration in the kidneys. The kidneys were found to be hypervascular, with atrophic tubules and glomerular fibrosis. The uterine cavity, endometrial glands, and myometrium all appeared normal in the controls. However, the chromium-restricted mice exhibited anomalies, such as hemosiderin buildup, the formation of cholesterol clefts, and bleeding (anti-islet 1 antibody). The kidneys (anti-Ksp-cadherin staining) were characterized by dilated renal tubules and glomerular fibrosis, as well as hyalinization and tubule dilation. IHC anti-MLH antibody staining of uterus tissues indicated that the uterine lining, glands, and myometrium were normal, whereas abnormal cytoplasmic staining of endometrial cells was observed in chromium-restricted mice. In this study, the pancreas, kidneys, adipose tissue, and uterus were histologically and immunologically analyzed for the first time in the offspring of mice fed a diet deficient in the trace mineral chromium. Histological and immunohistochemical examinations detected anomalies that demonstrated the long-term impacts of a chromium-restricted diet during fetal life, which could result in adult illnesses.

## Conclusion

Abnormalities were observed in terms of behavior and mobility in chromium-restricted mice offspring. These offspring also had higher blood glucose, serum insulin, cholesterol, and HDLc levels compared with the controls. Anti-islet-1, anti-Ksp-cadherin, and anti-MLH antibody staining confirmed the findings obtained by H&E staining. Biochemical, histological, and immunolabeling studies indicated that chromium restriction had adverse impacts on the pancreas, kidneys, and uterus parenchyma, which ultimately led to metabolic changes and disorders later in life.

## Study limitations

This animal study involved the generation of restricted models, and thus further evaluations are required in other species and with different levels of chromium restriction. In addition, the blood samples were only collected after two intervals and they did not fully represent the effects throughout the study period. Knockout models could not be generated due to limited resources.

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## Conflict of interest

The authors have no conflicts of interest to declare.

## Ethical approval

This study was approved by the research ethics committee at LUMHS, Jamshoro (No. LUMHS/REC/-95 dated October 3, 2013) in compliance with the recommendations set forth by the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 85–23, revised 1985).

## Authors contributions

Samreen Memon: study concept, article preparation, supervision, revision. Umbreen Bano: performing the experiments, article writing, data compilation. Syna Singha: experimentation, data collection and compilation. MYS: laboratory work, data collection. Pashmina shaikh: laboratory work, article writing. Shazia Shahani: statistical analysis, draft review. SSM: laboratory experiments, data collection and compilation, revision of draft manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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