Chromium and modulation of adiposity

Chronic maternal dietary chromium restriction modulates visceral adiposity: probable underlying mechanisms

Running title: Chromium and modulation of adiposity

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Objective- We demonstrated earlier that chronic maternal micronutrient restriction altered the body composition in rat offspring and may predispose them to adult onset diseases. Chromium regulates glucose and fat metabolism. The aim of this study is to determine the long term effects of maternal Cr restriction on adipose tissue development and function in a rat model.

Research design and Methods- Female, weanling WNIN rats received ad libitum, a control diet or the same with 65% restriction of Cr (CrR) for three months and mated with control males. Some pregnant CrR mothers were rehabilitated from conception or parturition and their pups weaned to control diet. While some CrR offspring were weaned to control diet, others continued on CrR diet. Various parameters were monitored in the offspring at three monthly intervals up to 15-18 months of age.

Results- Maternal Cr restriction significantly increased body weight and fat %, specially the central adiposity in both male and female offspring. Further the expression of leptin and 11β-HSD1 genes were significantly increased in CrR offspring of both the genders. Adipocytokine levels were altered in plasma and adipose tissue, circulating triglyceride and FFA levels were increased albeit in female offspring only. Rehabilitation regimes did not correct body adiposity but restored the circulating levels of lipids and adipocytokines.

Conclusions- Chronic maternal chromium restriction increased body adiposity probably due to increased stress and altered lipid metabolism in WNIN rat offspring, which may predispose them to obesity and associated diseases in their later life.
The fetal origins of adult disease hypothesis proposes that environmental factors can redirect the developmental path of the fetus such that the fetus adapts for survival in an environment in which the resources are limited (1). These adaptations contribute to poor fetal health outcomes resulting in the “thrifty phenotype”. Exposure of such a thrifty phenotype to excessive nutrition postnatally, overloads its reduced metabolic capacity which could manifest in metabolic disorders such as obesity, cardiovascular disease and type II diabetes in later life (2). Robust evidence (epidemiological and experimental) suggests that manipulation of maternal nutrition (macro or micronutrient restriction) during pregnancy leads to metabolic abnormalities, develop insulin resistance (IR) and its associated complications in the offspring (3; 4). Many studies report that central adiposity correlates strongly with IR (5).

Accumulation of adipose tissue, a major storage site for fat deposition leads to obesity. Adipose tissue differentiation is a highly regulated process, taking place from birth throughout adult life. The transcriptional factors: peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element-binding proteins (SREBPs) regulate the expression of genes involved in adipogenesis and lipid metabolism (6; 7). Expression of PPARγ in adipose tissue promotes the differentiation of preadipocytes and regulates the expression of fat cell-specific genes (8). SREBPs modulate lipogenesis and cholesterol homeostasis and SREBP-2 overexpression increases fatty acid synthase (FAS) gene expression (9). Adipose tissue differentiation is also regulated by glucocorticoid hormone (10). Glucocorticoid over secretion results in the manifestation of central adiposity, visceral obesity, IR, hypertension and dyslipidemia (11; 12).

Glucocorticoid-mediated effects in target tissues are regulated by the 11β hydroxysteroid dehydrogenase 1 (11β-HSD1), a NADPH dependent bidirectional enzyme (13). It reduces cortisone to active cortisol and is expressed in many tissues including the liver, adipose and skeletal muscles. Adipose tissue, currently considered as the biggest endocrine organ, secretes adipocytokines like adiponectin, leptin, PAI, IL-6, TNFα etc (14), which regulate energy metabolism, insulin sensitivity and play a vital role in the pathogenesis of obesity, atherosclerotic vascular disease, hypertension and diabetes mellitus (15).

Chromium, an important trace element, regulates carbohydrate and fat metabolism (16). Many investigations in humans and animal models suggest that Cr supplementation reduces body weight, regulates hunger and also decreases body fat (16-18). Cr supplementation is reported to decrease plasma total cholesterol and triglycerides, increase HDL-cholesterol and lower body weight in diabetics (16). However the effect of Cr deficiency per se on lipid / fat metabolism or obesity has not been studied, let alone the effect of peri / postnatal dietary Cr restriction on the development and function of the adipose tissue in the offspring. Based on the available evidence we hypothesized that perinatal and postnatal dietary Cr restriction modulates body adiposity and adipose tissue function in the offspring. The present study has been conducted in Wistar/NIN (WNIN) rats to validate / negate this hypothesis and to elucidate the associated mechanisms.

RESEARCH DESIGN AND METHODS

Study design. All experimental procedures were approved by the “Institute’s ethical committee on animal experiments” at National Institute of Nutrition, Hyderabad, India and were performed in accordance with
the ‘principles of laboratory animal care’ (NIH publication no. 85-23, revised 1985). Wistar/NIN (WNIN) female, weanling rats (n=30) were obtained from the National Centre for Laboratory Animal Sciences (NCLAS) at National Institute of Nutrition, Hyderabad, India.

The animals were divided into two groups of 6 and 24 rats, housed individually in wire mesh bottomed polypropylene cages and maintained under standard lighting conditions (12-hour light/dark cycle). Temperature and relative humidity were kept constant at 22 ± 2°C and 55 ± 10%, respectively. The diets (casein-based, 18 % protein) were prepared according to AIN-93G formulation and analyzed for Cr content in an Atomic Absorption Spectrometer (Varian Atomic Absorption Spectrometer, Spectra AA 220, Walnut Creek, CA) using reduced flame (19). Cr restricted diet was prepared by excluding the chromium salt from the mineral mixture that was added to the diet. The group of 24 rats received ad libitum, the Cr restricted (CrR) diet (0.51 mg Cr / kg diet) for 12 weeks while the group of 6 rats received the control diet (1.56 mg Cr / kg diet) with free access to de-ionized water. Daily food intake and weekly body weights were monitored in these rats till the end of the feeding regimen. Plasma Cr levels were determined in them at the end of 12 weeks of feeding the respective diets.

The animals were then mated with control males (two females with one male) and the day a vaginal plug was detected, was counted as day one of pregnancy. From this day of conception, six of CrR pregnant dams were switched to control diet (CrRC) and their offspring weaned on to control diet. Another six CrR mothers were rehabilitated with control diet from parturition (CrRP) and their offspring weaned on to control diet. The remaining twelve CrR mothers continued on CrR diet during lactation. Litter size was adjusted to seven in all groups on postnatal day three and maintained so throughout lactation. At weaning (postnatal day 21), half of CrR offspring were weaned onto control diet (CrRW) while the remaining pups were continued on CrR diet (CrR) throughout their life. Considering the high mortality in the female offspring beyond 15 months of their age, female offspring were followed up to 15 months of age and for similar reasons the male offspring were studied up to 18 months of age. The feeding protocol used in this experiment is presented schematically (Fig. 1).

**Plasma chromium status.** Plasma Cr levels were monitored once every 3 months in the offspring by atomic absorption spectrometer using graphite furnace (Thermo electron corporation, GFS97 SOLAAR AA Series, Cheshire, CW) according to Mahalingam et al (20).

**Body composition.** Body composition of the offspring was determined from three months of their age using TOBEC (Total Body Electrical Conductivity), a small animal body composition analysis system (EM – SCAN, Model SA – 3000 Multi detector) (Springfield, IL, USA) as described by us earlier (21; 22). Total body fat % was obtained mathematically by following Morbach and Brans’ method (23).

**Adiposity Index (AI).** Adiposity index, an index of visceral adiposity was computed according to Taylor et al (24). The wet weights of the retroperitoneal, mesenteric and epididymal / gonadal fat pads were determined and the adiposity index was computed using the following formula:

\[
\text{Adiposity index} = \left( \text{sum of the weights of the three fat deposits} / \text{body weight} \right) \times 100
\]

**Plasma lipid analysis.** Total cholesterol, triglycerides and HDL-cholesterol levels were determined in plasma using enzymatic assay kits from Biosystems (Barcelona, Spain). Plasma free fatty acids (FFAs) were determined using the enzymatic kit from Randox (Antrim, United Kingdom).
Adipocytokines in plasma and adipose tissue. Concentrations of adiponectin, leptin, PAI, IL-6 and TNF \( \alpha \) were determined in fasting plasma and adipose tissue using Lincoplex research kits (Linco Research, St. Louis, MO) on a BIOPLEX platform (BioRad). Adipose tissue homogenate was prepared as described by us earlier (25). The protein content in plasma and adipose tissue lysate was determined using bicinchoninic acid assay (26).

Quantitative analysis of genes by RT-PCR in the offspring. Retroperitoneal fat tissue was dissected from the male and female offspring of all the groups at the time of their sacrifice and stored frozen immediately at - 80°C. Total RNA was isolated from ~100mg of the adipose tissue using TRIzol reagent according to manufacturer’s instructions (Invitrogen Life technologies, Carlsbad, CA). cDNA was synthesized from 2 \( \mu \)g of total RNA using Invitrogen kit (Invitrogen Life technologies, Carlsbad, CA). Primers were designed with the aid of primer quest software (Integrated DNA Technologies, Coralville, IOWA). Semi-quantitative PCR was conducted to analyze the expression of PPAR\( \gamma \) (5’CCCATCTCTTGTACATCAAACC3’; 5’ATTGTGACACTCCCCCAAGC3’), SREBP 2 (5’AAGTCTGGCGTTCTGAGGAA3’; 5’CAGCCAAGGTGGAGGACAT3’), 11\( \beta \)-HSD1 (5’GCCCTGTGTTCTCTAGAATC3’; 5’AGTCCACATCGCCACTAC3’), adiponectin (5’CTACTGTTGCAAGCTCTCC3’; 5’CTTCACATCTTTCATGCCACTAC3’), leptin (5’GAGACCTCTCCATCTGCTG3’; 5’CTTCAGGGCTAAGGTCCAA3’) and FAS (5’TGGACATGTGCTGAGGAC3’; 5’TCAAAAATGTGCAATGCAGAC3’) with the internal control 18S rRNA (5’CCAGAGCGAAAGCATTTGCCAAGA3’; 5’AACCAACGCAAGCTTATGACCAGC3’).

The amplicons were resolved electrophoretically on 1.2% agarose gels prestained with ethidium bromide. The image was captured in a Chemidoc system (Bio-Rad Laboratories, Hercules, CA) and quantitated using Quantity One software (Bio-Rad). Results have been expressed as the ratio of the intensities of the band of the target gene to that of the 18S rRNA.

Statistical analysis. All values are presented as means ± S.E. Data was analyzed using unpaired student’s t test to identify differences between control and restricted mothers. One-way ANOVA followed by the multiple range test or least significant difference method was used appropriately to analyze data in the offspring. Wherever heterogeneity of variance was observed, differences between groups were tested using non-parametric Mann-Whitney U test. The differences were considered significant at \( p < 0.05 \).

RESULTS

Growth characteristics, chromium status and lipid profile in WNIN rat dams. As expected the plasma Cr levels were significantly decreased (\( p<0.05 \)) in CrR rats compared to CrC (Table 1). Food intake was significantly (\( p<0.05 \)) but marginally higher in CrR than CrC rats (Table 1). Body weight gain was comparable among the two groups. Indeed, there were no significant differences between the two groups of rats in the levels of plasma total cholesterol, HDL-cholesterol, triglycerides and FFAs (Table 1).

Growth characteristics of the offspring. Food intake was comparable among the offspring (of both the genders) of different groups at all the time points studied. However, CrR offspring (male and female) weighed significantly higher (\( p < 0.05 \)) than CrC from 12 months of age (Fig. 2A and B) till the time of their sacrifice. In male offspring all three rehabilitation regimes corrected the change at 12 months of age, whereas CrRP but not CrRC or CrRW restored the change to control levels at 18 months (Fig. 2A). In female offspring CrRP but not CrRC and CrRW could correct the insult at 12 months whereas none of them
could do so at 15 months of their age (Fig. 2B). Plasma Cr levels were significantly (p < 0.05) decreased in CrR than CrC offspring at all time points studied and the rehabilitation regimes restored them to control from as early as three months of their age (Fig. 2C and D).

**Body fat % and adiposity index of the offspring.** Body fat % of the male CrR offspring was significantly higher than CrC (Fig. 3A) at 18 months of age but not earlier. CrRC but not CrRP or CrRW corrected this insult. Similarly female CrR offspring had significantly (p<0.05) higher body fat % than CrC, albeit from 3 months of age (Fig. 3C). Although all three rehabilitation regimes appeared to correct the change at 12 months of their age only CrRC could correct the change at 15 months. Wet weights of the epididymal, mesenteric and retroperitoneal fat deposits were significantly (p<0.05) higher in CrR than CrC offspring (of both genders) and no rehabilitation regime could correct these changes (Fig. 3B and D). Accordingly, the computed values of adiposity index were significantly (p<0.05) higher in CrR than CrC offspring and rehabilitation in general did not correct the increased adiposity index.

**Plasma lipid profile.** Plasma lipid profile (triglycerides, total cholesterol, HDL cholesterol and FFAs) was comparable among male offspring of different groups at all the time points tested (data not given). However in female offspring, plasma triglycerides and FFAs were significantly higher (p<0.05) in CrR than CrC from 9 months of age but not earlier and all three rehabilitation regimes restored them to control levels (Fig. 5A and B). Total and HDL cholesterol levels were comparable among the female offspring of different groups at all time points studied (data not given).

**Adipocytokine levels in plasma.** Plasma adiponectin levels were comparable among male offspring of all the groups. In female offspring, although plasma adiponectin levels were comparable among CrR and CrC groups, CrRC and CrRP significantly (p<0.05) increased them (Table 2), while CrRW had no effect. Leptin levels were significantly higher (p<0.05) in CrR than CrC albeit in female offspring only; and all three rehabilitation regimes restored them to control levels. Interestingly, TNFα levels were significantly higher (p<0.05) in CrR than CrC offspring of both the genders; and all three rehabilitation regimes corrected the change (Table 2). However the levels of circulating IL-6 and PAI (active) were comparable among the groups in both male and female offspring (Table 2).

**Adipocytokines in adipose tissue.** Adiponectin and PAI (active) levels in adipose tissue homogenate were significantly (p<0.05) reduced and increased respectively in male CrR than CrC offspring (Table 3). Surprisingly, adiponectin levels were corrected in CrRW but not CrRC or CrRP offspring, whereas PAI levels were corrected in CrRC and CrRP but not CrRW offspring. The levels of other cytokines i.e. leptin, TNFα and IL-6 in the adipose tissue homogenate were comparable among all the groups (Table 3). Unlike male offspring, in females neither maternal Cr restriction nor rehabilitation affected the expression of the adipocytokines studied (Table 3).

**Quantitative analysis of genes involved in adipose tissue development and function.** Expression of leptin and 11β-HSD1 genes were significantly increased in the adipose tissue of CrR offspring of both the genders compared to the corresponding controls (Fig. 4A and B). In male offspring, change in 11β-HSD1 expression was corrected by all three rehabilitation regimes, whereas CrRC and CrRP but not CrRW could correct the change in leptin expression. On the other hand in female offspring, CrRP but not CrRC and CrRW showed comparable effect on the expression of both leptin and 11β-HSD1 genes. However, expression of PPARγ, SREBP2, adiponectin and FAS genes
did not show any significant change among the offspring of different groups (Fig. 4A and B).

DISCUSSION

We demonstrated earlier that restriction of micronutrients in utero increased body fat % and central adiposity in the offspring (21; 22; 25; 27; 28). Considering the importance of chromium in maintaining carbohydrate / lipid metabolism and modulating body composition in diabetics (29), we investigated the effects of peri / postnatal Cr restriction on the development of adiposity and the associated mechanisms in the WNIN rat model.

The marginally higher diet intake CrR than CrC rats is in disagreement with increased food intake reported on Cr picolinate supplementation (30). Consistent with earlier reports (27; 31) we observed no changes in the plasma lipid profile of CrR rats despite increased food intake. This could be due to moderate Cr deficiency and / or insufficient duration of Cr restriction.

Although it did not affect food intake in the offspring, peri / postnatal Cr restriction decreased plasma Cr in CrR pups from 3 months of age and all rehabilitation regimes restored them to control levels besides increasing their body weights. However, rehabilitation corrected body weight changes in male offspring partially but not in females. Though in line with earlier studies on the effects of Cr and vitamin A (17; 32) on body weight, these findings contradict reports on maternal micronutrient restriction lowered body weight in rat offspring (21; 27) and Cr supplementation increased body weight in pigs (29). However, some studies showed that chromium picolinate did not affect the body weight (33). Indeed no studies till date reported the effect of maternal Cr restriction on the offspring’s body weight.

Most often, high body adiposity precedes insulin resistance (34). In this study, maternal Cr restriction increased body fat % in males at 18 months of age whereas in females it increased from 3 months of age and only CrRC could correct these changes. These findings concur with the decreased fat% reported in obese subjects on Cr supplementation (17) and our previous reports that maternal mineral restriction had similar effects in rat offspring (21; 22; 25; 27). These observations suggest the importance of Cr during gestation and lactation in modulating body fat in offspring.

Increased visceral fat is usually associated with obesity and attendant metabolic disorders (35; 36). That chronic maternal Cr restriction significantly increased adiposity index in the offspring and rehabilitation did not correct it, stress the importance of Cr during growth, gestation and lactation in modulating visceral adiposity in offspring. These results agree with those reported in vitamin A restriction (32) and our findings in offspring of Mg restricted rats (25; 27) indicate that maternal Cr restriction increased adiposity, specially visceral adiposity in offspring and may hence predispose them to IR and associated diseases in later life.

Fasting hypertriglyceridemia and / or low HDL cholesterol levels are associated with IR (37). The increased plasma triglycerides and FFAs in female CrR offspring are in line with high plasma triglycerides reports in the offspring of micronutrient restricted rats (21; 22; 27), altered lipid metabolism in pups of protein or iron restricted rats (38) and effect of Cr supplementation on lipid metabolism and fat deposition in lambs (39).

Altered adipocytokines underlie the development of adiposity and IR (15) Our observations that maternal Cr restriction increased plasma TNFα in male and female CrR offspring but increased leptin only in females agree with similar reports in offspring of vitamin restricted rats (21).
Further, they corroborate reports: i) hypocaloric diet reduced leptin and TNFα but not adiponectin and PAI-1 levels in plasma (40) and ii) mice fed conjugated linoleic acid and chromium along with high fat diet had lower plasma leptin levels (41). That rehabilitation could correct changes in plasma adipocytokines but not body fat % or visceral adiposity suggest that rehabilitation may correct the associated biochemical changes but not the maternal Cr restriction induced visceral adiposity in rat offspring.

That maternal Cr restriction decreased adiponectin and increased PAI levels in adipose tissue of male offspring and rehabilitation partially corrected the changes suggest its role in modulating adipose tissue function in offspring which may predispose them to IR and associated diseases in later life. However our observation, that the lack of changes seen in leptin and TNFα levels in adipose tissue despite their increased levels in circulation was perplexing. Taken together, these observations suggest that maternal Cr restriction may affect the expression of adipocytokines differentially and variably in male and female offspring. The possible reasons for these discrepant observations on adipocytokines are not clear at present.

Expression of PPARγ, SREBP2 and 11β-HSD1 in adipose tissue modulate obesity / visceral adiposity, dyslipidemia, IR and associated complications (8; 42; 43). Our observation that 11β-HSD1 and leptin expression upregulation but not of PPARγ, SREBP2, adiponectin and FAS in CrR offspring and their partial correction by rehabilitation are in agreement with the upregulation of leptin and 11β-HSD1 reported in diabetics (44) and adipose tissue dysregulation in rats through high fat induced over expression of 11β-HSD1 (45). It thus appears that increased expression of 11β-HSD1 and leptin may underlie enhanced body adiposity (fat % & visceral adiposity) in the offspring.

In conclusion, this study has demonstrated for the first time to the best of our knowledge that chronic maternal Cr restriction increased visceral adiposity and modulated adipose tissue function in rat offspring. The up-regulation of 11β-HSD1 and leptin may underlie increased adiposity in these offspring. Finally this study reiterates the importance of Cr during peri / postnatal period in the development and function of adipose tissue in the offspring which may predispose them to obesity and IR in later life.

ACKNOWLEDGEMENTS

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REFERENCES


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**TABLE 1**
Diet intake, physical and lipid profile in WNIN female rats fed control and chromium restricted diets for 12 weeks before mating.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CrC (gms)</th>
<th>CrR (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake</td>
<td>9.73 ± 0.295</td>
<td>10.7 ± 0.107*</td>
</tr>
<tr>
<td>Body weight gain</td>
<td>107 ± 5.46</td>
<td>116 ± 2.31</td>
</tr>
<tr>
<td>Plasma Cr conc. (µg/L)</td>
<td>1.18 ± 0.181</td>
<td>0.648 ± 0.058*</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>1.46 ± 0.068</td>
<td>1.52 ± 0.098</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.16 ± 0.078</td>
<td>1.12 ± 0.084</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.433 ± 0.037</td>
<td>0.492 ± 0.035</td>
</tr>
<tr>
<td>Free fatty acids (mmol/L)</td>
<td>0.803 ± 0.064</td>
<td>0.813 ± 0.050</td>
</tr>
</tbody>
</table>

Values are mean ± SE (n=6). Control Vs Restricted *p<0.05 using student ‘t’ test

**TABLE 2**
Plasma adipocytokine levels of different groups of male and female offspring

<table>
<thead>
<tr>
<th></th>
<th>CrC (µg/ml)</th>
<th>CrR</th>
<th>CrRC</th>
<th>CrRP</th>
<th>CrRW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>31.5 ± 3.00</td>
<td>31.5 ± 3.90</td>
<td>33.8 ± 5.31</td>
<td>35.4 ± 8.48</td>
<td>36.2 ± 3.18</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>4.22 ± 0.407</td>
<td>4.24 ± 0.809</td>
<td>4.57 ± 0.582</td>
<td>2.73 ± 0.685</td>
<td>5.17 ± 0.676</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>0.820 ± 0.150a</td>
<td>2.28 ± 0.657b</td>
<td>1.46 ± 0.367a</td>
<td>1.34 ± 0.329a</td>
<td>0.905 ± 0.221a</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>0.014 ± 0.007</td>
<td>0.018 ± 0.006</td>
<td>0.015 ± 0.007</td>
<td>0.015 ± 0.000</td>
<td>0.015 ± 0.004</td>
</tr>
<tr>
<td>PAI (ng/ml)</td>
<td>0.653 ± 0.030</td>
<td>0.762 ± 0.137</td>
<td>0.948 ± 0.374</td>
<td>0.291 ± 0.133</td>
<td>0.597 ± 0.112</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CrC (µg/ml)</th>
<th>CrR</th>
<th>CrRC</th>
<th>CrRP</th>
<th>CrRW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>17.8±2.84 a</td>
<td>17.5±3.00 a</td>
<td>30.2±1.05 b</td>
<td>27.3±3.59 b</td>
<td>13.2±2.22 a</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.11±0.241 a</td>
<td>2.95±0.318 b</td>
<td>1.71±0.259 a</td>
<td>1.46±0.313 a</td>
<td>1.89±0.199 a</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.04±0.174 a</td>
<td>2.59±0.669 b</td>
<td>1.67±0.392 a</td>
<td>1.98±0.364 a</td>
<td>0.996±0.106 a</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>0.304±0.059</td>
<td>0.246±0.008</td>
<td>0.255±0.008</td>
<td>0.265±0.019</td>
<td>0.281±0.020</td>
</tr>
<tr>
<td>PAI (ng/ml)</td>
<td>0.417±0.092</td>
<td>0.500±0.128</td>
<td>0.541±0.115</td>
<td>0.476±0.201</td>
<td>1.02±0.316</td>
</tr>
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</table>

Values are mean± SE (n=6)
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA
<table>
<thead>
<tr>
<th></th>
<th>CrC</th>
<th>CrR</th>
<th>CrRC</th>
<th>CrRP</th>
<th>CrRW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (µg/mg)</td>
<td>8.56±0.865&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.94±0.425&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.89±0.637&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.71±0.958 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.58±0.492&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leptin (ng/mg)</td>
<td>5.12±0.972</td>
<td>5.48±1.03</td>
<td>3.51±0.195</td>
<td>4.82±0.703</td>
<td>4.19±0.585</td>
</tr>
<tr>
<td>TNF-α (pg/mg)</td>
<td>0.511±0.048</td>
<td>0.602±0.070</td>
<td>0.568±0.040</td>
<td>0.531±0.110</td>
<td>0.606±0.113</td>
</tr>
<tr>
<td>IL-6 (ng/mg)</td>
<td>0.073±0.008</td>
<td>0.129±0.031</td>
<td>0.120±0.032</td>
<td>0.149±0.069</td>
<td>0.122±0.031</td>
</tr>
<tr>
<td>PAI (ng/mg)</td>
<td>0.291±0.050&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33±0.154&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.582±0.076&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.480±0.171&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.07±0.239&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Male offspring**

<table>
<thead>
<tr>
<th></th>
<th>CrC</th>
<th>CrR</th>
<th>CrRC</th>
<th>CrRP</th>
<th>CrRW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (µg/mg)</td>
<td>5.97±0.434</td>
<td>4.71±.664</td>
<td>5.77±0.317</td>
<td>6.16±0.762</td>
<td>5.25±0.642</td>
</tr>
<tr>
<td>Leptin (ng/mg)</td>
<td>2.75±0.844</td>
<td>3.05±0.590</td>
<td>2.32±0.467</td>
<td>2.34±0.945</td>
<td>1.87±0.132</td>
</tr>
<tr>
<td>TNF-α (pg/mg)</td>
<td>0.628±0.119</td>
<td>0.799±0.115</td>
<td>0.718±0.289</td>
<td>0.587±0.085</td>
<td>0.670±0.075</td>
</tr>
<tr>
<td>IL-6 (ng/mg)</td>
<td>0.200±0.035</td>
<td>0.185±0.062</td>
<td>0.120±0.021</td>
<td>0.212±0.052</td>
<td>0.114±0.039</td>
</tr>
<tr>
<td>PAI (ng/mg)</td>
<td>0.428±0.078</td>
<td>0.561±0.208</td>
<td>0.381±0.060</td>
<td>0.659±0.333</td>
<td>0.503±0.170</td>
</tr>
</tbody>
</table>

**Female offspring**

Values are mean± SE (n=6)
Means without a common superscript are significantly different at p< 0.05 by one way ANOVA
LEGENDS TO THE FIGURES

FIG. 1. Schematic representation of the feeding protocol of different groups of WNIN rat mothers and their offspring. CrC – chromium control group; CrR – chromium restricted group; CrRC – chromium rehabilitation from conception; CrRP – chromium rehabilitation from parturition; CrRW – chromium rehabilitation from weaning.

FIG. 2. Body weights and plasma chromium levels in the male (panel A & C) and female (panel B & D) offspring at different ages; black bars = CrC group, white bars = CrR group, horizontal stripe bars = CrRC group, black and white square bars = CrRP group, vertical stripe bars = CrRW group. Values are mean ± SE (n=6). Bars without a common superscript are significantly different at p< 0.05 by one way ANOVA.

FIG. 3. Effect of maternal Cr restriction and rehabilitation on fat% in male (panel A) and female (panel C) offspring at different ages; wet weights of three fat deposits and adiposity index in male (panel B, at 18 months of age) and female (panel D, at 15 months of age) offspring; black bars = CrC group, white bars = CrR group, horizontal stripe bars = CrRC group, black and white square bars = CrRP group, vertical stripe bars = CrRW. Values are mean ± SE (n=6). Bars without a common superscript are significantly different at p< 0.05 by one way ANOVA.

FIG. 4. Effect of maternal Cr restriction and rehabilitation on expression of genes involved in adipogenesis and synthesis of adipocytokines by semi-quantitative PCR in adipose tissue in male (A) offspring (at 18 months of age) and female (B) offspring (at 15 months of age); black bars = CrC group, white bars = CrR group, horizontal stripe bars = CrRC group, black and white square bars = CrRP group, vertical stripe bars = CrRW. Gel picture for each gene is the representation of different groups. Values are mean ± SE (n=6). Bars without a common superscript are significantly different at p< 0.05 by one way ANOVA.

FIG. 5. Effect of maternal Cr restriction and rehabilitation on plasma lipid profile of female offspring. Panel A: Triglycerides, and panel B: Free fatty acids, at different ages; black bars = CrC group, white bars = CrR group, horizontal stripe bars = CrRC group, black and white square bars = CrRP group, vertical stripe bars = CrRW. Values are mean ± SE (n=6). Bars without a common superscript are significantly different at p< 0.05 by one way ANOVA.
Figure 3

Figure 4

A. Male offspring

B. Female offspring

Chromium and modulation of adiposity

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Figure 5

A

B

free fatty acids (mmol/l)

triglycerides (mmol/l)

9 moa

15 moa

CrC  CrR  CrRC  CrRP  CrRW