Chronic Maternal Dietary Chromium Restriction Modulates Visceral Adiposity

Probable Underlying Mechanisms

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OBJECTIVE—We demonstrated previously that chronic maternal micronutrient restriction altered the body composition in rat offspring and may predispose offspring to adult-onset diseases. Chromium (Cr) regulates glucose and fat metabolism. The objective 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 3 months and mated with control males. Some pregnant CrR mothers were rehabilitated from conception or parturition and their pups weaned to control diet. Whereas 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 percentage, especially the central adiposity in both male and female offspring. Further, the expression of leptin and 11 β-hydroxysteroid dehydrogenase 1 genes were significantly increased in CrR offspring of both the sexes. 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 Cr 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 later life. ***Diabetes*** **59: 98–104, 2010**

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The fetal origin 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 2 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 as well as development of insulin resistance and its associated complications in the offspring (3,4). Many studies report that central adiposity correlates strongly with insulin resistance (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 *SREBP2* overexpression increases fatty acid synthase (FAS) gene expression (9). Adipose tissue differentiation is also regulated by glucocorticoid hormone (10). Glucocorticoid oversecretion results in the manifestation of central adiposity, visceral obesity, insulin resistance, hypertension, and dyslipidemia (11,12). Glucocorticoid-mediated effects in target tissues are regulated by 11 β-hydroxysteroid dehydrogenase 1 (*11β-HSD1*), an 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 the biggest endocrine organ, secretes adipocytokines like adiponectin, leptin, plasminogen activator inhibitor (PAI), interleukin (IL)-6, tumor necrosis factor (TNF)-α, etc. (14), which regulate energy metabolism and insulin sensitivity and play a vital role in the pathogenesis of obesity, atherosclerotic vascular disease, hypertension, and diabetes (15).

Cr, an important trace element, regulates carbohydrate and fat metabolism (16). Many investigations in humans

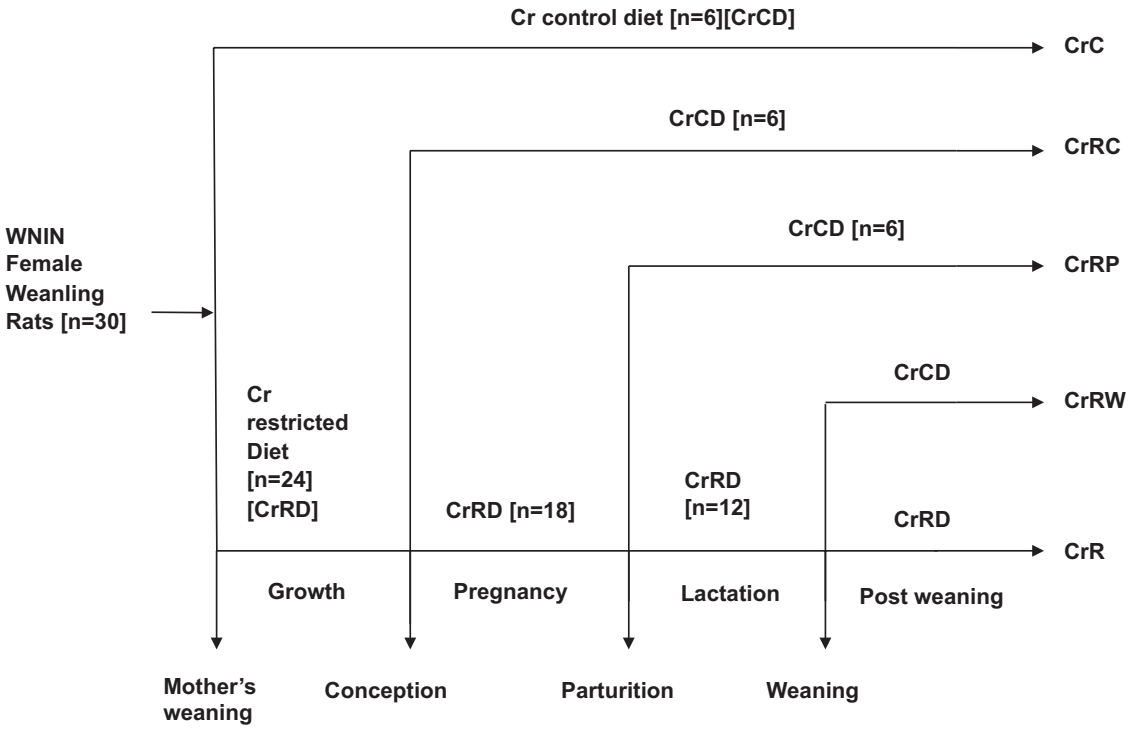

FIG. 1. Schematic representation of the feeding protocol of different groups of WNIN rat mothers and their offspring.

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 diabetic subjects (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 WNIN rats to validate/negate this hypothesis and to elucidate the associated mechanisms.

RESEARCH DESIGN AND METHODS

All experimental procedures were approved by the ethics committee on animal experiments at the National Institute of Nutrition, Hyderabad, India, and were performed in accordance with the principles of laboratory animal care (19). WNIN female weanling rats ($n = 30$) were obtained from the National Centre for Laboratory Animal Sciences (NCLAS) at the 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-h 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 American Institute of Nutrition–93G formulation and analyzed for Cr content in an atomic absorption spectrometer (Varian Spectra AA220; Varian, Walnut Creek, CA) using reduced flame (20). Cr-restricted diet was prepared by excluding the Cr 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, whereas the group of 6 rats received the control diet (1.56 mg Cr/kg diet) with free access to deionized water. Daily food intake and weekly body weights were monitored in these rats until the end of the feeding regimen. Plasma Cr levels were determined 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 1 of pregnancy. From this day of conception, six of CrR pregnant dams were switched to control diet (CrRC) and their offspring weaned to control diet. Another six CrR mothers were rehabilitated with control diet from parturition (CrRP) and their offspring weaned to control diet. The remaining 12 CrR mothers continued on CrR diet during lactation. Litter size was adjusted to seven in all groups on postnatal day 3 and maintained throughout lactation. At weaning (postnatal day 21), half CrR offspring were weaned to control diet (CrRW), whereas the remaining pups were continued on CrR throughout life. Considering the high mortality in the female offspring beyond 15 months of 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 Cr status. Plasma Cr levels were monitored once every 3 months in the offspring by atomic absorption spectrometer using a graphite furnace (GFS97 SOLAAR AA Series; Thermo Electron, Cheshire, CT) according to Mahalingam et al. (21).

Body composition. Body composition of the offspring was determined from 3 months of age using total body electrical conductivity, a small animal body composition analysis system (model SA 3000 multidetector; EMSCAN, Springfield, IL) as described previously (22,23). Total body fat percentage was obtained mathematically by following the method of Morbach and Brans (24).

Adiposity index. Adiposity index, an index of visceral adiposity, was computed according to Taylor et al. (25). The wet weights of the retroperitoneal, mesenteric, and epididymal/gonadal fat pads were determined, and the adiposity index was computed using the following formula: adiposity index = (sum of the weights of the three fat deposits / body weight) × 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, U.K.).

Adipocytokines in plasma and adipose tissue. Concentrations of adiponectin, leptin, PAI, IL-6, and TNFα 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 previously (26). The protein content in plasma and adipose tissue lysate was determined using bicinchoninic acid assay (27).

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 they were killed and was stored frozen immediately at −80°C. Total RNA was isolated from ~100 mg of the adipose tissue using TRIzol reagent according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA). cDNA was synthesized from 2 μg of total RNA

TABLE 1
Diet intake as well as physical and lipid profile in WNIN female rats fed control and Cr-restricted diets for 12 weeks before mating

	CrC	CrR
Food intake (g)	9.73 ± 0.295	10.7 ± 0.107*
Body wt gain (g)	107 ± 5.46	116 ± 2.31
Plasma Cr concentration (μg/l)	1.18 ± 0.181	0.648 ± 0.058*
Total cholesterol (mmol/l)	1.46 ± 0.068	1.52 ± 0.098
HDL cholesterol (mmol/l)	1.16 ± 0.078	1.12 ± 0.084
Triglycerides (mmol/l)	0.433 ± 0.037	0.492 ± 0.035
FFAs (mmol/l)	0.803 ± 0.064	0.813 ± 0.050

Data are means ± SE ($n = 6$). *$P < 0.05$ using Student t test.

using an Invitrogen kit (Invitrogen Life technologies, Carlsbad, CA). Primers were designed with the aid of primer quest software (Integrated DNA Technologies, Corolville, IA). Semiquantitative PCR was conducted to analyze the expression of *1*) *PPARγ* (5′CCCATTCCTTTGACATCAAACC3′; 5′ATTGTGAGACATCCCCACAGC3′), *2*) *SREBP2* (5′AAGTCTGGCGTTCTGAGGAA3′; 5′ CCAGGAAGGTGAGGACACAT3′), *3*) *11β-HSD1* (5′ GCCCTGGTGCTCTAGAACTG3′; 5′ AGTTCCACATCGGCCACTAC3′), *4*) adiponectin (5′ CTACTGTTGCAAGCTCTCC3′; 5′CTTCACATCTTTCATGTACACC3′), *5*) leptin (5′ GAGACCTCCTCCATCTGCTG3′; 5′ CATTCAGGGCTAAGGTCCAA3′), and *6*) *FAS* (5′ TCGAGACACATCGTTTGAGC3′; 5′ TCAAAAAGTGCATCCAGCAG3′) with the internal control 18S rRNA (5′CCAGAGCGAAAGCATTTGCCAAGA3′; 5′AATCAACGCAAGCTTATGACCCGC3′). 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, Herculus, 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 ± SE. Data were analyzed using unpaired Student 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 nonparametric Mann-Whitney U test. The differences were considered significant at $P < 0.05$.

RESULTS

Growth characteristics, Cr status, and lipid profile in WNIN rat dams. As expected the plasma Cr levels were significantly decreased ($P < 0.05$) in CrR rats compared with CrC rats (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 sexes) of the various groups at all the time points studied. However, CrR offspring (male and female) weighed significantly more ($P < 0.05$) than CrC from 12 months of age (Fig. 2*A* and *B*) until the time they were killed. In male offspring all three rehabilitation regimens corrected the change at 12 months of age, whereas CrRP but not CrRC or CrRW restored the change to control levels at 18 months of age (Fig. 2*A*). In female offspring CrRP but not CrRC and CrRW could correct the insult at 12 months of age, whereas none of them could do so at 15 months of age (Fig. 2*B*). Plasma Cr levels were significantly ($P < 0.05$) decreased in CrR than CrC offspring at all time points studied, and the rehabilitation regimens restored them to control from as early as 3 months of age (Fig. 2*C* and *D*).

Body fat percentage and adiposity index of the offspring. Body fat percentage of the male CrR offspring was significantly higher than that of CrC rats (Fig. 3*A*) 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 percentage than CrC rats, albeit from 3 months of age (Fig. 3*C*). Although all three rehabilitation regimens appeared to correct the change at 12 months of 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

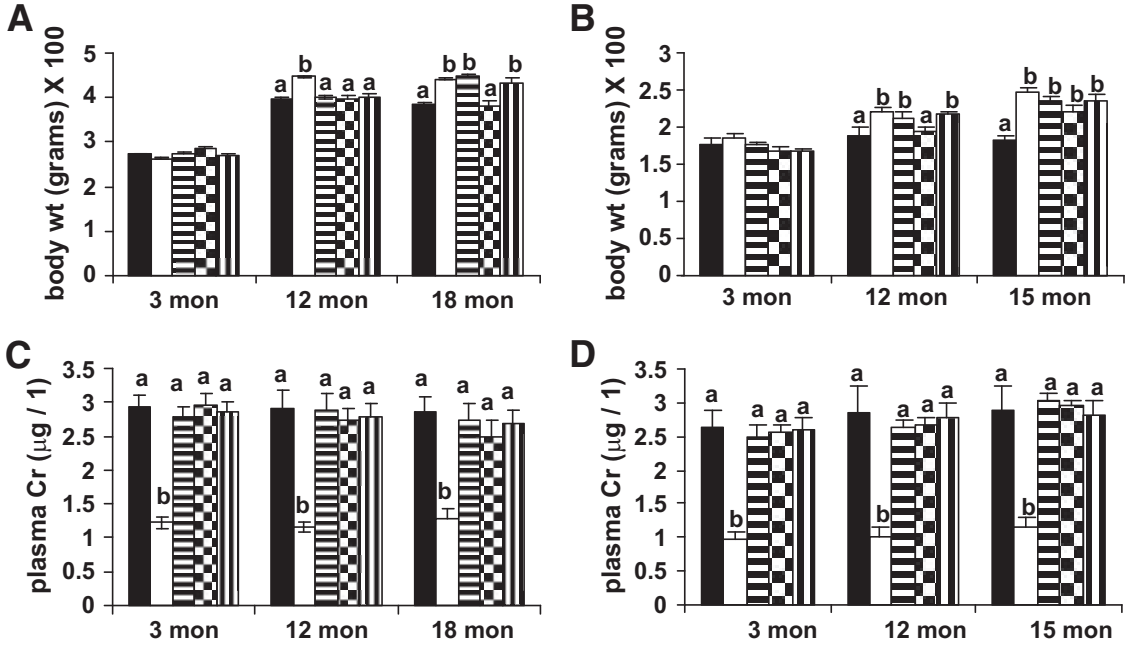

FIG. 2. Body weights and plasma chromium levels in the male (*A* and *C*) and female (*B* and *D*) offspring at different ages. CrC, ■; CrR, □; CrRC, ▤; CrRP, ▩; CrRW, ▥. Values are mean ± SE ($n = 6$). Bars without a common letter are significantly different at $P < 0.05$ by one-way ANOVA.

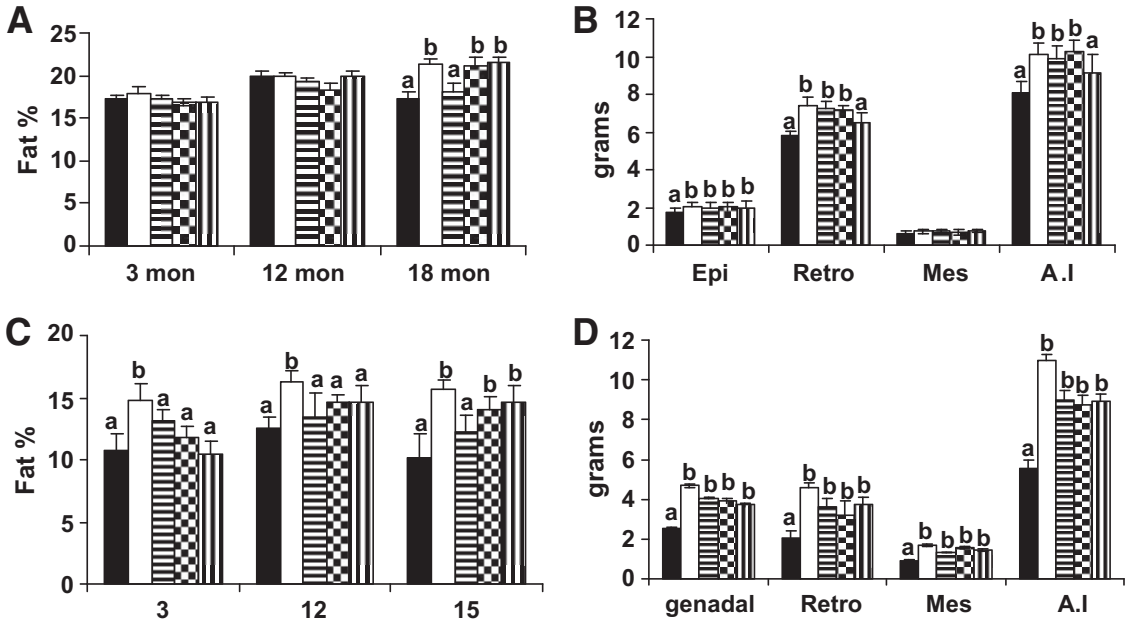

FIG. 3. Effect of maternal Cr restriction and rehabilitation on fat % in male (*A*) and female (*C*) offspring at different ages; wet weights of three fat deposits and adiposity index in male (*B*, at 18 months of age) and female (*D*, at 15 months of age) offspring. CrC, ■; CrR, □; CrRC, ▤; CrRP, ▩; CrRW, ▥. Values are mean ± SE (n = 6). Bars without a common letter are significantly different at $P < 0.05$ by one-way ANOVA.

sexes), and no rehabilitation regimen could correct these changes (Fig. 3*B* 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 rats from 9 months of age but not earlier, and all three rehabilitation regimens restored these rats to control levels (Fig. 4*A* 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 rats had significantly ($P < 0.05$) increased levels (Table 2), whereas CrRW rats showed no effect. Leptin levels were significantly higher ($P < 0.05$) in CrR than CrC rats, albeit in female offspring only, and all three rehabilitation regimens restored rats to control levels. Interestingly, TNFα levels were significantly higher ($P < 0.05$) in CrR than CrC offspring of both the sexes, and all three rehabilitation regimens 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 was significantly increased in the adipose tissue of CrR offspring of both sexes compared with the corresponding controls (Fig. 5*A* and *B*). In male offspring change in *11β-HSD1* expression was corrected by all three rehabilitation regimens, whereas CrRC and

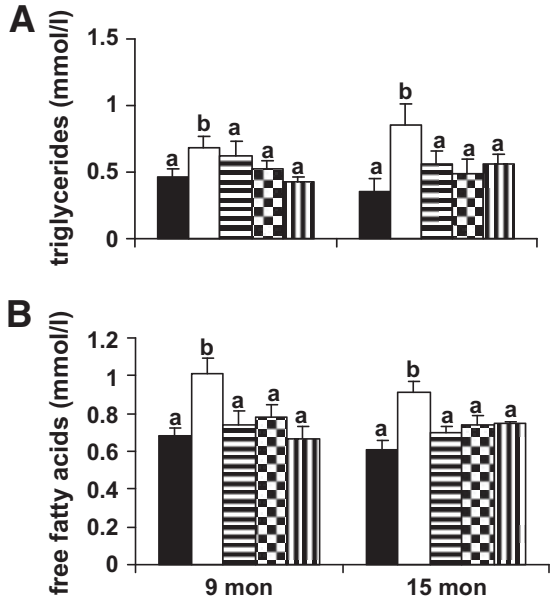

FIG. 4. Effect of maternal Cr restriction and rehabilitation on plasma lipid profile of female offspring. *A*: Triglycerides. *B*: Free fatty acids, at different ages. CrC, ■; CrR, □; CrRC, ▤; CrRP, ▩; CrRW, ▥. Values are mean ± SE (n = 6). Bars without a common letter are significantly different at $P < 0.05$ by one-way ANOVA.

TABLE 2
Plasma adipocytokine levels of various groups of male and female offspring

	CrC	CrR	CrRC	CrRP	CrRW
Male offspring					
Adiponectin (μg/ml)	31.5 ± 3.00	31.5 ± 3.90	33.8 ± 5.31	35.4 ± 8.48	36.2 ± 3.18
Leptin (ng/ml)	4.22 ± 0.407	4.24 ± 0.809	4.57 ± 0.582	2.73 ± 0.685	5.17 ± 0.676
TNFα (pg/ml)	0.820 ± 0.150*	2.28 ± 0.657†	1.46 ± 0.367*	1.34 ± 0.329*	0.905 ± 0.221*
IL-6 (ng/ml)	0.014 ± 0.007	0.018 ± 0.006	0.015 ± 0.007	0.015 ± 0.000	0.015 ± 0.004
PAI (ng/ml)	0.653 ± 0.030	0.762 ± 0.137	0.948 ± 0.374	0.291 ± 0.133	0.597 ± 0.112
Female offspring					
Adiponectin (μg/ml)	17.8 ± 2.84*	17.5 ± 3.00*	30.2 ± 1.05†	27.3 ± 3.59†	13.2 ± 2.22*
Leptin (ng/ml)	1.11 ± 0.241*	2.95 ± 0.318†	1.71 ± 0.259*	1.46 ± 0.313*	1.89 ± 0.199*
TNFα (pg/ml)	1.04 ± 0.174*	2.59 ± 0.669†	1.67 ± 0.392*	1.98 ± 0.364*	0.996 ± 0.106*
IL-6 (ng/ml)	0.304 ± 0.059	0.246 ± 0.008	0.255 ± 0.008	0.265 ± 0.019	0.281 ± 0.020
PAI (ng/ml)	0.417 ± 0.092	0.500 ± 0.128	0.541 ± 0.115	0.476 ± 0.201	1.02 ± 0.316

Data are means ± SE ($n = 6$). Data without a common symbol are significantly different at $P < 0.05$ by one-way ANOVA.

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 effects 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. 5*A* and *B*).

DISCUSSION

We demonstrated previously that restriction of micronutrients in utero increased body fat perdentage and central adiposity in offspring (22,23,26,28,29). Considering the importance of Cr in maintaining carbohydrate/lipid metabolism and modulating body composition in diabetic subjects (30), 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 of CrR compared with CrC rats is in disagreement with increased food intake reported on Cr picolinate supplementation (31). Consistent with earlier reports (28,32), 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 regimens restored rats to control levels, besides increasing their body weights. However, rehabilitation corrected body weight changes in male offspring partially but not in female offspring. Although in line with previous studies on the effects of Cr and vitamin A (17,33) on body weight, these findings contradict reports on maternal micronutrient restriction–lowered body weight in rat offspring (22,28) and Cr supplementation–increased body weight in pigs (30). However, some studies showed that Cr picolinate did not affect body weight (34). Indeed, no studies to date reported the effect of maternal Cr restriction on the offspring body weight.

Most often, high body adiposity precedes insulin resistance (35). In this study, maternal Cr restriction increased body fat percentage 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 percentage reported in obese subjects on Cr supplementation (17) and our previous reports that maternal mineral restriction had similar effects in rat offspring (22,23,26,28). 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 (36,37). That chronic maternal Cr restriction significantly increased adiposity index in the offspring and rehabilitation did not correct it stresses 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 (33) and our findings in offspring of Mg-

TABLE 3
Adipocytokine levels in adipose tissue of different groups of male and female offspring

	CrC	CrR	CrRC	CrRP	CrRW
Male offspring					
Adiponectin (μg/mg)	8.56 ± 0.865*	5.94 ± 0.425†	5.89 ± 0.637†	5.71 ± 0.958†	6.58 ± 0.492*
Leptin (ng/mg)	5.12 ± 0.972	5.48 ± 1.03	3.51 ± 0.195	4.82 ± 0.703	4.19 ± 0.585
TNFα (pg/mg)	0.511 ± 0.048	0.602 ± 0.070	0.568 ± 0.040	0.531 ± 0.110	0.606 ± 0.113
IL-6 (ng/mg)	0.073 ± 0.008	0.129 ± 0.031	0.120 ± 0.032	0.149 ± 0.069	0.122 ± 0.031
PAI (ng/mg)	0.291 ± 0.050*	1.33 ± 0.154†	0.582 ± 0.076*	0.480 ± 0.171*	1.07 ± 0.239†
Female offspring					
Adiponectin (μg/mg)	5.97 ± 0.434	4.71 ± 0.664	5.77 ± 0.317	6.16 ± 0.762	5.25 ± 0.642
Leptin (ng/mg)	2.75 ± 0.844	3.05 ± 0.590	2.32 ± 0.467	2.34 ± 0.945	1.87 ± 0.132
TNFα (pg/mg)	0.628 ± 0.119	0.799 ± 0.115	0.718 ± 0.289	0.587 ± 0.085	0.670 ± 0.075
IL-6 (ng/mg)	0.200 ± 0.035	0.185 ± 0.062	0.120 ± 0.021	0.212 ± 0.052	0.114 ± 0.039
PAI (ng/mg)	0.428 ± 0.078	0.561 ± 0.208	0.381 ± 0.060	0.659 ± 0.333	0.503 ± 0.170

Data are means ± SE ($n = 6$). Data without a common symbol are significantly different at $P < 0.05$ by one-way ANOVA.

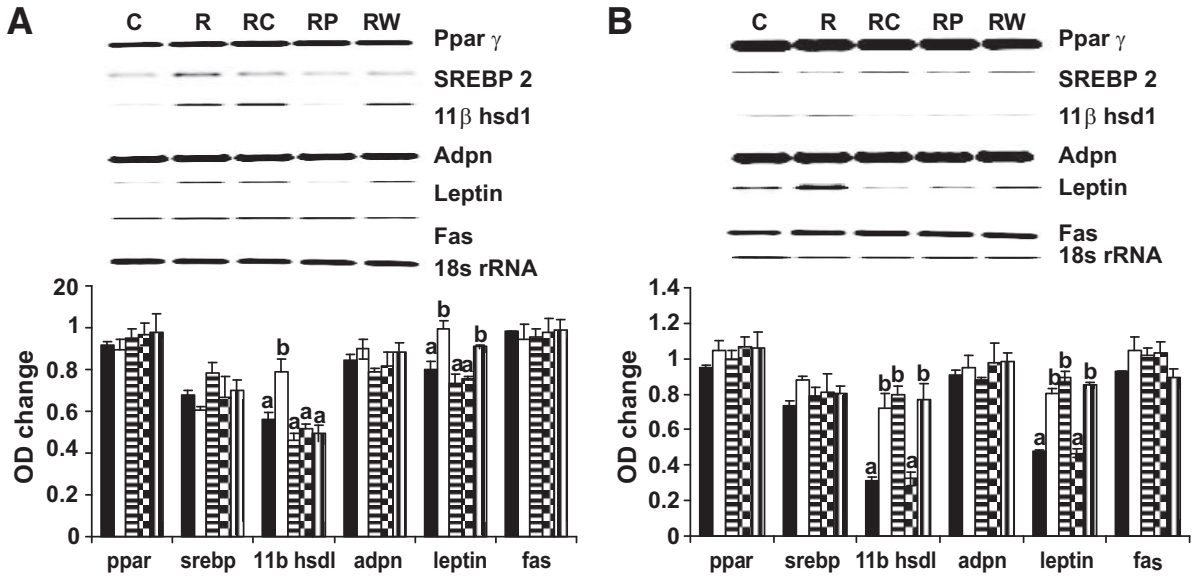

FIG. 5. Effect of maternal Cr restriction and rehabilitation on expression of genes involved in adipogenesis and synthesis of adipocytokines by semiquantitative PCR in adipose tissue in male (*A*) offspring (at 18 months of age) and female (*B*) offspring (at 15 months of age). CrC, ■; CrR, □; CrRC, ▤; CrRP, ▨; CrRW, ▥. Gel picture for each gene is the representation of different groups. Values are mean ± SE ($n = 6$). Bars without a common letter are significantly different at $P < 0.05$ by one-way ANOVA.

restricted rats (26,28), which indicate that maternal Cr restriction increased adiposity, especially visceral adiposity, in offspring and may therefore predispose to insulin resistance and associated diseases in later life.

Fasting hypertriglyceridemia and/or low HDL cholesterol levels are associated with insulin resistance (38). The increased plasma triglycerides and FFAs in female CrR offspring are in line with reports of high plasma triglyceride in the offspring of micronutrient-restricted rats (22,23,28), altered lipid metabolism in pups of protein- or iron-restricted rats (39), and effects of Cr supplementation on lipid metabolism and fat deposition in lambs (40).

Altered adipocytokines underlie the development of adiposity and insulin resistance (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 (22). Further, they corroborate reports that *1*) hypocaloric diet reduced leptin and TNFα but not adiponectin and PAI-1 levels in plasma (41) and *2*) mice fed conjugated linoleic acid and chromium along with high-fat diet had lower plasma leptin levels (42). That rehabilitation could correct changes in plasma adipocytokines but not body fat percentage or visceral adiposity suggests 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 suggests its role in modulating adipose tissue function in offspring, which may predispose them to insulin resistance and associated diseases in later life. However, our observation that the lack of changes seen in leptin and TNFα levels in adipose tissue despite 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 modulates obesity/visceral adiposity, dyslipidemia, insulin resistance, and associated complications (8,43,44). We observe that *11β-HSD1* and leptin expression upregulation, but not that of *PPARγ*, *SREBP2*, adiponectin, and *FAS*, in CrR offspring and the partial correction by rehabilitation are in agreement with the upregulation of leptin and *11β-HSD1* reported in diabetic subjects (45) and the adipose tissue dysregulation reported in rats through high fat–induced overexpression of *11β-HSD1* (46). It thus appears that increased expression of *11β-HSD1* and leptin may underlie enhanced body adiposity (fat percentage and 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 upregulation of *11β-HSD1* and leptin may underlie increased adiposity in these offspring. Finally, this study reiterates the importance of Cr during the peri-postnatal period in the development and function of adipose tissue in the offspring that may predispose them to obesity and insulin resistance in later life.

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