Impact of maternal chromium restriction on glucose tolerance, plasma insulin and oxidative stress in WNIN rat offspring

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Abstract

Robust evidence suggests that nutritional insult during fetal development could program the offspring to glucose intolerance, impaired insulin response and insulin resistance (IR). Considering the importance of chromium (Cr) in maintaining carbohydrate metabolism, this study determined the effect of maternal Cr restriction (CrR) on glucose metabolism and plasma insulin in Wistar/NIN (WNIN) rat offspring and the associated biochemical and/or molecular mechanisms. Female, weanling WNIN rats received *ad libitum* for 12 weeks, a control diet or the same with 65% restriction of Cr and mated with control males. Some of the Cr-restricted dams were rehabilitated from conception or parturition and their pups weaned on to control diet. At the time of weaning, half of the Cr restricted offspring were rehabilitated to control diet while others continued on Cr-restricted diet. Maternal CrR increased fasting plasma glucose, fasting insulin, homeostasis model assessment of IR, and area under the curve of glucose and insulin during oral glucose tolerance test in the offspring. Expression and activity of rate-limiting enzymes of glucose metabolism were comparable among different groups and expression of genes involved in insulin secretion was increased albeit in male offspring whereas antioxidant enzyme activities were decreased in offspring of both genders. Rehabilitation, in general, corrected the changes albeit partially. Maternal dietary CrR induced IR, impaired glucose tolerance in WNIN rat offspring and was associated with increased oxidative stress, which may predispose them to type 2 diabetes in their later life.

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Introduction

Fetal growth retardation caused by maternal undernutrition results in obesity, insulin resistance (IR), and associated adult onset diseases such as cardiovascular disease, type 2 diabetes mellitus, and hypertension (Barker *et al.* 1993). A possible reason for this could be a primary impairment of β-cell development (Dahri *et al.* 1991) and an overall underdevelopment of endocrine pancreas (Holness 1996). Several animal models have demonstrated that malnutrition during fetal development programs the endocrine pancreas and insulin-sensitive tissues, resulting in IR and diabetes in later life (Snoeck *et al.* 1990). *In utero* protein restriction reduces pancreatic β-cell number in rat offspring and blunts insulin secretion in response to oral glucose challenge (Berney *et al.* 1997, Reusens & Remacle 2001).

We showed earlier that maternal micronutrient restriction increased body fat% especially the central adiposity, impaired plasma lipids, altered glucose tolerance, impaired insulin secretion, increased oxidative stress, and appeared to predispose the Wistar/NIN (WNIN) rat offspring to IR and associated diseases in later life (Venu *et al.* 2004*a*,*b*, 2005, 2008, Padmavathi *et al.* 2009).

Trivalent chromium (Cr), also called glucose tolerance factor, regulates glucose and lipid metabolism (Mertz 1969). Acting as a cofactor for insulin, it enhances glucose utilization by insulin target tissues (Anderson 1992). It facilitates insulin binding to its receptors, activates insulin receptor kinases and inhibits insulin receptor phosphatases (Vincent 2000). Chromodulin, the Cr-binding oligopeptide activities tyrosine kinase activity of insulin receptor in response to insulin (Davis & Vincent 1997). Although epidemiological evidence for the incidence of Cr deficiency *per se* is limited, several studies in humans and experimental animals report the beneficial effects of Cr supplementation on glucose tolerance and insulin sensitivity (Anderson 1989, Amoikon *et al.* 1995, Kitchalong *et al.* 1995, Ravina *et al.* 1995). Recently, we reported that maternal Cr restriction (CrR) increased body fat% (central adiposity) and was associated with decreased muscle development in WNIN rat offspring (Padmavathi *et al.* 2010*a*,*b*).

Despite the known impact of Cr supplementation on glucose tolerance and insulin sensitivity, effects if any of maternal CrR in programming the offspring to altered glucose metabolism and plasma insulin levels have not yet been reported. Therefore, this study determined the effect of maternal CrR on

glucose metabolism and plasma insulin levels in WNIN rat offspring.

Materials and methods

Study design

Weanling (21-day old), female rats ($n=30$) WNIN strain were obtained from National Centre for Laboratory Animal Sciences (NCLAS) with the approval of the Ethics Committee on animal experiments at National Institute of Nutrition, Hyderabad, India. They were housed individually in polypropylene cages with wire mesh bottom and maintained at $22\pm2\,°C$, under standard lighting conditions (12 h light:12 h darkness cycle). Diets were prepared according to AIN-93G formulation and their Cr content analyzed by atomic absorption spectrometer (Varian Atomic Absorption Spectrometer, Spectra AA 220; Varian India, Agilent Technologies, Forest Hills, VIC, Australia) using reduced flame. The animal feeding and experimental protocol for this study has been described previously (Padmavathi *et al.* 2010*a*).

Oral glucose tolerance test (OGTT) was performed (as given below) in WNIN female rats (mothers to be) after 3 months on their respective diets (i.e. before mating) and in the offspring of different groups at quarterly intervals from 3 months of their age. The offspring were sacrificed at 15 months of age after performing the OGTT, the liver and pancreas were dissected out, weighed, snap frozen in liquid nitrogen, and stored at $-80\,°C$ till analyzed.

Glucose tolerance and plasma insulin levels

Blood samples were collected from supra-orbital sinus of overnight fasted rats. Glucose was then administered orogastrically at a dose of 2.5 g/kg body weight and blood samples were collected from the tail vein at 30, 60, and 120 min under light ether anesthesia. Plasma glucose was estimated using the commercial kit (Biosystem, Barcelona, Spain) and insulin by the RIA kit (BRIT, Mumbai, India). The area under the curve (AUC) of glucose and insulin during OGTT were computed by the trapezoidal method (Mathews *et al.* 1990). The indices of IR such as homeostasis model assessment of IR (HOMA-IR) index and the ratio of glucose AUC to insulin AUC during OGTT were calculated as mentioned earlier (Padmavathi *et al.* 2009).

Activity of enzymes of glucose metabolism

Liver was homogenized and processed as described earlier (Venu *et al.* 2005) to get the microsomal and cytosolic fractions. Protein content was estimated by the bicinchoninic acid method (Smith *et al.* 1985) and the activities of the rate-limiting enzymes of glucose

metabolism, antioxidant enzymes, and oxidative stress markers were determined as follows.

Hepatic glucokinase (GCK) activity was assayed in the cytosolic fraction by the reduction of NADP at 340 nm (Pilkis 1975). One unit of GCK represents the phosphorylation of 1 μmol of glucose per minute. Pyruvate kinase (PK) activity was determined in liver cytosol by the oxidation of NADH at 340 nm (Bucher & Pfleiderer 1955) and one unit of PK is the nanomoles of phosphoenolpyruvate hydrolyzed per minute.

Glucose-6-phosphatase (G6PC) activity was monitored at 510 nm in the microsomal fraction by the formation of quinoneimine. One unit of G6PC is defined as 1 μmol of glucose-6-phosphate formed per minute (Gierow & Jergil 1982). Fructose-1,6-bisphosphatase (FBP) was determined in the cytosolic fraction by following the rate of NADPH formation at 340 nm (Marcus *et al.* 1982) and one unit of this enzyme represents the formation of 1 μmol of fructose-6-phosphate per minute.

Gene expression in liver and pancreas by semi-quantitative PCR

Total RNA was isolated from 100 mg each of liver and pancreatic tissues using Qiazol reagent. This was followed by the synthesis of cDNA from 2 μg of total RNA using Invitrogen Kit (Invitrogen Life Technologies). Primers were designed with the aid of primer quest software (Integrated DNA Technologies, Coralville, IA, USA). Semi-quantitative PCR was conducted to analyze the expression of *Gck* (5′-CCTTAGACCTGG-GAGGAACC-3′; 5′-ACGATGTTGTTCCCTTCTGC-3′), *Pklr* (5′-CCAAGGGACCTGAGATACGA-3′; 5′-AGGTCC-ACCTCAGTGTTTGG-3′), phosphoenolpyruvate carboxykinase (*Pck*; 5′-CCCAGGAGTCACCATCACTT-3′; 5′-TTCGTAGACAAGGGGGACAC-3′), *G6pc* (5′-AGCT-CCGTGCCTCTGATAAA-3′; 5′-AAAGTGAGCCGCAAG-GTAGA-3′), preproinsulin 1 (*Ins1*; 5′-CACCTTTGTGGT-CCTCACCT-3′; 5′-CCAGTTGGTAGAGGGAGCAG-3′), and *Ins2* (5′-CAGCACCTTTGTGGTTCTCA-3′; 5′-CAG-TGCCAAGGTCTGAAGGT-3′) with the internal standard 18S rRNA (5′-CCAGAGCGAAAGCATTTGCCAA-GA-3′; 5′-AATCAACGCAAGCTTATGACCCGC-3′). The amplified products were resolved on 1.2% agarose

Table 1 Glucose tolerance and insulin resistance indices in female Wistar/NIN rats. Values are mean$\pm$s.e.m. ($n=6$)

Parameter	CrC	CrR
Fasting glucose (mmol/l)	4.73 ± 0.387	4.84 ± 0.167
Fasting insulin (pmol/l)	398 ± 83.8	411 ± 54.5
HOMA-IR	11.6 ± 1.36	12.7 ± 1.50
Glucose AUC (mmol/l per h)	11.9 ± 0.714	11.7 ± 0.508
Insulin AUC (pmol/l per h)	815 ± 146	801 ± 88.0
Glucose AUC/insulin AUC	0.015 ± 0.002	0.015 ± 0.001

Table 2 Daily food intake in male and female offspring of different groups at 9 and 15 months of their age. Values are mean±s.e.m. (*n*=6)

|	Months	CrC	CrR	CrRC	CrRP	CrRW
Males						
Food intake (g)	9	15·0±0·341	15·7±0·209	15·5±0·362	15·1±0·316	15·4±0·306
	15	15·1±0·341	15·9±0·209	15·4±0·326	14·9±0·316	15·5±0·306
Females						
Food intake (g)	9	12·4±0·476	13·4±0·158	12·7±0·519	12·5±0·421	12·2±0·374
	15	12·2±0·478	13·0±0·160	12·0±0·518	12·8±0·422	12·4±0·379

gel electrophoresis and the image was quantified with the Bio-Rad gel documentation system using Quantity One Software (Bio-Rad Laboratories). Results are expressed as the ratio of the intensities of the band of the gene of interest to that of the 18S rRNA.

Oxidative stress and antioxidant defense markers

Oxidative stress and antioxidant status were determined in liver homogenate. Thiobarbituric acid reactive substances was determined as a measure of lipid peroxidation (Balasubramanian *et al.* 1988) and protein carbonyls were quantified spectrophotometrically at 370 nm using 2,4-dinitrophenylhydrazine (Uchida *et al.* 1998). Reduced glutathione (GSH) and oxidized glutathione (GSSG) were estimated spectrofluorimetrically at excitation and emission wavelengths of 350 and 420 nm, respectively, using ortho-phthalaldehyde (Hissin & Hilf 1976).

Superoxide dismutase (SOD) activity was estimated in the cytosolic fraction (100 000 g supernatant;
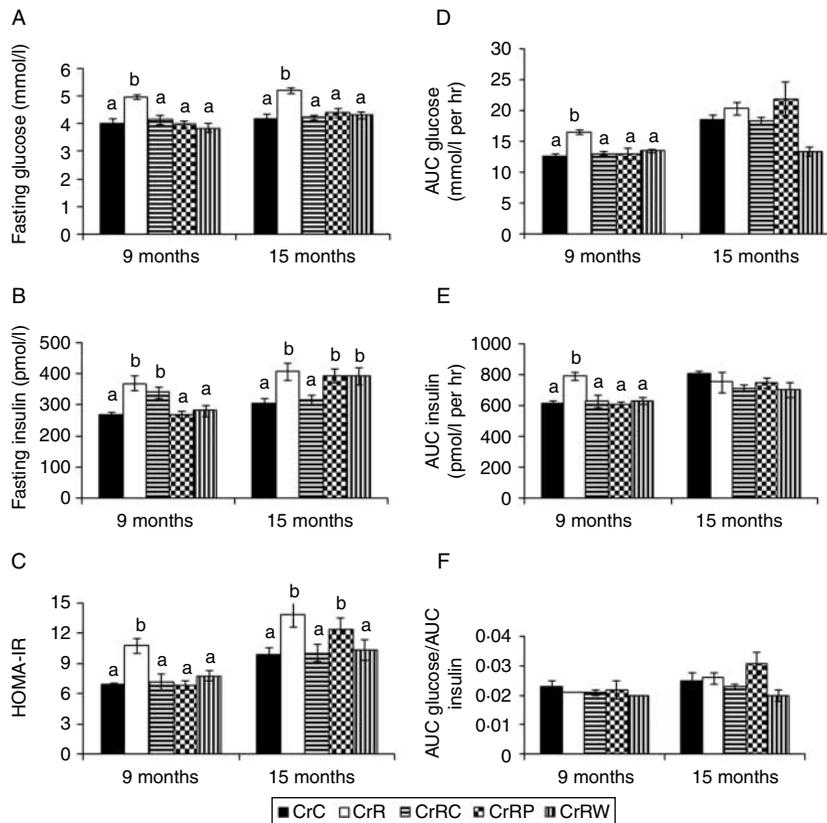
Figure 1 Effect of maternal Cr restriction and rehabilitation on glucose tolerance and plasma insulin levels in WNIN male offspring at different ages. Panel A, fasting glucose; panel B, fasting insulin; panel C, HOMA-IR; panel D, glucose AUC during OGTT; panel E, insulin AUC during OGTT; panel F, glucose AUC/insulin AUC during OGTT. Values are mean±s.e.m. (*n*=6). Bars without a common superscript ('a and b') are different at $P < 0.05$ by one-way ANOVA followed by *post hoc* least significant difference (LSD) test.

Marklund & Marklund 1974) and one unit of SOD activity is the amount of the enzyme that inhibits the rate of auto-oxidation of pyrogallol by 50% per minute. The activity of catalase was measured by the reduction of hydrogen peroxide (Aebi 1984) whereas glutathione peroxidase (GPx) was determined in the cytosolic fraction by the oxidation of reduced glutathione by cumene hydroperoxide (Paglia & Valentine 1967). One unit of GPx is the micromoles of NADPH oxidized per minute.

Statistical analysis

All values are presented as mean $\pm$ S.E.M. Data were 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 the groups were tested by non-parametric Mann–Whitney U test. The differences were considered significant at $P < 0.05$.

Results

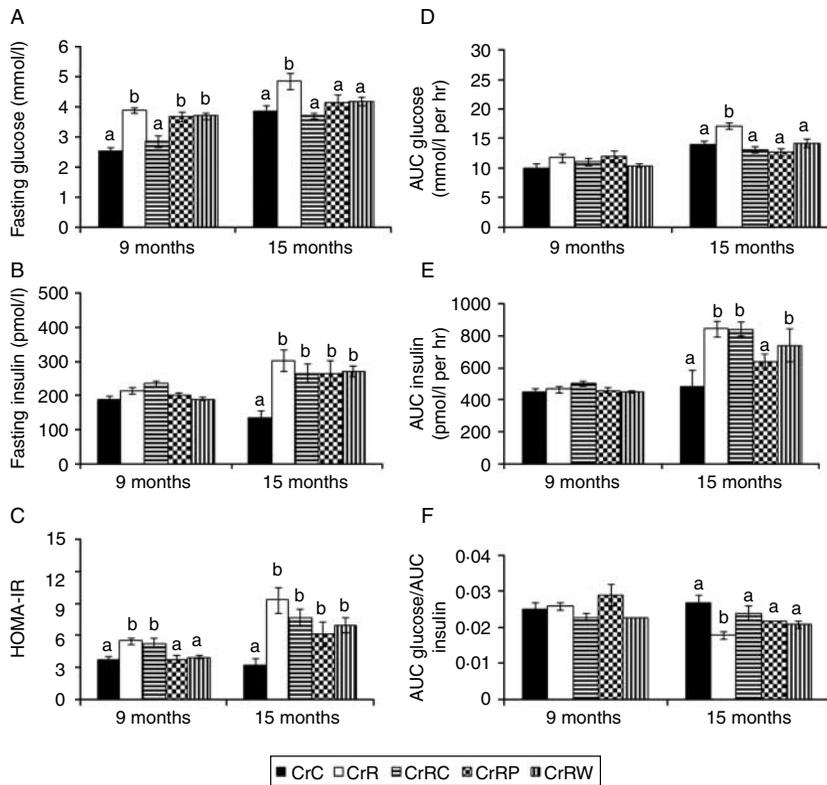## Glucose tolerance and plasma insulin levels in female WNIN rats on CrR

Plasma Cr was lower ($P < 0.05$) in CrR than CrC (chromium control) rats (Padmavathi *et al.* 2010*a*). However, there was no difference between CrC and CrR rats in the levels of fasting plasma glucose and insulin or glucose AUC and insulin AUC during OGTT (Table 1). In line with these observations, IR as assessed by HOMA index or the ratio of glucose AUC to insulin AUC during OGTT was comparable between the female CrC and CrR rats (mother to be) before mating (Table 1).

Growth characteristics of control, Cr-restricted and rehabilitated offspring

Food intake was comparable among the offspring of different groups at all the time points studied (Table 2). Plasma Cr levels were lower ($P < 0.05$) in CrR than CrC offspring and three rehabilitation regimes restored the levels to control from 3 months of their age (Padmavathi *et al.* 2010*a*). However, body weight was higher
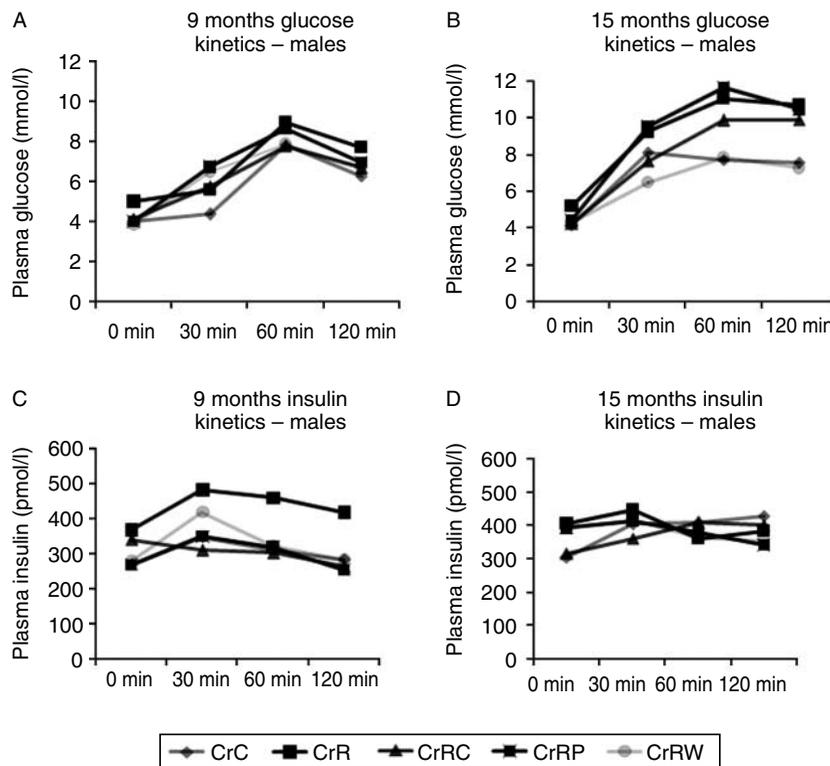
Figure 2 Effect of maternal Cr restriction and rehabilitation on plasma glucose and insulin levels at various time points (0, 30, 60, and 120 min) during oral glucose tolerance test in WNIN male offspring. Panel A, plasma glucose kinetics at 9 months of age; panel B, plasma glucose kinetics at 15 months of age; panel C, plasma insulin kinetics at 9 months of age; panel D, plasma insulin kinetics at 15 months of age. Values are mean ($n = 6$).

Figure 3 Effect of maternal Cr restriction and rehabilitation on glucose tolerance and plasma insulin levels in WNIN female offspring at different ages. Panel A, fasting glucose; panel B, fasting insulin; panel C, HOMA-IR; panel D, glucose AUC during OGTT; panel E, insulin AUC during OGTT; panel F, glucose AUC/insulin AUC during OGTT. Values are mean $\pm$ S.E.M. ($n=6$). Bars without a common superscript ('a and b') are different at $P<0.05$ by one-way ANOVA followed by *post hoc* least significant difference (LSD) test.

($P<0.05$) in CrR than CrC offspring of both the sexes from 12 months of age and rehabilitation appeared to correct the body weight change partially albeit at 12 months of age but not later (Padmavathi *et al.* 2010*a*).

## Glucose tolerance and plasma insulin levels to glucose challenge

Males

CrR offspring had higher ($P<0.05$) fasting plasma glucose and insulin at 9 and 15 months of age (Figs 1A and B and 2) than controls. While rehabilitation restored glucose levels, it had varied effects on plasma insulin levels. At 9 months of age, Cr rehabilitated from parturition (CrRP) and Cr rehabilitated from weaning (CrRW) appeared to mitigate the increased plasma insulin levels whereas Cr rehabilitated from conception (CrRC) had only a partial effect. On the other hand, at 15 months of age CrRC but not CrRP and CrRW restored these changes (Figs 1A and B and 2). HOMA-IR was higher ($P<0.05$) in CrR at 9 and 15 months

of age and the changes in general appeared to be corrected by rehabilitation (Fig. 1C).

Glucose AUC and insulin AUC during OGTT were higher ($P<0.05$) in CrR than CrC at 9 but not 15 months of age (Figs 1D and E and 2) and rehabilitation in general mitigated the changes. Nevertheless, the ratio of glucose AUC to insulin AUC was comparable among different groups at both the time points (Fig. 1F).

Females

Fasting plasma glucose was higher ($P<0.05$) in CrR than CrC both at 9 and 15 months of age. Only CrRC appeared to correct the change at 9 months of age while all rehabilitation regimes did so at 15 months of age (Figs 3A and 4). On the other hand, fasting insulin was higher ($P<0.05$) in CrR than CrC offspring albeit at 15 months of age only and rehabilitation did not appear to correct the change (Figs 3B and 4). As a consequence, HOMA-IR was higher ($P<0.05$) in CrR than CrC both at 9 and 15 months of age. Curiously,
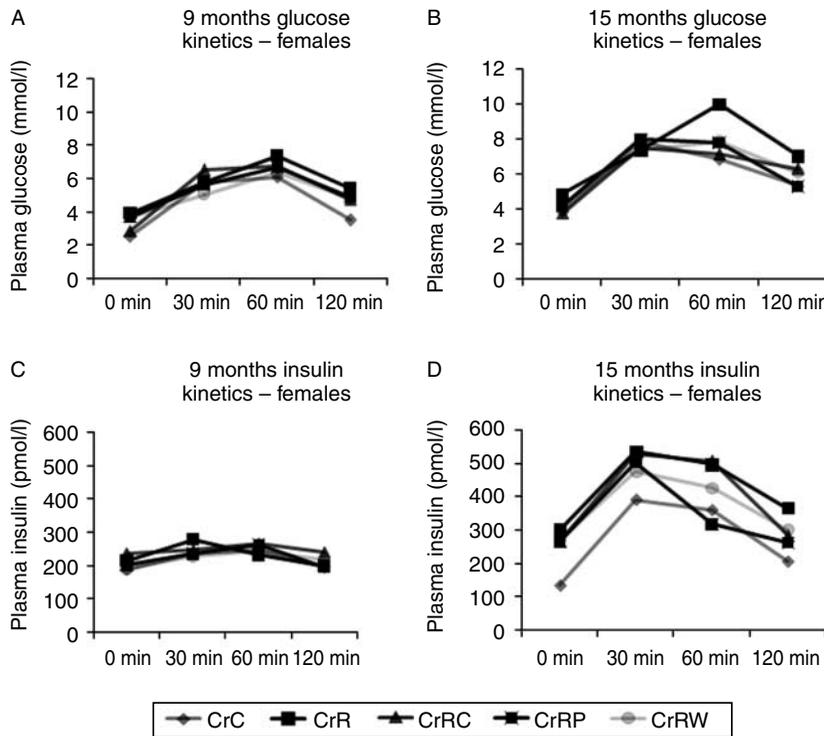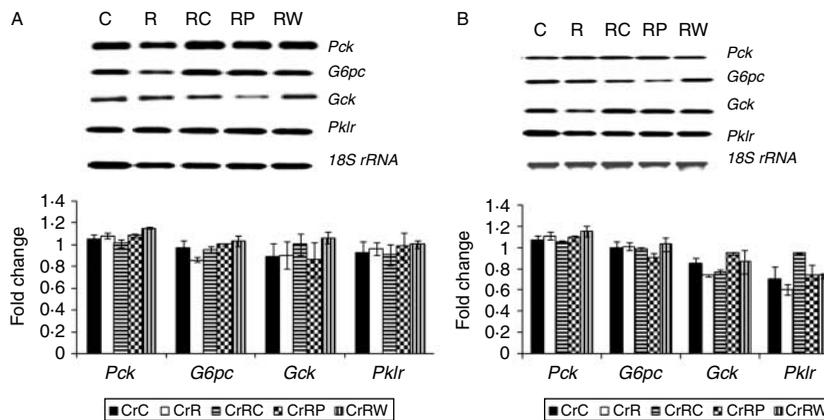
Figure 4 Effect of maternal Cr restriction and rehabilitation on plasma glucose and insulin levels at various time points (0, 30, 60, and 120 min) during OGTT in WNIN female offspring. Panel A, plasma glucose kinetics at 9 months of age; panel B, plasma glucose kinetics at 15 months of age; panel C, plasma insulin kinetics at 9 months of age; panel D, plasma insulin kinetics at 15 months of age. Values are mean ($n=6$).

CrRP and CrRW but not CrRC appeared to correct the change partially at 9 months, while no rehabilitation regime appeared to mitigate the change at 15 months of age (Fig. 3C).

Glucose AUC and insulin AUC during OGTT were higher ($P<0.05$) in CrR than CrC offspring at 15 but not 9 months of age. While all three rehabilitation regimes appeared to restore the glucose AUC to control levels, only CrRP but not CrRC and CrRW appeared to correct insulin AUC (Figs 3D and E and 4). The ratio of glucose AUC to insulin AUC was decreased ($P<0.05$) in CrR offspring compared with CrC but only at 15

Figure 5 Effect of maternal Cr restriction and rehabilitation on expression of genes of glucose metabolism by semi-quantitative PCR in liver in WNIN male (A) offspring (at 18 months of age) and female (B) offspring (at 15 months of age); gel picture for each gene is the representation of different groups. Values are mean ± S.E.M. ($n=6$).

Table 3 Enzymatic activity in liver of Wistar/NIN rat offspring fed different diets. Values are mean±s.e.m. (*n*=6)

	CrC	CrR	CrRC	CrRP	CrRW
Males					
G6PC (units/mg)	0·014±0·004	0·020±0·002	0·022±0·002	0·014±0·005	0·024±0·002
FBP (units/mg)	0·017±0·001	0·020±0·001	0·018±0·000	0·021±0·002	0·019±0·001
GCK (units/mg)	0·006±0·000	0·005±0·001	0·007±0·000	0·005±0·000	0·007±0·000
PK (units/mg)	0·141±0·030	0·109±0·011	0·181±0·003	0·127±0·007	0·102±0·003
Females					
G6PC (units/mg)	0·034±0·002	0·028±0·002	0·036±0·006	0·043±0·005	0·038±0·000
FBP (units/mg)	0·020±0·001	0·019±0·002	0·022±0·001	0·021±0·001	0·021±0·000
GCK (units/mg)	0·012±0·001[a]	0·005±0·001[b]	0·011±0·000[a]	0·009±0·002[a]	0·012±0·003[a]
PK (units/mg)	0·105±0·005	0·100±0·008	0·096±0·007	0·109±0·010	0·108±0·009

Means without a common superscript ('a and b') are significantly different at $P<0.05$ by one-way ANOVA followed by *post hoc* least significant difference (LSD) test.

months of age and rehabilitation in general mitigated this change (Fig. 3F).

Gene expression and enzyme activity

Gene expression of hepatic GCK, PK, Pck, and G6PC, the enzymes important in glycolysis and gluconeo-genesis was comparable among offspring of different groups (in both genders; Fig. 5A and B).

The activity of hepatic GCK was lower ($P<0.05$) in female CrR than CrC offspring and all rehabilitation regimes corrected this change. Unlike females, GCK activity was comparable among male offspring of different groups. However, activities of PK, G6PC, and FBP were comparable among offspring of both sexes in different groups (Table 3).

Expression of *Ins1* and *Ins2* genes was higher ($P<0.05$) in the pancreas of male CrR than CrC

offspring. While no rehabilitation regime could restore the change in *Ins1* expression, CrRP and CrRW but not CrRC corrected the change in *Ins2* expression (Fig. 6A). In female offspring, expression of *Ins1* was comparable among groups while curiously, expression of *Ins2* was lower ($P<0.05$) in CrR than CrC offspring. However, rehabilitation in general corrected changes in *Ins2* gene expression (Fig. 6B).

Oxidative stress and antioxidant status

Males

Malondialdehyde (MDA) levels were higher ($P<0.05$) in CrR than CrC offspring and CrRC and CrRP restored this change (Table 4). However, protein carbonyls, glutathione (reduced and oxidized) levels, and catalase activity were comparable among different groups.
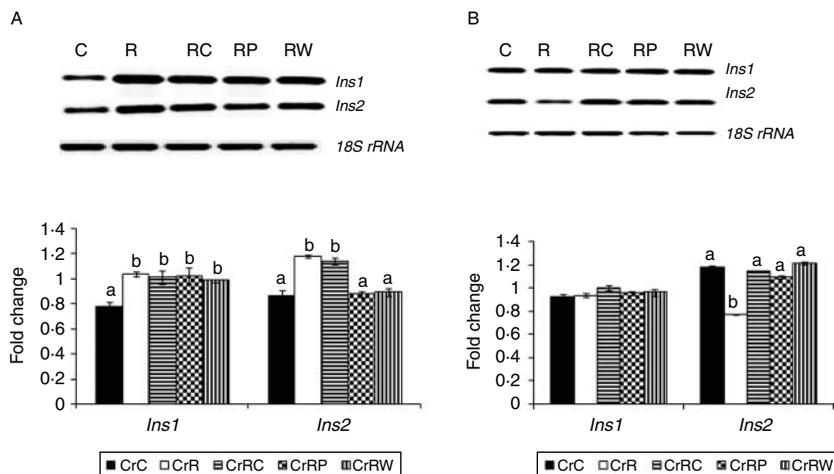
Figure 6 Effect of maternal Cr restriction and rehabilitation on expression of genes involved in insulin secretion by semi-quantitative PCR in pancreas in WNIN male (A) offspring (at 18 months of age) and female (B) offspring (at 15 months of age); gel picture for each gene is the representation of different groups. Values are mean±s.e.m. (*n*=6).

Table 4 Oxidative stress and antioxidant status in liver of Wistar/NIN rat offspring of different groups. Values are mean ± S.E.M. (n=6)

	CrC	CrR	CrRC	CrRP	CrRW
Males					
MDA (nmol/mg)	0·540 ± 0·025[a]	0·848 ± 0·044[b]	0·565 ± 0·077[a]	0·606 ± 0·048[a]	0·821 ± 0·025[a]
Protein carbonyls (nmol/mg protein)	2·32 ± 0·153	2·09 ± 0·117	2·17 ± 0·071	2·11 ± 0·094	2·40 ± 0·102
GSH (μmol/mg)	3·58 ± 0·157	4·11 ± 0·212	3·39 ± 0·458	3·24 ± 0·186	4·03 ± 0·096
GSSG (μmol/mg)	6·98 ± 0·452	8·18 ± 0·682	7·56 ± 0·821	7·97 ± 0·803	7·13 ± 0·745
Catalase (units/mg)	0·110 ± 0·006	0·102 ± 0·008	0·107 ± 0·008	0·114 ± 0·006	0·099 ± 0·006
SOD (units/mg)	7·79 ± 0·428[a]	6·12 ± 0·553[b]	6·21 ± 0·249[b]	7·30 ± 0·553[a]	6·94 ± 0·299[a]
GPx (units/mg)	0·228 ± 0·014[a]	0·165 ± 0·024[b]	0·184 ± 0·016[a]	0·259 ± 0·029[a]	0·215 ± 0·017[a]
Females					
MDA (nmol/mg)	0·358 ± 0·053	0·347 ± 0·020	0·405 ± 0·033	0·340 ± 0·023	0·327 ± 0·024
Protein carbonyls (nmol/mg protein)	2·44 ± 0·143	2·53 ± 0·054	2·38 ± 0·135	2·65 ± 0·061	2·52 ± 0·103
GSH (μmol/mg)	3·74 ± 0·140	3·40 ± 0·268	3·60 ± 0·201	3·60 ± 0·361	3·17 ± 0·367
GSSG (μmol/mg)	6·93 ± 0·758	8·00 ± 0·660	6·64 ± 0·656	7·01 ± 0·413	8·63 ± 0·575
Catalase (units/mg)	0·074 ± 0·004[a]	0·049 ± 0·002[b]	0·070 ± 0·003[a]	0·066 ± 0·006[a]	0·051 ± 0·002[b]
SOD (units/mg)	7·79 ± 0·423[a]	5·97 ± 0·393[b]	6·18 ± 0·296[b]	6·49 ± 0·366[b]	6·30 ± 0·184[b]
GPx (units/mg)	0·293 ± 0·018[a]	0·207 ± 0·026[b]	0·177 ± 0·012[b]	0·179 ± 0·019[b]	0·208 ± 0·067[b]

Means without a common superscript ('a and b') are significantly different at P<0·05 by one-way ANOVA followed by *post hoc* least significant difference (LSD) test.

Interestingly, SOD and GPx activities were lower (P<0·05) in CrR than CrC offspring (Table 4). While CrRP and CrRW corrected the change only in SOD activity, all rehabilitation regimes corrected the reduced GPx activity.

Females

Malondialdehyde, protein carbonyls and glutathione (reduced and oxidized) levels were comparable among female offspring of different groups. However, catalase, SOD, and GPx activities were lower (P<0·05) in CrR than CrC. While no rehabilitation regime could correct the decrease in SOD and GPx activities, CrRC and CrRP but not CrRW could correct the reduced catalase activity (Table 4).

Discussion

We showed earlier that micronutrient restriction *in utero* predisposed rat offspring to glucose intolerance and altered insulin secretion (Venu *et al.* 2004a,b, 2005, 2008, Padmavathi *et al.* 2009). Cr supplementation is known to modulate body composition, improve glucose tolerance and insulin sensitivity (Anderson 1989, Mertz 1993). Recently, we reported that chronic maternal dietary CrR (65% restriction) irreversibly increased body fat% (especially visceral adiposity) and decreased muscle mass (Padmavathi *et al.* 2010a,b) in WNIN rat offspring, despite comparable (to controls) food intake suggesting the effects to be due to maternal CrR-induced programming of the fetal body composition

(Padmavathi *et al.* 2010c, Vincent & Rasco 2010). Although there was some difference in plasma Cr levels between mothers and offspring, at the levels of dietary CrR employed, they were significantly lower in CrR than controls in both the mothers (35%) and the offspring (57%). In this study, effects of CrR *per se* and of maternal CrR on glucose metabolism and plasma insulin levels were elucidated in the WNIN rat model.

In line with our reports in maternal magnesium or zinc restricted rat offspring (Venu *et al.* 2005, Padmavathi *et al.* 2009), this study showed that chronic dietary CrR *per se* did not affect glucose tolerance and IR in WNIN female rats. However, they are at variance with the reported effects of Cr deficiency in human subjects (Offenbacher & Pi-Sunyer 1980). This lack of effect of CrR *per se* could be due to moderate Cr deficiency observed in CrR rats and/or insufficient duration of Cr deficiency and/or the use of a different species (rat).

Chronic maternal dietary CrR induced fasting hyperglycemia, hyperinsulinemia increased HOMA-IR, altered the kinetics of blood glucose and insulin during the OGTT, impaired glucose tolerance and resulted in postprandial hyperinsulinemia to glucose challenge and mitigation of these changes by rehabilitation was albeit partial. These observations are in agreement with studies reporting impaired glucose tolerance in the later life of diet-restricted rat offspring (Dahri *et al.* 1991, Langley *et al.* 1994). However, they disagree with the decreased capacity of insulin secretion to a glucose challenge in the offspring born to WNIN rat dams on magnesium and zinc deficiencies (Venu *et al.* 2005, 2008, Padmavathi *et al.* 2009). Considering that IR and/or altered insulin secretion exist before the onset of fasting and

postprandial hyperglycemia which lead to type 2 diabetes (Weyer *et al.* 1999, Dostou & Gerich 2001), our findings suggest the predisposal of the CrR offspring to type 2 diabetes. The finding that the changes observed at 9 months of age were not seen later in male offspring is in agreement with our similar findings in Mg restricted rat offspring (Venu *et al.* 2008). Although the reasons for the transient nature of the effect remains to be understood, the findings do indicate the importance of maternal Cr status in regulating glucose tolerance and plasma insulin levels in later life of the offspring.

In general, blood glucose levels are regulated by a balance between glucose uptake by peripheral tissues and glucose secretion by the liver. An imbalance between systemic glucose delivery (endogenous glucose production) and glucose utilization leads to hyperglycemia (Mevorach *et al.* 1998). Despite the fasting hyperglycemia and impaired glucose tolerance observed in the CrR offspring, that there were no changes in the gene expression of the rate-limiting enzymes of glycolytic and gluconeogenic pathways, probably indicates that maternal CrR may not alter the expression of these enzymes at transcription level and the observed phenotypic changes could be due to post-transcriptional and/or translational and/or post-translational modifications.

Although enzyme protein expression was not assessed, the observation that activities of the key enzymes of glycolysis and gluconeogenesis were in general comparable among different groups (but for lower GCK in CrR than CrC female offspring and rehabilitation corrected the defect) appear to suggest that maternal CrR may not affect glucose metabolism in the offspring. On the other hand, decreased GCK activity in CrR female offspring could lower glucose catabolism raising intracellular glucose levels that in turn could impair the clearance of circulating glucose and result in hyperglycemia. Such increase in plasma glucose levels may not be conducive to hepatic gluconeogenesis and hence the comparable activities of gluconeogenic enzymes in these offspring. These findings not only agree with mRNA expression data but may also suggest impaired glucose metabolism (decreased glycolysis?) as a possible reason for the fasting hyperglycemia. Although the actual mechanism(s) need to be studied, the present results are the first to the best of our knowledge to demonstrate the effect of maternal CrR on glucose tolerance in the offspring.

Insulin genes in rodents form a two-gene system (Soares *et al.* 1985, Wentworth *et al.* 1986) composed of preproinsulin 2 (*Ins2*), an ortholog to the insulin genes in the other mammals and *Ins1*, a rodent-specific retrogene. *Ins1* and *Ins2* are expressed in pancreas and both encode proinsulin peptides that include signal peptide, B chain, C-peptide, and A chain. In line with the observed hyperinsulinemia, expression of *Ins1* and *Ins2* genes were increased in CrR male offspring indicating that maternal CrR may affect the transcription of the insulin genes, resulting in hyperinsulinemia (fasting and post-glucose). However, it was surprising that in female CrR offspring *Ins2* expression was indeed downregulated compared with CrC while expression of *Ins1* was unaffected. The reasons for these discrepancies and gender differences need to be deciphered.

In line with our earlier reports on vitamin restricted rat offspring (Venu *et al.* 2004a), maternal CrR increased oxidative stress (MDA levels) and decreased activities of antioxidant enzymes in the offspring. These findings are in agreement with literature that maternal undernutrition is associated with stress in the offspring (Hostetler & Kincaid 2004) and agrees with increased corticosteroid stress we reported earlier in these offspring (Padmavathi *et al.* 2010a). The present results are in agreement with substantial evidence that increased oxidative stress and/or decreased antioxidant defense is associated with IR that could lead to type 2 diabetes at a later date (Giugliano *et al.* 1995, Vijayalingam *et al.* 1996, Urakawa *et al.* 2003). However, the actual mechanism(s) and/or the causal relationships among the increased oxidative stress, IR, and impaired glucose tolerance in the CrR offspring remain to be deciphered yet.

Overall, these findings stress the importance of maternal Cr status in modulating carbohydrate metabolism and plasma insulin levels in the offspring. Although much work needs to be done to decipher the underlying/associated mechanism(s), they have unequivocally demonstrated the effects of maternal CrR *per se* in programming the offspring to glucose intolerance and IR and the possible involvement of increased oxidative stress in the process. Taken together with our earlier demonstration of increased body fat%, especially the visceral adiposity (Padmavathi *et al.* 2010a) and decreased myogenesis and altered muscle function in CrR offspring (Padmavathi *et al.* 2010b), the present findings appear to suggest the probable predisposal of the CrR offspring to type 2 diabetes in their later life.

Declaration of interest

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