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RESEARCH LETTER

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Glucose metabolism is altered in the adequately-nourished grand-offspring (F_3 generation) of rats malnourished during gestation and perinatal life

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To the Editor:
A growing number of experimental animal studies have demonstrated the intergenerational effects of foetal/perinatal programming on birth weight, blood pressure and glucose metabolism. Potential explanations for these intergenerational effects include the following: (1) shared genetic attributes of parent and offspring; (2) adverse 'extrinsic' environmental conditions that persist across generations; and (3) adverse intrauterine environments that may be propagated across generations [1].

While a large number of animal studies have shown the effects of undernutrition during foetal/perinatal development on the glucose metabolism of exposed animals (F_1) in adulthood [2], several studies have shown that glucose metabolism is also altered in the offspring (F_2) of foetally malnourished F_1 females, even when the F_1 females have been well nourished since weaning [3, 4]. Here, we show, for the first time, that the glucose metabolism of the grand-offspring (F_3) of female rats malnourished during development is also adversely affected.

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Sprague–Dawley rats consumed either a nutritionally adequate diet (20% protein; TD 91352; Harlan Teklad, Madison, WI, USA), or an isoenergetic, low-protein diet (8% protein; TD 93033; Harlan Teklad) from day 1 of pregnancy through lactation. Pups that were protein malnourished in utero consumed an adequate unrestricted diet post-weaning. The dams of control pups were adequately nourished throughout pregnancy and their offspring consumed an adequate unrestricted diet post-weaning. To conserve animal resources, only one generation of control animals was bred. At ~70 days of age, control and experimental animals were deprived of food overnight and subjected to an i.p. glucose tolerance test. Animals were killed under CO_2 anaesthesia at 0, 30 and 120 min after glucose load (30% w/v; 2 g/kg body weight, i.p.). Blood was collected by cardiac puncture. Four female first-generation (F_1) rats, whose mothers were protein-malnourished both during pregnancy and while nursing, were randomly selected from the experimental group at ~70 days of age. The selected F_1 rats were mated with control breeder males and maintained on the adequate diet throughout gestation and lactation. Their offspring, the F_2 generation, also consumed an adequate diet post-weaning. At ~70 days of age, glucose tolerance tests were conducted on the F_2 rats as described above. A final generation of animals (F_3) was bred from control breeder males and four randomly selected F_2 dams whose mothers (F_1) had been protein-malnourished throughout pregnancy and lactation. F_3 animals were maintained on the adequate diet and tested as described. This research was approved by the Institutional Animal Care and Use Committee (IACUC) at Arizona State University. Animals were maintained in accordance with the 'Guide for the Care and Use of Laboratory Animals' of the National Research Council (7th ed., 1996).

As in previous reports [1, 2], the mean (±SEM) birthweight of F_1 animals in our study (5.29±0.082 g) was significantly lower ($p<0.05$) than that of control animals

Table 1 Plasma glucose and insulin concentrations in control rats and in rats whose dams consumed low-protein diets during gestation (F_1), offspring of F_1 rats (F_2), and offspring of F_2 rats (F_3)

		Fasting	30 min	120 min
Plasma glucose (mmol)				
Controls	Males	6.0±0.2[a] (5)	16.5±0.8[a]* (5)	10.4±0.5[a] (5)
	Females	5.5±0.2[a] (5)	12.7±0.6[a] (5)	8.8±0.5[a] (5)
F_1	Males	6.6±0.7[a] (4)	11.2±1.0[b] (5)	10.0±0.6[a] (5)
	Females	5.5±0.4[a] (5)	11.3±1.0[a] (4)	10.3±1.3[a] (4)
F_2	Males	7.4±0.4[a]* (5)	10.9±0.9[b] (5)	11.0±1.3[a] (8)
	Females	5.7±0.3[a] (10)	10.8±0.4[a] (11)	8.8±0.3[a] (6)
F_3	Males	7.6±0.7[a] (6)	10.8±0.6[b] (8)	8.8±0.2[a] (5)
	Females	7.0±0.1[b] (12)	11.0±0.7[a] (11)	11.3±1.1[a] (3)
Plasma insulin (mIU)				
Controls	Males	20.1±1.3[a] (5)	88.5±6.3[ab]* (5)	48.4±3.6[a] (5)
	Females	18.1±1.2[a] (5)	57.0±5.7[a] (5)	36.6±4.7[a] (5)
F_1	Males	16.9±1.7[a] (4)	52.1±19.8[a] (5)	59.4±22.1[a] (5)
	Females	15.0±2.2[a] (5)	58.6±18.3[a] (4)	42.6±17.4[a] (4)
F_2	Males	68.1±16.6[b]* (4)	130.4±8.3[b] (5)	148.4±25.1[b]* (8)
	Females	37.1±4.6[b] (10)	98.0±13.0[a] (11)	59.3±7.2[a] (5)
F_3	Males	51.1±9.5[b] (6)	106.4±13.6[b] (8)	73.4±4.7[a]* (5)
	Females	31.1±5.8[ab] (12)	78.7±12.7[a] (11)	47.4±7.3[a] (3)

Sample size shown in parentheses. Blood was collected from animals at fasting, at 30 min or at 120 min following a glucose challenge. Means (±SEM) in columns with different letters (within gender group) differ significantly ($p<0.05$, significant univariate ANOVA with least significant difference post hoc analysis). Asterisks indicate significant gender difference within generation

(6.43±0.22 g). The mean birthweights of the F_2 (6.60± 0.124 g) and F_3 (6.18±0.119 g) animals did not differ significantly from the mean birthweight of the control animals. Our results (Table 1; Fig. 1) also show that the effects of foetal/perinatal malnutrition on glucose homeostasis of F_1 animals and their F_2 offspring are consistent with previous studies: reduced insulin secretion in F_1 animals [5] and insulin resistance in F_2 animals [3, 4]. In addition, our results clearly indicate altered glucose homeostasis in the grand-offspring (F_3) of foetally/perinatally malnourished F_1 females. Fasting plasma glucose levels of F_3 female animals were significantly ($p<0.05$) higher than those of control females, while 30-min plasma glucose levels among F_1, F_2 and F_3 males were significantly lower than those of control males (Table 1). Although F_3 female insulin concentrations did not differ significantly from controls at any time point, F_3 male insulin levels were significantly higher than controls at fasting and 30 min after the glucose load. The data further show a significantly higher insulin:glucose ratio among F_3 males vs controls at 30 min. Since the diets of F_2 dams during pregnancy and the post-weaning diets of F_3 animals were 'adequate' (control), these observations suggest that the metabolic disturbances in the F_2 generation had some effect on metabolism in the F_3 generation. However, while fasting glucose in F_3 females was significantly higher than that in F_2 females, neither the insulin concentrations nor the insulin:glucose ratio were significantly different. Moreover, among F_3 males, although insulin levels at fasting and 30 min were significantly higher than those of controls animals, as was the insulin:glucose ratio at 30 min, all of these levels were lower than those of F_2 animals (Table 1; Fig. 1). Taken together, these data suggest some movement towards normalisation in the F_3 generation when the maternal diets of F_2 dams and post-weaning diets of F_3 animals were adequate, and may provide further evidence of an eventual intergenerational 'resolution' of altered glucose–insulin metabolism, as reported by Drake et al. [6]. Whether such an intergenerational normalisation can be accelerated by manipulating the diet of insulin-resistant F_2 dams remains to be seen. Further studies aimed at better understanding the intergenerational effects of nutritionally mediated foetal/perinatal programming are clearly warranted in light of the current global epidemic of human metabolic disorders.

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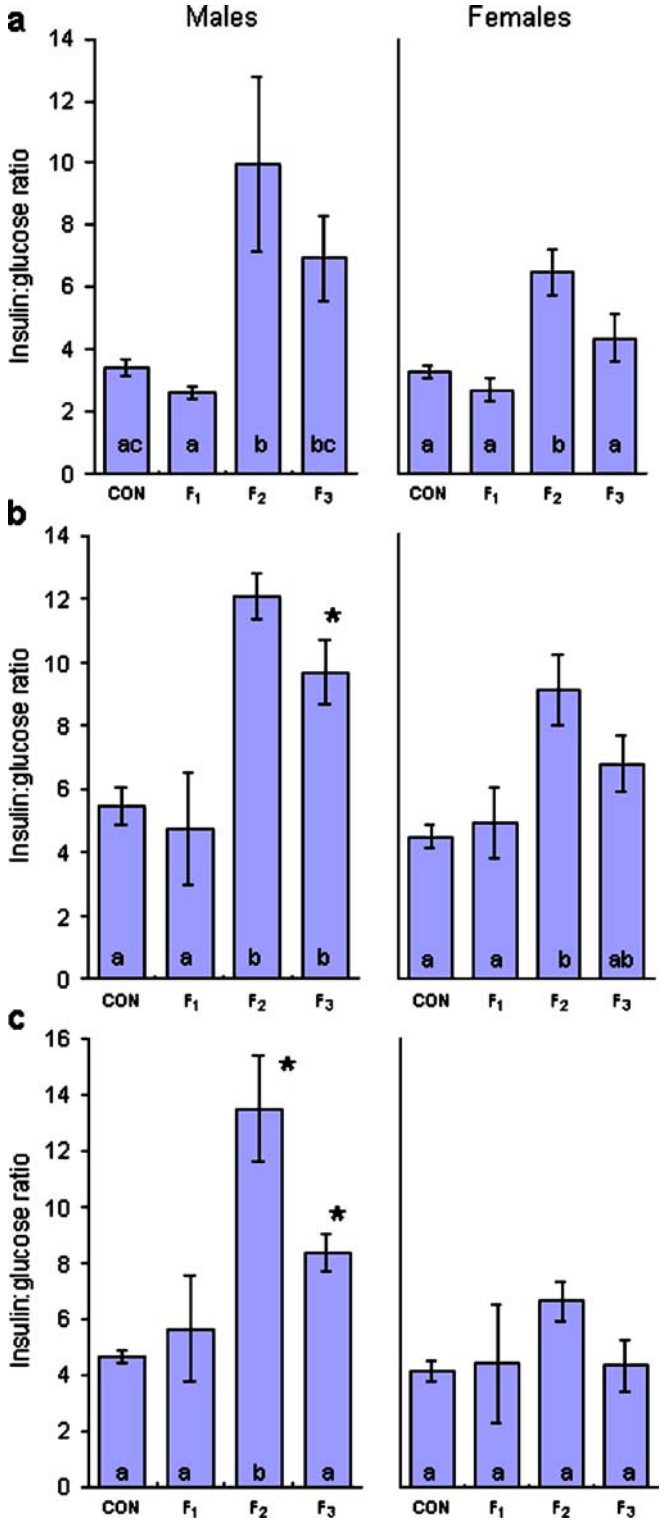

Fig. 1 Insulin:glucose ratios (means±SEM) in control (CON) rats, rats whose dams consumed low-protein diets during gestation (F_1), offspring of F_1 rats (F_2), and offspring of F2 rats (F_3). All rats consumed control diets post-weaning. Values are for fasting (**a**), 30 min post-glucose challenge (**b**), and 120 min post-glucose challenge (**c**). *Bars* with different letters differ significantly ($p<0.05$, significant univariate ANOVA with least significant difference post hoc analysis). *Asterisks* indicate significant gender difference within generation

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