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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Postnatal Weight Gain and Onset of Puberty in Rats Exposed to Aqueous Extract of *Hibiscus sabdariffa* in Utero

Eghosa, E. Iyare¹ and Olufeyi A. Adegoke²

¹Department of Physiology, College of Medicine,
University of Nigeria, Enugu Campus, Enugu State, Nigeria

²Department of Physiology, College of Medicine, University of Lagos, Idiaraba, Lagos State, Nigeria

Abstract: The present study was designed to investigate whether or not maternal consumption of aqueous extract of *Hibiscus sabdariffa* (HS) during pregnancy has any effect on the growth and onset of puberty in the female offspring. Eighteen in-bred virgin female Sprague-Dawley rats aged between 10-12 weeks and weighing 125±5.5g (mean±SEM) with two consecutive regular 4-day estrus cycle were randomly assigned to one of three groups of 6 rats/group. One group had tap water (control); another had 1.5g-extract/kg while the third group had 3.0g-extract/kg in their drinking water throughout pregnancy. Results showed that HS consumption during pregnancy significantly decreased maternal fluid and food intake, increased postnatal weight gain and delays the onset of puberty in the female offspring.

Key words: *Hibiscus sabdariffa*, postnatal weight gain, onset of puberty, *in utero*

Introduction

In 1989, Barker proposed a novel hypothesis (the "fetal origins of adult disease" hypothesis) to link nutritional insults during embryonic and foetal development not only to impaired maturation of physiological functions, but also to latent diseases in adulthood (Barker, 1989). Expanding upon this, Hales and Barker (1992) proposed the 'thrifty phenotype' hypothesis (coined from an earlier "thrifty genotype" hypothesis (Neel, 1962; 1999 that suggested that when the foetal nutritional environment is poor, there is an adaptive response, which optimizes the growth of key body organs to the detriment of others and leads to an altered postnatal metabolism, which is designed to enhance postnatal survival under conditions of intermittent or poor nutrition. It was proposed that these adaptations only became detrimental when nutrition was more abundant in the postnatal environment, than it had been in the prenatal environment.

A sweetened aqueous extract of *Hibiscus sabdariffa* (zobo drink) (family: malvaceae) is commonly produced and consumed indiscriminately in Nigeria and extracts of *Hibiscus sabdariffa* (HS) have been reported to decrease fluid and food intake through a mechanism not yet fully understood (Orisakwe *et al.*, 2003; Orisakwe *et al.*, 2004; Ojokoh, 2006). Thus when aqueous extract of HS is administered to pregnant animals, it may lead to decreased food consumption in these animals with a possible foetal malnutrition and the attendant developmental sequelae (Barker *et al.*, 1993; Gluckman and Hanson, 2004a Armitage *et al.*, 2005a,b) in accordance with the "fetal origins of adult disease" and "thrifty phenotype" hypotheses.

Whether or not maternal consumption of aqueous extract of HS during pregnancy has any effect on the

growth and onset of puberty in the female offspring is not known. The present study was therefore designed to investigate this.

Materials and Methods

Experimental animals: Eighteen in-bred virgin female Sprague-Dawley rats age between 10-12 weeks and weighing 125±5.5g (mean±SEM) with two consecutive regular 4-day estrus cycle were used for this study. These rats were housed individually in cages under standard environmental conditions. The estrous cycles were monitored and male rats of proven fertility were introduced into the cages of the female rats that were expected to get into the estrous phase within 12 hours to allow for mating. Day 1 of pregnancy was taken as the day sperm were seen in the vaginal smear of the rats. On day 1 of pregnancy, animals were divided randomly into three groups of six animals each. The first group (control) was given tap water to drink. The second group was placed on 1.5g-extract/Kg body weight/day in their drinking water while the third group received 3.0g-extract/Kg body weight/day in their drinking water. All groups received normal rat chow *ad libitum*. Fluid and food intake and dam weights were measured daily throughout pregnancy and at delivery. Gestational length, litter size and weight were also recorded. On the day of delivery, the HS solutions were withdrawn and replaced with tap water. Each dam in each group was allowed 9 pups to nurse throughout the lactational period so as to eliminate the effect of undernutrition or overnutrition of some of the pups. After 21 days, the pups were weaned to tap water. After weaning, the female pups were kept in groups of three per cage. Pups' weights were recorded at birth, weaning and weekly thereafter till onset of

Iyare and Adegoke: *In utero* HS and onset of puberty

Table 1: Mean fluid intake (ml/kg/day) during pregnancy

Group	1st trimester	2nd trimester	3rd trimester
Control	248.75±3.61	293.93±7.29	233.72±5.3
1.5g/Kg/day	146.99±2.07*	182.84±2.45* ^p	187.02±4.83*
3g/Kg/day	143.2±3.15*	150.77±3.58*	199.97±9.34*

N = 6 each. Values are expressed as Mean±SEM, *p<0.05 versus control rats, ^pp<0.05 versus 3.0g/kg rats

Table 2: Mean food consumption (g/kg/d) during pregnancy

Group	1st trimester	2nd trimester	3rd trimester
Control	174.0±4.59	217.02±8.94	199.36±6.12
1.5g/Kg/day	140.53±4.68*	176.38±3.66* ^p	168.5±5.93* ^p
3g/Kg/day	136.01±4.67*	136.76±3.93*	148.25±2.48*

N = 6 each. Values are expressed as Mean±SEM, *p<0.05 versus control rats, ^pp<0.05 versus 3.0g/kg rats

puberty. Pubertal development starts soon after weaning, so from postnatal day 30 onwards, the young female rats were inspected daily for vaginal opening since onset of puberty is defined as the age (in days) at which vaginal opening occurs (Engelbregt *et al.*, 2000).

Extraction procedure: Mature dry dark-red calyces of HS were purchased from a local market in Enugu, Nigeria and authenticated by Mr T.I. Adeleke of the department of Pharmacognosy, University of Lagos, Nigeria where a voucher specimen number PCG H455 was deposited. The extraction procedure used in our laboratory was as described previously (Adegunloye *et al.*, 1996; Mojiminiyi *et al.*, 2007) but with slight modification. Briefly, 30g of the dry petals of HS was brewed in 400ml of boiled tap water for 45min. The resulting decoction was filtered and evaporated to dryness giving a dark red powder (yield 48.87%).

Statistical analysis: The Student's *t*-test for paired data was used to analyze data from the same group of rats. For data comparison between the three groups, the one way analysis of variance (ANOVA) was used followed by a post-hoc Student's Newman-Keuls test. P<0.05 was taken as statistically significant.

Results

Maternal fluid and food intake

Fluid intake (ml/kg): Dams given HS consumed less (p<0.05) fluid per day compared with the control dams at all the trimesters of pregnancy. There was a significantly increased fluid intake in the 2nd trimester compared with the fluid intake in the 1st trimester in 1.5g/kg group whereas 3.0g/kg group and group C showed no difference. 3.0g/kg dams also drank less (p<0.05) fluid in the 2nd trimester compared with 1.5g/kg and Control groups. Fluid intake in the 3rd trimester in groups 1.5g/kg and 3.0g/kg was greater than the fluid intake in the 1st trimester whereas the control group showed no difference. There was a significant difference in fluid intake between the 3rd and the 2nd trimester in 3.0g/kg

and Control groups (3.0g/kg dams higher while Control dams were lower) while fluid intake in group 1.5g/kg dams in the 3rd and 2nd trimester were similar.

Food intake/day/rat: There was a significant dose-dependent reduction in food intake/day (p<0.05) in 1.5g/kg and 3.0g/kg groups dams compared with Control group dams at all stages of pregnancy except for the first trimester where there was no significant difference in food intake between 1.5g/kg and 3.0g/kg groups. There was a similar change in food intake in all the groups as pregnancy progressed except in 3.0g/kg group where the food intake by the dams in the 2nd trimester was not different from the 1st trimester but less than (p<0.05) that of the 3rd trimester.

Postnatal growth: Result of the present study show a significant increase in body weight from birth to postnatal day 28 in groups 1.5g/kg and 3.0g/kg compared with Control group. From postnatal day 35, however, there was a significant increase in body weight in offspring of 3.0g/kg group compared with both group 1.5g/kg and Control offspring. Body weight of group 1.5g/kg offspring was not significantly different from that of Control group offspring.

Age and body weight at onset of puberty: There was a significant delay in the age at onset of puberty in groups 1.5g/kg and 3.0g/kg offspring compared with the Control group offspring with group 1.5g/kg rat exhibiting more delay. The body weight at onset of puberty in groups 1.5g/kg and 3.0g/kg offspring were higher than Control group offspring.

Discussion

The reduced food intake in the dams given aqueous extract of HS (Table 2) did not result in low birth weight. This is in contrast to several reports that malnutrition in pregnancy results in low birth weight (Barker, 2000; Seckl, 1998; Edwards *et al.*, 1993; Phillips *et al.*, 1998; Lesage *et al.*, 2001; Fowden and Forhead, 2004). The reason for this may be that the malnutrition in the exposed dams that commenced early in pregnancy, may have caused placental development that favoured increased nutrient delivery to the foetus (Woodall *et al.*, 1996; Osgerby *et al.*, 2002; Fowden *et al.*, 2006) and/or possibly because the developing rat pups recruited adaptive mechanisms that protected its growth (Gluckman and Hanson, 2004a,b). It is also possible that the various constituents of the HS extract may have directly or indirectly affected the growth of the developing pups in utero.

The decreased food intake in the dams that drank HS may also have led to decreased leptin levels in these dams (Maffei *et al.*, 1995; Frederich *et al.*, 1995). Since leptin is transported from the mother to the foetus

Iyare and Adegoke: *In utero* HS and onset of puberty

Table 3: Mean offspring postnatal weights (g) from birth to postnatal day 42

Groups, n = 9	Postnatal day 0 (birth)	Postnatal day 21 (weaning)	Postnatal day 28	Postnatal day 35	Postnatal day 42
Control	5.61±0.14	22.78±0.65	32.78±.77	48.33±0.93	55.83±1.44
1.5g/Kg/day	6.0±.14*	26.94±1.99*	37.78±1.21*	44.44±2.24 ^P	54.17±3.06 ^P
3g/Kg/day	6.11±.12*	28.33±1.20*	40.56±1.34*	58.06±1.29*	76.94±1.07*

N = 9 each. Values are expressed as Mean±SEM, *p<0.05 versus control rats, ^Pp<0.05 versus 3g/Kg/day rats

Table 4: Age and Body weight of offspring at onset of puberty

Parameters at onset of puberty	Group		
	Control	1.5g/Kg/day	3g/Kg/day
Age (days)	43.11±1.84	62.39±1.84* ^P	47.56±0.73*
Body weight (g)	58.89±1.96	87.22±2.52*	94.44±2.94*

N = 9 each. Values are expressed as Mean±SEM, *p<0.05 versus control rats, ^Pp<0.05 versus 3g/Kg/day rats

(Hoggard *et al.*, 1997; Banks *et al.*, 1996), it may therefore have acted as the metabolic signal to the foetus of the status of maternal energy reserves and by extension, environmental food availability thus inducing in the foetus some of the metabolic adaptations that are designed to enhance postnatal survival under conditions of poor nutrition in accordance with the “thrifty phenotype” hypothesis. That the “thrifty phenotype” offspring are better able to acquire and utilize nutrients and demonstrate an increased risk of obesity as adults (Barker *et al.*, 1993) may have accounted for the higher weight increases in the pups from dams that were given HS extract during pregnancy compared with the control pups (Table 3).

Vagina opening is considered a good marker of the onset of puberty in the female rat (Engelbregt *et al.*, 2002). In the present study, there was a delayed onset of puberty (Table 4) in the pups from dams that were given HS extract during pregnancy through mechanisms that may not be unconnected with alterations in glucocorticoid and leptin signaling mechanisms.

Since dams that were given aqueous HS extract drank less fluid compared with the control dams during pregnancy (Table 1), the decreased fluid intake may have caused a state of water deprivation and a consequent plasma hypernatremia in these dams (Ross and Desai, 2005). Aqueous extract of HS has also been shown to be rich in Na⁺ (Adigun *et al.*, 2006). This, coupled with the diuretic effect of HS may have caused the plasma hypernatremia observed by Mojiminiyi and Coworkers (2000). The water deprivation in these dams and the accompanying plasma hypernatremia (osmotic stress) may have caused dehydration-anorexia (Ross and Desai, 2005) with the resultant decrease in food intake (nutrient stress). Osmotic and nutrient stresses are known to elevate plasma glucocorticoid level.

Elevation of plasma glucocorticoid level has been shown to cause a permanent resetting of endocrine systems, such as the somatotrophic and hypothalomo-pituitary adrenal axes (Barker, 2000; Seckl, 1998; Edwards *et al.*, 1993; Phillips *et al.*, 1998; Lesage *et al.*,

2001; Fowden and Forhead, 2004) and a delay in the onset of puberty in female offspring (Smith and Waddell, 2000) possibly by causing altered behaviour and abnormalities in hypothalamic-pituitary-gonadal function (Welberg *et al.*, 2001).

We conclude that maternal consumption of HS during pregnancy affect postnatal growth and delay the onset of puberty in female offspring. Whether or not it was the constituents of HS and/or the decreased food intake induced by the HS that caused the delay in the onset of puberty remains to be investigated.

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Iyare and Adegoke: *In utero* HS and onset of puberty

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