# Effects of sugar rich diet on brain serotonin, hyperphagia and anxiety in animal model of both genders

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**Abstract**: Lower levels of 5-hydroxytryptamine (5-HT; serotonin) in the brain elicit sugar craving, while ingestion of sugar rich diet improves mood and alleviates anxiety. Gender differences occur not only in brain serotonin metabolism but also in a serotonin mediated functional responses. The present study was therefore designed to investigate gender related differences on the effects of long term consumption of sugar rich diet on the metabolism of serotonin in the hypothalamus and whole brain which may be relevant with the hyperphagic and anxiety reducing effects of sugar rich diet. Male and female rats were fed freely on a sugar rich diet for five weeks. Hyperphagic effects were monitored by measuring total food intake and body weights changes during the intervention. Anxiolytic effects of sugar rich diet was monitored in light-dark transition test. The results show that ingestion of sugar rich diet decreased serotonin metabolism more in female than male rats. Anxiolytic effects were elicited only in male rats. Hyperphagia was comparable in both male and female rats. Finings would help in understanding the role of sugar rich diet-induced greater decreases of serotonin in sweet craving in women during stress.

**Keywords**: Serotonin, gender difference, sugar-diet, Hyperphagia, anxiety.

## INTRODUCTION

Carbohydrate craving are extremely common particularly among women. Craving is frequently reported for specific types of food including chocolate and food high in both sugar and fat (Corsica and Spring, 2008; Spring *et al*., 2008; Yanovski, 2003). Carbohydrate cravers reported feeling distressed prior to their craving and satisfied, happy/good and relaxed following carbohydrate consumption (Christensen and Pettijohn, 2001; Macht and Mueller, 2007). Craving for specific macronutrients such as carbohydrate has been postulated to result from a physiological need to alter neurotransmitter 5-hydroxytryptamine (5-HT; serotonin). Neurochemical research on experimental animals shows that brain 5-HT metabolism is increased following the ingestion of particularly carbohydrate rich diet (Christensen, 1997; Fernstrom and Fernstrom, 1995). Conversely, long-term consumption of sugar rich diet decreased 5-HT metabolism in the hypothalamus and elicited hyperphagia in rats (Haleem *et al*., 2000; Inam *et al*., 2008).

Gender differences in brain 5-HT have been reported in neurochemical (Dalla *et al*., 2005; Haleem *et al*., 1990; Mitsushima *et al*., 2006), pharmacological (Currie *et al*., 2004; Eckel *et al*., 2005) as well as behavioral studies (Haider and Haleem, 2000; Haleem, 1993; Walker *et al*., 2007). Present study was designed to test the hypothesis that long term consumption of sugar rich diet decreases brain serotonin metabolism more in female gender which leads to sweet craving in women, some other effects of sugar rich diet such as hyperphagic and an alleviation of anxiety are also compared in male female rats.

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## METHODS AND MATERIAL

### *Animals*

Locally bred male and female Albino Wistar rats weighing 200-220g were housed individually under 12 h light dark cycle (lights on at 6:00h) at 25±5°C. Animals had free access to cubes of standard rodent diet and tap water 3 days before experimentation. The animals were handled according to the U.K Animals Act of 1986 and the associated guidelines.

### *Preparation of sugar rich diet*

The diet was prepared essentially in the same way as described before (Haleem *et al*., 2000). Standard rodent diet available in the form of cubes was crushed finely. The crush mixed with sugar in the ratio of 3:1 (rat diet: Sugar; w/w) was used to prepare cubes of sugar diet. Cubes for normal diet were also prepared from the same crushed rodent diet without mixing sugar in it.

### *Experimental protocol*

Twelve male and female rats were randomly assigned into normal diet and sugar rich diet fed groups each containing six animals. Pre-weighed amount of respective diets were placed in the hopper of cages for five weeks. Total food intake and body weights were monitored. Anxiety level was monitored after five weeks in light-dark transition test. Experimental protocol was approved by the local animal ethics committee (IAEC).

***Food intakes and body weights***

Cumulative food intakes (g) were determined by taking the difference of food given on day 1, between 8:00 and 9:00h and food left next day and every day (between 8:00 and 9:00h). Body weights were also monitored at the same time and change in body weights were calculated (body weight on monitoring day/body weight on preceding day) × 100 as reported previously (Haleem *et al*., 2013; Ikram and Haleem, 2011).

***Light-dark activity***

Light-dark activity test was conducted as described before (Samad *et al*., 2005). A rat placed in light-dark box is expected to pass more time in the dark compartment, to determine the activity a rat was placed in the light-dark compartment of the box. Time spent in the light compartment was monitored for a cut off time of 5min (Mirza *et al*., 2013).

***Brain dissection***

After five weeks of treatment, animals were decapitated between 10:00-11:00h using a balanced design to collect brain samples. Whole brain or hypothalami dissected out as described before (Ikram *et al*., 2011; Haleem *et al*., 2000) were stored at -70ºC until analysis of tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by HPLC-EC.

***Neurochemical Analysis***

For the HPLC-EC determination of tryptophan, 5-HT and 5-HIAA samples were extracted as described before (Haleem and Haider, 1996). A 5µ ODS (ECHPHERE) separation column of 4.0 mm internal diameter, 250mm length was used. The mobile phase, comprising methanol (14%), octyl sodium sulphate (0.023%) and EDTA (0.0035%) in 0.1M phosphate buffer of pH=2.9 was passed through the column at an operating pressure of 2000-3000 psi with the help of Waters 510 HPLC pump. Electrochemical detection was achieved on Shimadzu L-ECD 6A detector (Kyoto, Japan). 5-HT and 5-HIAA were detected at an operating potential of 0.8 volts and tryptophan at 1.0 volts (Ikram *et al*., 2014; Ikram *et al*., 2012).

## STATISTICAL ANALYSIS

Values were represented as means ± SD (n=6). The data were analyzed by two-way ANOVA using SPSS software (ver17). Post hoc comparisons done by Newman-Keuls test. Values of $p<0.05$ were considered as significant.

## RESULTS

Effects of sugar diet on food intake of male and female rats are shown in fig. 1. Two-way ANOVA (df=1,20) shows significant effects of sugar diet ($F=220.7$, $p<0.01$), gender ($F=573.4$, $p<0.01$) and interaction between the two factors ($F=5.66$, $p<0.05$). Post-hoc analysis by Newman-Keuls test showed that sugar rich diet ingestion increased food intake in male and female rats. Normal and sugar rich diet treated female rats exhibited smaller values than normal and sugar rich diet treated male rats respectively.

Effects of sugar diet on growth rates of male and female rats are shown in fig. 2. Two-way ANOVA (df=1,20) revealed significant effects of sugar diet ($F=7$, $p<0.05$), gender ($F=30.5$, $p<0.01$) and interaction between the two factors ($F=4.5$, $p<0.05$). Post-hoc analysis by Newman-Keuls test showed that sugar rich diet ingestion decreased body weight in male rats but no effect was observed in

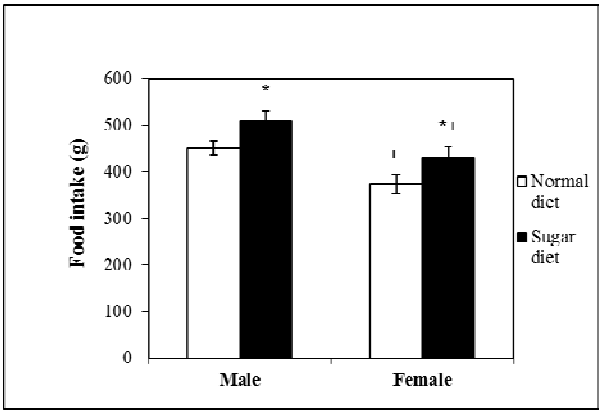



**Fig. 1**: Effects of sugar rich diet ingestion on food intake (g) in freely feeding male and female rats. Values are means ± SD (n=6). Significant difference by Newman-Keuls test *p<0.01 from respective normal diet treated rats, +p<0.01 from respective male rats following Two-way ANOVA.

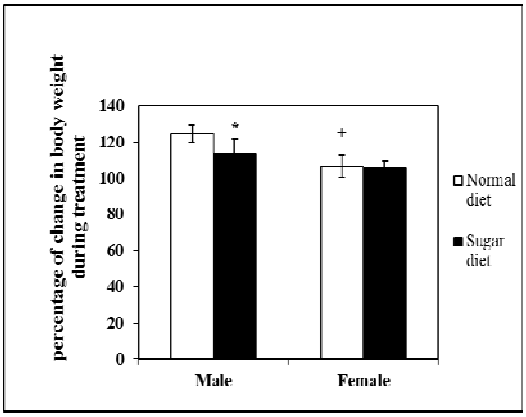



**Fig. 2**: Effects of sugar rich diet ingestion on growth rate (percentage of change in body weight during treatment) in male and female rats. Values are means ± SD (n=6). Significant difference by Newman-Keuls test *p<0.01 from respective normal diet treated rats, +p<0.01 from respective male rats following Two-way ANOVA.

female rats. Female rats given exhibited smaller values than male rats (normal diet group). While sugar rich diet treated male and female rats exhibited comparable values ($p<0.01$).

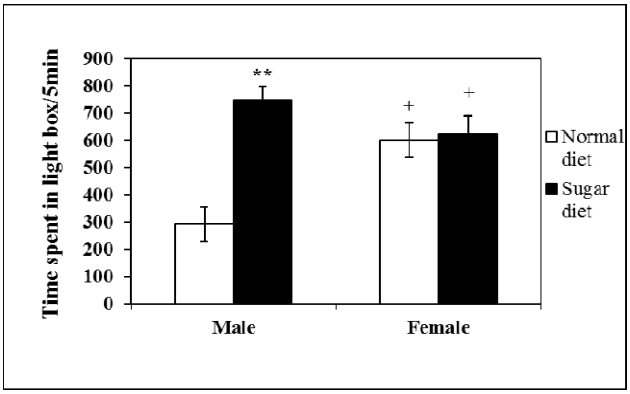
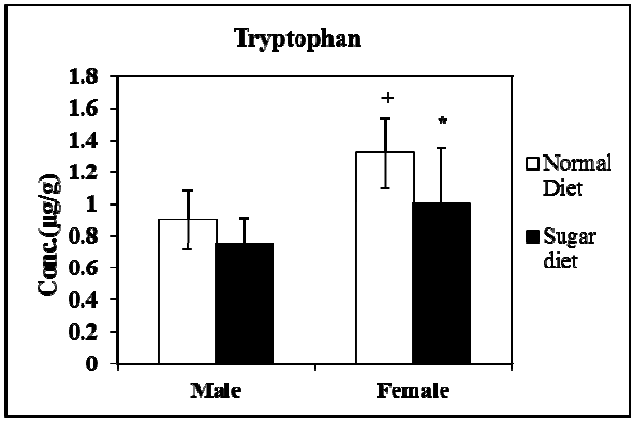
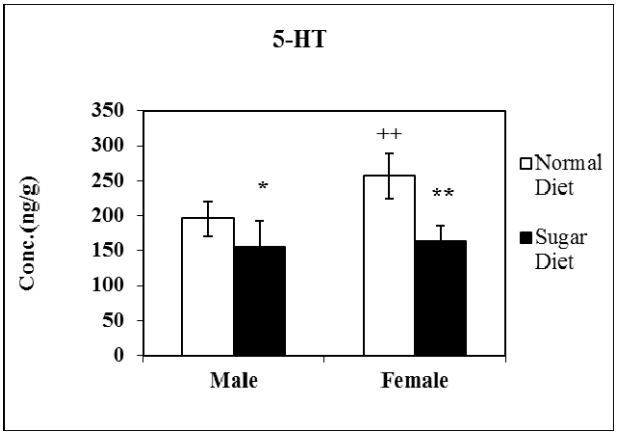



**Fig. 3**: Effects of sugar rich diet ingestion on time spent in light compartment of a light-dark box in male and female rats. Values are means ± S.D (n=6). Significant difference by Newman-Keuls test *p<0.01 from respective normal diet treated rats, +p<0.01 from respective male rats following Two-way ANOVA.

Anxiolytic effects of sugar diet in light-dark activity box in male and female rats are shown in fig. 3. Two-way ANOVA (df=1,20) revealed significant effects of sugar diet ($F=95.2$, $p<0.01$), gender ($F=13.8$, $p<0.01$) and interaction between the two factors ($F=77.9$, $p<0.01$). Post-hoc analysis by Newman-Keuls test showed that sugar rich diet ingestion increased time spent in light box in male but not in female rats. Control diet treated female rats exhibited greater values than control diet treated male rats. Sugar rich diet treated female rats exhibited smaller values than sugar rich diet treated male rats.

Effects of sugar diet on the levels of (a) tryptophan (b) 5-HT and (c) 5-HIAA in the hypothalamus of male and female rats are shown in fig. 4. Two-way ANOVA (df=1,20) revealed significant effects of sugar rich diet for tryptophan ($F=5.4$, $p<0.05$), 5-HT ($F=56.3$, $p<0.01$) and 5-HIAA ($F=56.4$, $p<0.01$). Effects of gender were significant for tryptophan ($F=11.6$, $p<0.01$), 5-HT ($F=15$, $p<0.01$) and 5-HIAA ($F=15$, $p<0.01$). Interaction of sugar rich diet and gender were not significant for tryptophan ($F=0.6$, $p>0.05$) but significant for 5-HT ($F=8.5$, $p<0.01$) and 5-HIAA ($F=8.5$, $p<0.01$). Post-hoc test showed that sugar rich diet ingestion decreased tryptophan levels in female rats. The decreases in male rats 15% were not significant possible due to large S.D. The levels of 5-HT and 5-HIAA decreased by sugar rich diet and the decreases were greater in female than male rats. Tryptophan, 5-HT and 5-HIAA levels were greater in normal diet treated female rats than normal diet treated male rats. Sugar rich diet treated male and female rats exhibited comparable values for tryptophan, 5-HT and 5-HIAA.



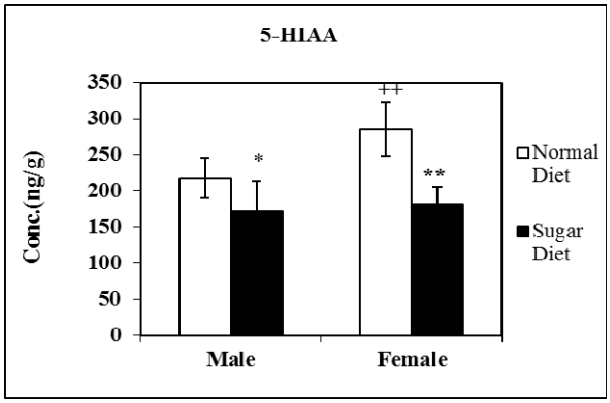




**Fig. 4**: Effects of sugar rich diet ingestion on the levels of tryptophan, 5-HT and 5-HIAA in the hypothalamus of male and female rats. Values are means ± S.D (n=6). Significant difference by Newman-Keuls test *p<0.05, **p<0.01 from respective normal diet treated rats, +p<0.05, ++p<0.01 from respective male rats following Two-way ANOVA.

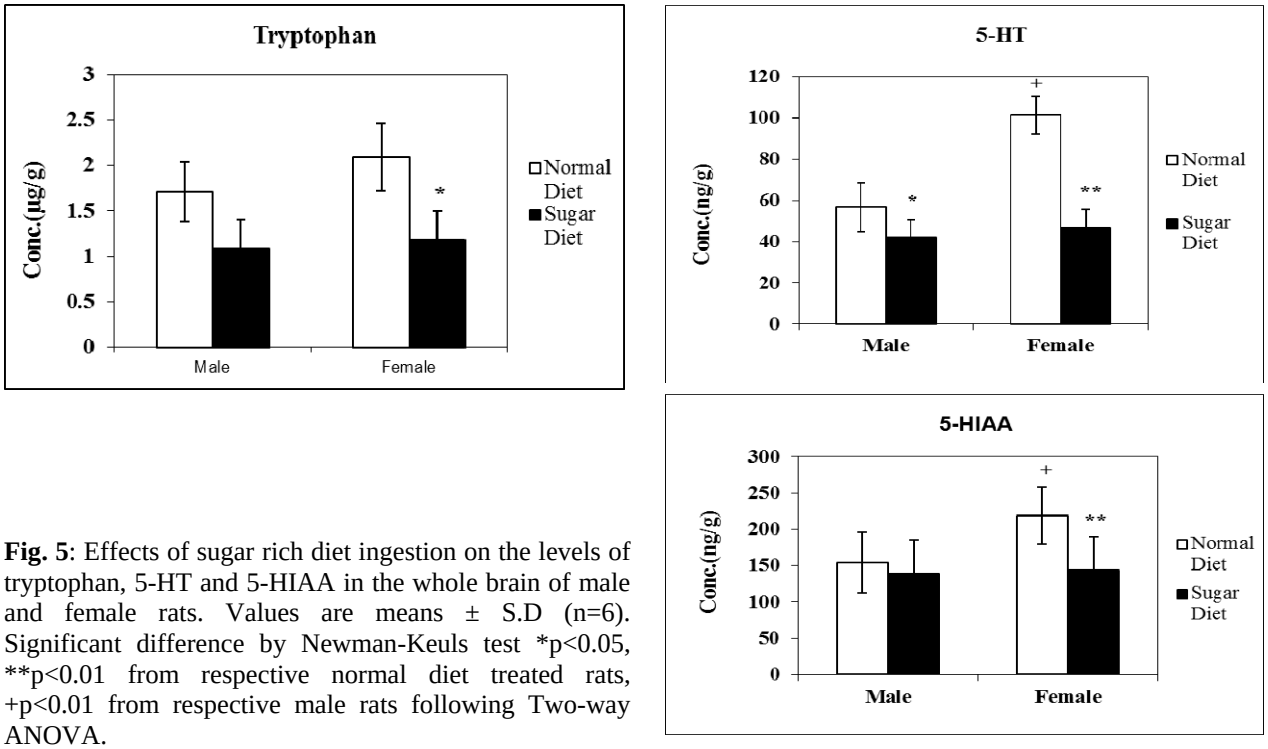



**Fig. 5**: Effects of sugar rich diet ingestion on the levels of tryptophan, 5-HT and 5-HIAA in the whole brain of male and female rats. Values are means ± S.D (n=6). Significant difference by Newman-Keuls test *p<0.05, **p<0.01 from respective normal diet treated rats, +p<0.01 from respective male rats following Two-way ANOVA.

Effects of sugar diet on the levels of (a) tryptophan (b) 5-HT and (c) 5-HIAA in the whole brain of male and female rats are shown in fig. 5.

Two way ANOVA (df=1,20) revealed significant effects of sugar rich diet for tryptophan ($F=13.8$, $p<0.01$), 5-HT ($F=107.6$, $p<0.01$) and 5-HIAA ($F=19.8$, $p<0.01$). Effects of gender not significant for tryptophan ($F=1.4$, $P>0.05$) were significant for 5-HT ($F=55$, $p<0.01$) and 5-HIAA ($F=11.5$, $p<0.01$). Interaction of sugar rich diet and gender not significant for tryptophan ($F=0.5$, $p>0.05$) but significant for 5-HT ($F=34.9$, $p<0.01$) and 5-HIAA ($F=8.5$, $p<0.01$). Post-hoc test showed that sugar rich diet ingestion decreased tryptophan and 5-HIAA levels in female rats. The decreases in male rats 63% were not significant possible due to large S.D. The levels of 5-HT greater decreased by sugar rich diet in female than in male rats. Normal diet treated male and female rats exhibited comparable values for tryptophan. 5-HT and 5-HIAA levels were greater in normal diet treated female rats than normal diet treated male rats. Sugar rich diet treated male and female rats exhibited comparable values for tryptophan, 5-HT and 5-HIAA.

## DISCUSSION

Important findings of the present study is that sugar rich-diet induced decreases of 5-HT metabolism in the whole brain as well as in the hypothalamus (fig. 4,5) were greater in female than male rats while hyperphagic effects (fig. 1) of sugar rich diet were comparable in them. Anxiolytic effects of sugar rich diet were elicited only in male rats (fig. 2). Tryptophan the precursor of 5-HT decreased significantly in the whole brain and hypothalamus in female rats only. Tryptophan decreases in male rats 63% in the whole brain and 15% in the hypothalamus were not significant (fig. 4,5).

Tryptophan is an essential amino acid, its source is only dietary. Studies performed on male rats show that acute ingestion of carbohydrate diet increased brain tryptophan as well 5-HT (Christensen, 1997; Fernstrom and Fernstrom, 1995), but long-term consumption of sugar rich diet did not alter brain tryptophan, while brain 5-HT decreased (Haleem *et al*., 2000; Inam *et al*., 2008). The present study shows that long term consumption of sugar rich diet decreased both tryptophan and 5-HT levels in female rats, tryptophan levels decreased non significantly in male rats and decreased of 5-HT were greater in the female rats, suggesting that serotonin pathway in female gender is more vulnerable to sugar diet. Similarly long term food restriction decreased plasma and brain tryptophan more in female than male rats (Haider and Haleem, 2000).

The present study shows that long-term consumption of sugar rich diet decreased significantly tryptophan in the whole brain and hypothalamus in female rats only. Previous studies shows that circulating tryptophan is largely metabolized in liver via hepatic kynurenine pathway (Allegri *et al*., 2003;Bertazzo *et al*., 2001). The metabolism is regulated by tryptophan pyrrolase (Saeed and Bano, 2007). Higher circulating levels of corticosteroids increase tryptophan pyrrolase activity in the liver (Bano *et al*., 1996; Macho *et al*., 1991), leading

to a decrease in tryptophan concentration in circulation (Badawy *et al*., 2009; Bano *et al*., 1996). Increases of corticosteroids by different factors were greater in female than male rats (Verma *et al*., 2010). Corticosteroid levels were not determined in this study. It is possible that decreased brain tryptophan levels are due to stimulatory effects of corticosteroids on tryptophan pyrrolase activity.

The decreased of brain 5-HT in male rats in the absence of a decrease in precursor level as observed in the present investigation, therefore, suggests a decrease in the activity of rate limiting enzyme tryptophan hydroxylase because evidence suggests that kinetic properties of tryptophan hydroxylase are also sometimes modulated *in vivo* and may be responsible for changing the rate of 5-HT synthesis in the absence of any change in precursor level (Haleem and Haider, 1996; Herrera *et al*., 2005). A greater decrease in serotonin levels in the hypothalamus and in the whole brain of sugar rich diet treated female than male rats may therefore occur because of a significant decrease in the availability of tryptophan in female but not in male rats (fig 4,5).

Hyperphagic effects (fig. 1) of sugar rich diet in the present study can be explained in terms of sugar rich diet-induced decreases of brain serotonin. Indeed pharmacological manipulations that tend to increase 5-HT functions in the hypothalamus are hypophagic (Leibowitz and Alexander, 1998; Reis *et al*., 2005). On the other hand comparable hyperphagic effects of sugar rich diet in male and female rats cannot be explained in terms of 5-HT decreases because 5-HT levels in the hypothalamus as well as in the whole brain (fig. 4,5) were decreases more in female than male rats. It is however possible that sensitivity of hypophagic serotonin receptors is greater in female gender (Haleem, 1993; Steffens *et al*., 2008) and a larger decrease of 5HT in female than males produces comparable hyperphagia in the two genders.

It may be noted that sugar rich diet-induced hyperphagia was not associated with a corresponding increase in body weight (fig. 2). These later results are explainable in terms of the adaptability of metabolic routes to decrease calorie storage due to an increase in the mobilization of metabolic fuel (Woods *et al*., 1998). Thus an increase in the activity of sympathetic nervous system occurred in rats drinking sucrose solution (Freitas *et al*., 2007). Evidence shows that enhanced activity of sympathetic nervous system could produce hyper catabolism and reduction in body weight occurs without an effect on feeding center (Tanida *et al*., 2009).

The present study shows that consumption of sugar rich diet for five weeks elicited anxiolytic effects (fig. 3) in male animals, which are consistent with the decrease in 5-HT neurotransmission. Drugs that tend to increase 5-HT functions are anxiogenic (Salchner and Singewald, 2006) while blockade of serotonergic neurotransmission produced antianxiety effects (Shields and King, 2008). Consumption of sugar rich diet that decreased whole brain 5-HT more in females than males cannot explain sugar rich diet-induced lack of anxiolysis. It is however possible that circulating corticosteroids modulate 5-HT function in the brain (Hensler *et al*., 2010; Tokarski *et al*., 2009) to alleviate anxiolytic effects of sugar rich diet.

## CONCLUSION

In conclusion, the present study shows that females are more vulnerable to long-term consumption of sugar rich diet. Sugar rich diet-induced decreases of brain serotonin were greater in females. However, antianxietic effects of sugar rich diet were not observed in females. Hyperphagia is also an adverse effect of sugar consumption, this effect was not possible to be greater in female because of more sensitive hypophagic serotonin receptors. It is suggested that excessive consumption of sugar may put female sex at a greater risk of sugar craving and anxiety.

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