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Effect of sugar, protein and fat rich diet on cumulative food intake and brain serotonin metabolism in rats

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Abstract: Central 5-hydroxytryptamine (5-HT; serotonin) system plays a key role in the regulation of eating behaviour; cerebral level of serotonin in animal models is inversely related to food intake and body weight. The present study concerns the effects of long-term consumption of sugar, protein and fat rich diet on food intake, growth rate and brain 5-HT metabolism in rats. The study was conducted on 24 male albino Wistar rats. Rats were divided into three groups, and each group was given a different macronutrient-specific diets including carbohydrate-, protein-, and fat-rich diets. Sugar, protein and fat rich diet were prepared by mixing standard rodent diet with table sugar, minced beef and beef fat in the ratio of 2:1 (w/w) respectively. Control rats were fed freely on standard rodent diet whereas the three test group rats were fed freely on their respective diet for 5 weeks. Cumulative food intakes and growth rates were monitored weekly. After 5 weeks of treatment, animals were decapitated to collect the brain samples for the analysis of tryptophan (TRP), 5-HT and 5-Hyroxyindoleacetic acid (5-HIAA) by HPLC-EC. After 5 weeks of treatment, growth rates were significantly decreased ($p<0.01$) in sugar rich diet treated rats and not altered in protein and fat rich diet treated animals. Brain 5-HT levels were significantly decreased in sugar ($p<0.05$) and protein ($p<0.01$) rich diet treated rats and significantly increased ($p<0.05$) in fat rich diet treated rats. Brain 5-HIAA and TRP levels were significantly decreased following the intake of sugar ($p<0.05$), protein ($p<0.01$) or fat ($p<0.01$) rich diet. Results showed that ingestion of sugar rich diet induced hyperphagia while ingestion of protein and fat rich diet induced hypophagia. The findings are discussed in the context of a role of serotonin in the regulation of appetite.

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INTRODUCTION

Large number of data on animals links brain serotonergic system to the regulation of appetite and feeding behavior. Neurochemical research shows that serotonin has a role in the normal termination of feeding[1, 2] and in disorders of appetite[3]. 5-HT is believed to have an inhibitory influence over feeding behavior because drugs that tend to increase 5-HT functions in synaptic cleft decrease food intake in experimental animals[1,4,5]. Meal consumption depending on the proportion of carbohydrate, protein and fat can enhance or inhibit brain serotonin metabolism in animals thereby affecting appetite regulation[6].

Neurochemical research for a relationship between central serotonergic system and feeding were stimulated as certain dietary manipulations were found to alter brain serotonin metabolism[7, 8]. The synthesis of serotonin depends on the availability of its precursor tryptophan[9]. It was found that a protein rich diet increased plasma levels of aromatic amino acids and decreased brain serotonin metabolism[10, 11]. Previous studies reported that fat rich diet increased food intake and elicits weight gain[12, 13]. Carbohydrate rich diet decreased food intake and had more satiating capacity by increasing 5-HT metabolism[13, 14]. It has also been reported that sugar rich diet produced hyperphagia and decreased serotonin metabolism in rats[15]. Decrease in brain serotonin metabolism could increase food intake while an increase in brain serotonin metabolism could suppress appetite to decrease the normal consumption of food[8].

The present study was therefore designed to investigate the effects of long-term intake of dietary carbohydrate, protein and fat rich diet on total food consumption, body weight changes and brain 5-HT metabolism in rats. The aim was to investigate the possible neurochemical mechanism behind the macronutrient rich meal, food consumption and serotonin metabolism.

MATERIALS AND METHODS

Animals

Twenty four locally bred albino Wistar rats (180-200g) purchased from Aga Khan University Hospital were used in the study. All animals were housed individually under a 12 h light-dark cycle (light on at 6:00h) and controlled room temperature (22 ± 2°C) with free access to cubes of standard rodent diet and tap water for at least 3-4 days before experimentation. All experiments were conducted according to a protocol approved by Local Animal Care Committee.

Experimental procedure

Animals were randomly divided into control and 3 test groups (carbohydrate, protein and fat rich diet treated groups). Sugar, protein and fat rich diet were

prepared by mixing standard rodent diet with table sugar, minced beef and beef fat in the ratio of 2:1 (w/w) respectively. Control rats were fed freely on standard rodent diet whereas the three test group rats were fed freely on their respective diet for 5 weeks. Cumulative food intakes and growth rates were monitored weekly. Rats were decapitated after 5 weeks to collect brain samples. The experiments were performed in a balanced design in such a way that the control and test rats were killed alternatively to avoid the order effect. Brain samples were excised very quickly from the cranial cavity within 30 seconds of the decapitation. Fresh brains were dipped in chilled saline and stored at low temperature (-70°C) until analysis of 5HT and 5HIAA by HPLC-EC.

Neurochemical estimations

HPLC-EC determination was carried out as standard[8]. A 5-II Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by a mobile phase containing methanol (14%), octyl sodium sulfate (0.023%) and EDTA (0.0035%) in 0.1 M phosphate buffer at pH 2.9 at an operating pressure of 2000-3000psi on Schimadzu LEC 6A detector at an operating potential of 0.8 volts for biogenic amines.

Statistical analysis

Neurochemical and behavioral data were analyzed by one-way ANOVA. Post hoc comparisons were made using the Newman-Keuls test; p values <0.05 were considered significant.

RESULTS

Figure 1 shows weekly food intake of rats consuming normal, sugar rich, and protein rich and fat rich diet. One way ANOVA revealed a significant effect of diet on food intake (F=380, p<0.01). Post hoc comparison by Neuman Keuls test showed that food intake was significantly greater in sugar rich diet (p<0.01; 23%) treated rats and significantly smaller in protein (p<0.01; 17%) and fat rich diet (p<0.01; 30%) treated rats.

Figure 2 shows growth rate of rats consuming normal, sugar rich, protein rich and fat rich diet. One way ANOVA revealed a significant effect of diet on growth rate (F=78, p<0.01). Post hoc comparison by Neuman Keuls test showed that intake of sugar rich diet significantly decreased (p<0.01; 16%) the body weight of rats. Effect of protein (p>0.05) and fat (p>0.05) rich diet on growth rates was not significant.

Figure 3 shows the effect of normal, sugar rich, protein rich and fat rich diet on brain TRP levels in rats. One way ANOVA revealed a significant effect of diet on brain TRP levels (F=78.8, p<0.01). Post hoc comparison by Neuman Keuls test showed that intake of sugar (p<0.01; 30%), protein (p<0.01; 32%), and fat (p<0.01; 19%) rich diet significantly decreased the TRP levels of rats. Protein rich diet decreased brain TRP levels to a greater extent.

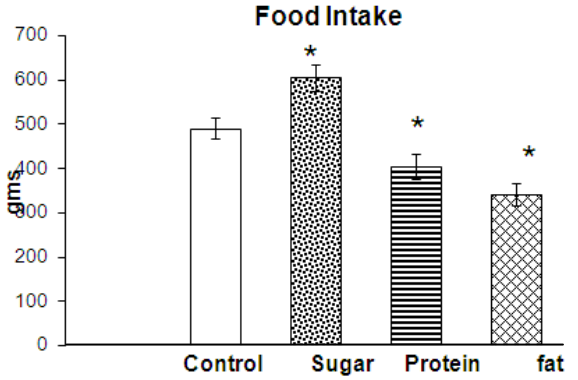

Figure 1: Effect of sugar rich, protein rich and fat rich diet on food intake in rats. Values are mean±SD (n=6). Significant difference by Newman Keuls test; *P<0.01 vs control group following one way ANOVA.

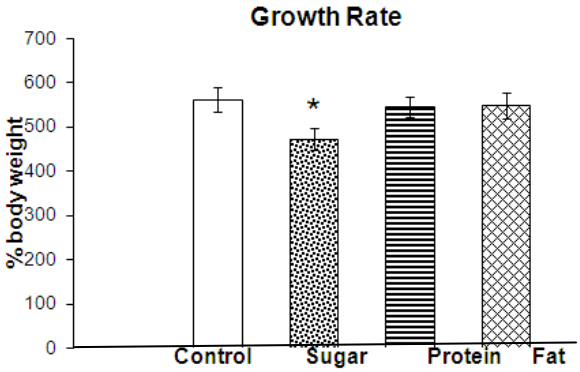

Figure 2: Effect of sugar rich, protein rich and fat rich diet on growth rate of rats. Values are mean±SD (n=6). Significant difference by Newman Keuls test; *P<0.01 vs control group following one way ANOVA.

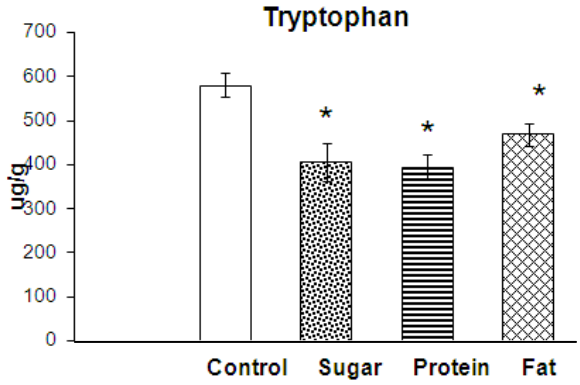

Figure 3: Effect of sugar rich, protein rich and fat rich diet on brain tryptophan levels in rats. Values are mean±SD (n=6). Significant difference by Newman Keuls test; *P<0.01 vs control group following one way ANOVA.

Figure 4 shows the effect of normal, sugar rich, protein rich and fat rich diet on brain 5-HT levels in rats. One way ANOVA revealed a significant effect of diet on brain 5-HT levels (F=16.8, p<0.01). Post hoc comparison by Neuman Keuls test showed that intake of sugar (p<0.05; 24%) and protein (p<0.01; 29%) rich diet significantly decreased the brain 5-HT levels of rats. However fat rich diet significantly increased the brain 5-HT levels in rats (p<0.05; 23%).

Figure 5 shows the effect of normal, sugar rich, protein rich and fat rich diet on brain 5-HIAA levels in rats. One way ANOVA revealed a significant effect of diet on brain 5-HIAA levels (F=12.0, p<0.01). Post hoc comparison by Neuman Keuls test showed that intake of sugar (p<0.05; 19%), protein (p<0.01; 30%), and fat (p<0.01; 34%) rich diet significantly decreased the 5-HIAA levels of rats.

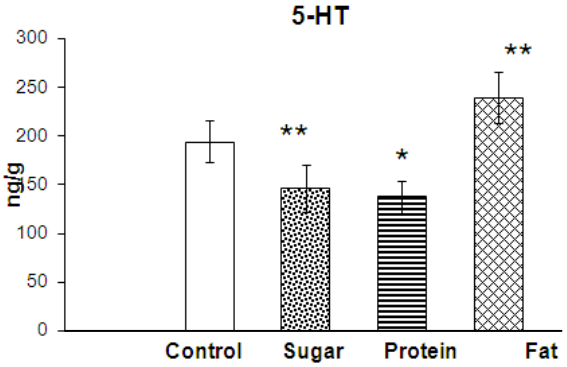

Figure 4: Effect of sugar rich, protein rich and fat rich diet on brain 5-HT levels in rats. Values are means±SD (n=6). Significant differences by Newman keuls test:*P<0.01; **P<0.05 vs control group following one way ANOVA.

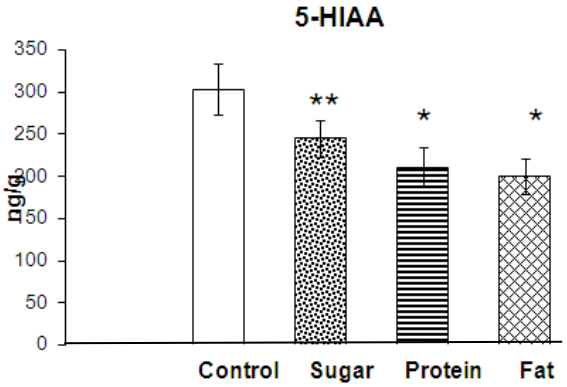

Figure 5: Effect of sugar rich, protein rich and fat rich diet on brain 5-HIAA levels in rats. Values are means ± SD (n=6). Significant differences by Newman keuls test:*P<0.01; **P<0.05 vs control group following one way ANOVA.

DISCUSSION

Evidence accumulated over more than one decade indicates the existence of several neurochemical systems that may influence food intake, appetite for specific nutrients and meal taking behavior[16]. Serotonin, an indoleamine neurotransmitter is thought to play a key role in the suppression of appetite by reducing the rate of eating and size of meal through the intensification of satiety[17]. The present study showed that long-term intake of sugar diet for 5 weeks produced hyperphagia which was associated with a decrease of body weight and 5-HT metabolism. It was reported previously that a decrease in 5-HT metabolism but not an increase in the responsiveness of somatodendritic 5-HT1A receptors is involved in sugar rich diet induced hyperphagia[18- 20.]

Previously it was reported that carbohydrate rich diet increases TRP and 5-HT levels[21]. In the present study levels of brain TRP, 5-HT and 5-HIAA were significantly decreased in rats fed on sugar rich diet for 5 weeks. It has been reported that long-term intake of sugar rich diet produced hyperphagia in rats that was associated with a decrease in brain serotonin metabolism[15]. Pharmacological research shows that drugs that tend to increase serotonin functions via post synaptic 5-HT2C receptors decrease appetite[4, 17]. Conversely stimulation of somatodendritic 5-HT1A receptors elicits hyperphagia in experimental animals[18- 22] because the availability of 5-HT at post synaptic 5-HT2C receptors sites is decreased[23]. The results of the present study are explainable in terms of long-term intake of sugar rich diet decreasing the availability of TRP for the synthesis of 5-HT[15]. It has been reported that metabolism of 5-HT could also be altered by changes of plasma and brain TRP concentration[24]. It could be that TRP is an essential amino acids and its source is only dietary[25], so long-term intake of carbohydrate rich diet decrease plasma and brain TRP concentration due to less availability of precursor. It may be relevant that carbohydrate craving is the result of decreased 5-HT levels.

In this study, protein consumption for 5 weeks elicited hypophagia and significantly decreased brain TRP, 5-HT and 5-HIAA levels in rats. The hypophagia was not associated with a decrease in body weight. Previous studies reported that protein

consumption provides the plasma with sources of all amino acids[26]. One of the effects of high protein meal is that less TRP is able to reach the brain. Most protein present in the diet contains amino acids other than TRP. It has been reported previously that protein rich diet increased the concentration of large neutral amino acids (LNAAs) other than TRP[10, 11]. These LNAAs compete with TRP for uptake to the brain, thus decreasing the transport of TRP to the brain for 5-HT synthesis and its conversion to 5-HIAA[27, 28]. Another explanation could be that the protein rich diet increases the synthesis of catecholamines by increasing the availability of its precursor tyrosine.

Hypophagic effect of fat rich diet as observed in the present study is explainable in terms of an increase in brain 5-HT levels although brain TRP and 5-HIAA levels were also significantly decreased. TRP in the blood is found in two forms, one is free and other is bound to albumin[24]. The free fraction of TRP in circulation plays a major role in the regulation of available TRP for transport across blood brain barrier[29]. It is well known that intake of high fat diet increases the lipolysis as a result more free fatty acids released in the plasma, that compete with TRP for binding to albumin as a result more TRP is released into the plasma[30]. TRP is more likely to cross the BBB, where it is taken up by the serotonergic neurons to activate the synthesis of 5-HT. TRP hydroxylase, rate limiting enzyme of 5-HT synthesis is half saturated with its substrate[31, 32], and therefore an increase in 5-HT synthesis occurs. In the present study an increase in brain TRP concentration did not occur in rats treated with fat rich diet for 5 weeks. The present results are largely explainable in terms of an increase in the activity of TRP hydroxylase in rats treated with fat rich diet.

The present finding suggests that long-term intake of sugar rich diet produced hyperphagia which is associated with a decrease in 5-HT metabolism, while intake of protein rich diet produced hypophagia which was not associated with greater 5-HT metabolism. Long-term intake of fat rich diet also produced hypophagia which was associated with the increased 5-HT levels. It is therefore concluded that long-term consumption of a specific type of diet may produce chemical changes not directly relevant to serotonin-induced satiety signals.

REFERENCES

1. Curzon G. Serotonin and appetite. *Ann. NY Acad. Sci.,* 1990; 600: 521-530.
2. Leibowitz SF and Alexander JT. Hypothalamic serotonin in control of eating behavior, meal size and body weight. *Biol. Psychiatry*, 1988; 1, 44: 851-864.
3. Kaye WH, Bailer UF, Frank JK, Wagner A and Henry SE. Brain imaging of serotonin after recovery from anorexia and bulimia nervosa. *Physiol. Behavior*, 2005; 15, 86: 7-15.
4. Leibowitz SF. The role of serotonin in eating disorders. *Drugs*, 1990; 39, 3: 33-48.
5. Haleem DJ. Function specific super sensitivity of m-CPP induced seotonergic neurotransmission in females compared to male rats. *Life Sci.*, 1993; 52: 279-284.
6. Fernstrom MH and Fernstrom JD. Brain tryptophan concentration and serotonin synthesis remain responsive to food consumption after the ingestion of sequential meals. *Am. J. Clinical Nutr*., 1995; 61: 312-319.
7. Wurtman RJ. Nutrient effecting brain composition and behavior. *Integer. Psychiatry*, 1987; 5: 226.
8. Haleem DJ and Haider S. Food restriction decrease serotonin and its synthesis rate in hypothalamus. *Neuroreports*, 1996; 7: 1153-1156.
9. Fernstrom JD. Effect of diet on brain function. *Acta. Astronaut*, 1981; 8: 1035-1042
10. Blundell J. Pharmacological approaches to appetite suppression. *TIPS*, 1991; 12: 147-157.
11. Rowland NE, Morein A and Li B. The physiology and brain mechanism of feeding. *Nutrition*, 1996; 12: 626-639.
12. Golay A and Bobbioni E. Dietary fats and obesity, Teaching diabetic division, Geneva University Hospital. 1998.
13. Blundell JE and Stubbs RJ. High and low carbohydrate and fat intakes: limits imposed by appetite and palatability and their implications for energy balance. *Eur. J. Clin. Nutr*., 1999; 53: 148-165.
14. Haleem DJ, Zafar A, Azam S and Yasmeen A. Tolerance to diacetylmorphine: effect on brain serotonin. Neuroreport, 1994; 5: 781-784.
15. Haleem DJ, Haider S, Perveen T, Inam Q, Kidwai IM and Haleem MA. Hyperphagia and decrease of brain serotonin in rats fed freely on sugar rich diet for three weeks. *Nutritional Neurosci*., 2000; 3: 399-405.
16. Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav*., 2005; 86: 773-95.
17. Smith BM, Thomsen WJ, Grottick AJ. The potential use of selective 5-HT2C agonists in treating obesity. *Expert. Opin. Investig. Drugs*, 2006; 15: 257-66.
18. Inam QU, Jabeen B, Haleem MA, Haleem DJ. Long-term consumption of sugar-rich diet decreases the effectiveness of somatodendritic serotonin-1A receptors. *Nutr. Neurosci*., 2008; 11:277-82.
19. Inam QU, Haleem MA, Haleem DJ. Attenuation of somatodendritic responses to 8-hydroxy-2-di-npropylamino tetralin following long-term dietary sugar consumption in rats. *J. Coll. Physicians Surg. Pak*., 2009; 19: 401-5.
20. Jabeen B and Haleem DJ. Desensitization of pre and post synaptic 5-HT-1A receptor responses following long term consumption of sugar rich diet: implications for sugar-induced obesity. *Pak J Pharm Sci.*, 2008 ; 21: 327-32.
21. Takeda E, Terao J, Nakaya Y, Miyamoto K, Baba Y, Chuman H, Kaji R, Ohmori T, Rokutan K. Stress control and human nutrition. *J. Med. Invest.*, 2004; 51: 139-45.
22. DeVry J and Schrieber R. Effects of selected serotonin 5-HT1 and 5-HT2 receptor agonist on feeding behavior: Possible mechanism of action. Neuroscience *Behav. Rev*., 2000; 24: 341-353.
23. Liu RJ, Lambe EK and Aghajanian GK. Somatodendritic autoreceptors regulation of serotonergic neurons:

dependence on L-Tryptophan and tryptophan hyroxylase activating enzyme. *Eur. J. Neuroscience*, 2005; 21: 945-958.
24. Hernandez-Rodriguez MD and Gabriel MG. Macronutrients and neurotransmitter formation during brain development. *Nutrition Review*, 2001; 59: 49-59.
25. Cangiano C, Ceci F, Cascino A, Del Ben M, Laviano A, Muscaritoli M, Antonucci F, Rossi-Fanelli F. Eating behavior and adherence to dietary prescriptions in obese adult subjects treated with 5-hydroxytryptophan.*Am. J. Clin. Nutr*., 1992; 56: 863-7.
26. Carolyn D and Berdanier. Advanced nutrition: *Macronutrients.* 1995; 8: 8439-8500.
27. Fernstrom JD. Branched-chain amino acids and brain function. *J. Nutr*., 2005;135:1539S-46S
28. Bloxam DL, Tricklebank MD, Patel AJ and Curzon G. Effect of albumin, amino acids and clafibrate on the uptake of tryptophan by rat brain. *J. Neurochem*., 1980; 34: 43-49.
29. Zeisel SH, Mauron C, Watkins CJ and Wurtman RJ. Developmental changes in brain indoles, serum tryptophan and other serum neutral amino acids in the rat. *Brain Res*., 1981; 227:551-64.
30. Curzon G, Friedel J and Knott PJ. The effect of fatty acids on the binding of tryptophan to plasma protein. *Nature*, 1973; 16; 242:198-200.
31. Hamon M, Bourgoin S, Artaud F and El Mestikawy S. The respective roles of tryptophan uptake and tryptophan hydroxylase in the regulation of serotonin synthesis in the central nervous system. *J. Physiol*., 1981; 77: 269-79.
32. Young SN and Gauthier S . Tryptophan availability and the control of 5-hydroxytryptamine and tryptamine synthesis in human CNS. *Adv. Exp. Med. Biol*., 1981; 133: 221-30.