


Behavioral, Hormonal, and Serotonergic Responses to Different Restricted Feeding Schedules in Rats

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ABSTRACT: To determine the effect of long-term restricted feeding schedules on behavior, serotonergic responses, and neuro-endocrine functions, metabolism of serotonin (5-HT) in the striatum, expression of serotonin-1A (5-HT_{1A}) auto-receptor in the raphe nuclei and circulating levels of leptin and corticosterone were determined in female Wistar rats kept on excessive food restriction schedule. Due to a role of dietary deficiency of tryptophan (Trp) in influencing serotonergic neurotransmission, circulating levels of Trp were also determined. Estimations were done in 2 different restricted feeding models: time-restricted feeding (TRF) and diet restricted (DR). TRF animals were given access to food ad libitum only for 2 hours/day. The DR animals were given a small calculated amount of food each day. We found that chronic food restriction for 5 weeks cause a significant decrease in the body weight and produced hyperactivity in both, TRF and DR animals. Levels of Trp were declined in circulation and in the striatum. Similarly, the levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were decreased in the striatum. Also, the expression of 5-HT_{1A} auto-receptor was declined in the raphe nuclei. These changes in 5-HT metabolism and 5-HT_{1A} auto-receptor expression were more profound in DR animals as compare to TRF animals. Similarly, hypoleptinemia and increased corticosterone found in both models was higher in DR animals. Effect of dietary deficiency of Trp in the modulation of striatal 5-HT metabolism and its consequences on circulating leptin and corticosterone are discussed.

KEYWORDS: Food restriction, hyperactivity, tryptophan deficiency, hypoleptinemia, corticosterone, serotonin-1A

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Introduction

Food restriction is shown to decrease the availability of Trp, an essential amino acid.¹ Following which, brain 5-HT levels are also decreased.² Extreme food restriction leads to hyperactivity³ and hormonal dysregulation.⁴ Studies on animal models show that excessive food restriction results in weight-loss and stress.⁵ Studies suggest that most of the behavioral symptoms associated with diet restriction are due to decreased 5-HT neurotransmission in the brain.⁶

Together with 5-HT, leptin—the satiety hormone, and glucocorticoid—the stress hormone, have a critical role in food restriction induced stress.⁷ Circulating cortisol levels are elevated and those of leptin are declined in underweight stress patients.⁸ Studies suggest a potential relationship between 5-HT, leptin and cortisol.⁹ 5-HT regulates its endocrine functions through its several receptor types in brain. Administration of 5-HT precursors has shown to influence leptin levels.¹⁰ The possible mechanism behind this increase is the involvement of serotonergic system in the release of leptin.^{6,11} Leptin-mediated regulation of appetite and energy expenditure takes place by inhibiting 5-HT synthesis and release in the brain stem neurons.^{12,13}

The study was designed to determine which feeding schedule can produce behavioral, hormonal, and serotonergic deficits in rats. We assessed 2 rat models of food restriction to

determine the effects of diet restriction, particularly on, hyperactivity, circulating leptin and corticosterone, and 5-HT metabolism in the striatum. In view of an important role of 5-HT_{1A} auto-receptor in altering the availability of 5-HT in terminal regions, 5-HT_{1A} receptor expression in the raphe nuclei is also determined.

Materials and Methods

Animals

Female Albino Wistar rats were used in the study. Rats were provided by the Animal Research Facility, Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi. Animal study protocol was designed and submitted for approval to the Institutional Ethics and Animal Care Committee. Study was performed after approval from this committee (animal study protocol no. 0059). Rats were housed according to the institutional in-house guidelines provided by this committee. These guidelines are adapted from “Guide for the Care and Use of Laboratory Animals” by the Institute for Laboratory Animal Research, USA.

Female rats weighing 80 to 120 g were separated and housed in individual cages 5 days before the start of experiment, for acclimatization. Rats were provided free access to the standard



chow diet and tap water. Temperature of the housing room was kept at 25°C and 12 hours light and dark cycle was also maintained.

Experimental protocol

24 female Albino Wistar rats weighing 80 to 120 g were randomly divided into 3 groups: (i) Freely feeding (FF), (ii) Time restricted feeding (TRF), and (iii) Diet restricted (DR), each containing 8 animals. Length of the study was 5 weeks. FF rats were provided free access to standard chow diet throughout the day, whereas, the TRF group was given access to same diet only for 2 hours per day, from 15:00 to 17:00 hours. The DR groups were given a limited access to the same diet. Daily food-intake by each rat was monitored during acclimatization phase, which lasted for 5 days. For the next 5 weeks, each DR rat was daily given 50% of the diet it consumed on a single day during the acclimatization period. Food was given at 15:00 hours.

Bodyweight and food intake was monitored weekly. Behavior of rats was monitored in both familiar and novel environment in activity box and open-field respectively. These tests were performed only once on 35th and 36th day.

After 5 weeks (on day-37), animals were sacrificed. Brain was removed and micro-dissected to isolate the patches of striatum and raphe nuclei (including both dorsal and median raphe). Serum was also separated from the whole blood samples. Brain regions and serum samples were stored at -80°C till further processing.

Trp, leptin, and corticosterone concentrations were determined in the serum. Levels of 5-HT and 5-HIAA were determined in the striatum using HPLC-EC (High Performance Liquid Chromatography with Electrochemical Detector). Concentration of Trp was determined in the striatum and circulation through HPLC coupled with UV detector. mRNA Expression of the 5-HT_{1A} auto-receptor was also determined in the raphe nuclei through quantitative real-time PCR.

Activity in familiar environment

Activity of animals in familiar environment was monitored in activity box using the method adopted by Cheema et al.¹⁴ Rat was placed in a box, similar to the transparent cage, in which it was housed. Before monitoring the activity, 10 minutes were given to the rat for familiarization with the environment. For the next 10 minutes, numbers of cage crossings, chewing, grooming, and vertical movements were monitored. Each one of these movements were counted as a single activity of that rat.

Open field test

Activity in novel environment was monitored in open-field, which is an open squared box with an area of 76 cm² and walls that are 42 cm high. The floor of the box has 25 equal squares

drawn on it. The test was performed by introducing the rat in the mid box and allowing it to explore the field for the next 5 minutes. During this time, locomotor activity was noted, in terms of number of squares crossed. As the study primarily focuses on dietary restriction induced hyperactivity, therefore, only number of squares crossed were recorded.

Neurochemical analysis

Extraction of 5-HT, 5-HIAA, and Trp from tissue samples was done using a solution containing 0.1% sodium meta bisulfate, 0.4M perchloric acid, 0.01% cystein, and 0.01% EDTA. After extraction through homogenization and separation of liquid solution by centrifugation, 20 µL of extracted sample solution was used for quantification through reversed phase HPLC using Waters Alliance HPLC System (Milford, US). Waters Spherisorb 5 µm ODS-2, 4.6 × 150 mm analytical column was used as stationary phase.

For the separation of 5-HT and 5-HIAA from striatum samples, a mobile phase containing 14% methanol, 0.023% sodium octyl sulfate (SOS), and 0.005% EDTA in 0.1M sodium phosphate buffer at pH 2.9, was used. Waters 2465 electrochemical detector at an operating potential of +0.8 to +1.0 V and an operating pressure of 2000 to 3000 psi was used. For the separation of Trp from serum and striatum samples, 10% methanol was used as mobile phase at an operating pressure of 2000 to 3000 psi. Detection of Trp was done using UV detector at a wavelength of 273 nm.

Message from the detector was recorded by computer software (Empower 3 Chromatography Data Software by Waters). Each component of sample was identified and quantified by comparing its retention time and area under the peak with that of the standard.

Analytical grade 5-HIAA, 5-HT, sodium octyl sulfate (SOS), and L-Trp were procured from Sigma (St. Louis, MO, USA). All chemicals and reagents used in HPLC analysis were of HPLC grade. All other chemicals and reagents were also of highest purity grade.

Hormonal analysis

Serum concentrations of leptin and corticosterone were determined using commercially available ELISA kits by Glory Science Co., Ltd (catalog # 30492 and 30590). The kits work on the principle of sandwich ELISA. Manufacturer's protocol was followed. Final absorbance of each well was determined spectrophotometrically using MultiscanGO by ThermoScientific.

mRNA expression studies

Total RNA was isolated from the patches of raphe nuclei using guanidinium thiocyanate, brand name TRIzol reagent (purchased from Ambion-Life Technologies). Along with TRIzol,

pure chloroform, iso-propanol, and ethanol were also used and manufacturer's protocol was followed. After isolation, RNA samples were treated with DNases using DNases-I by ThermoFisher Scientific (catalog # EN0521). Manufacturer's protocol was followed. Concentration and purity of RNA was estimated using Nanodrop 2000c by ThermoScientific. The quality of RNA samples was assessed using ratio of the absorbance at 260 and 280 nm. The value of 2 was considered as pure.

cDNA synthesis from the RNA samples was done through Revert Aid First Strand cDNA synthesis kit by ThermoScientific (catalog # K1622). About 1 µg of RNA was used to synthesize cDNA using manufacture's protocol. Temperature conditions were maintained using thermal cycler (XP Cyclor by BIOER).

For amplification and quantification of 5-HT1A gene, the method defined by Ali et al¹⁵ was adopted. Expression of β-actin, a constitutively expressed gene was used as control in RNA expression studies. β-actin is well known as a house keeping gene showing stable expression in similar experiments we performed previously.¹⁶ Primers for *Rattus norvegicus* β-actin gene (forward: 5'-ACCCACACTGTGCCCATCTA-3' and reverse: 5'-CGGAACCGCTCATTGCC-3') and that of 5-HT1A (forward: 5'-CCCCCAAGAAGAGCCTGAA-3' and reverse: 5'-GGCAGCCAGCAGAGGATGAA-3') were used for amplification of both the genes.

For qPCR, Maxima SYBR Green qPCR Master Mix by ThermoScientific (catalog # K0221), and qRT-PCR machine (AriaMx Real-time PCR System) was used. Thermal amplification cycling conditions included an initial denaturation step at 95°C for 10 minutes and the run for 40 cycles for 15 seconds at 95°C, annealing at 60°C for 30 seconds, and final extension at 72°C for 30 seconds.

Relative abundance of 5-HT1A was calculated in relation to the abundance of β-actin gene. ΔCt values were calculated using AriaMx Agilent HRM software. The levels of the 5-HT1A mRNA were normalized by β-actin.

Statistical analysis

All results are specified as mean ± SD. Variance among the groups were analyzed by applying ANOVA. Data on weekly food intake and body weight were analyzed by 2-way ANOVA with repeated measure design, taking treatment as the between group factor and weekly monitoring (repeated measure) as within group factor. Neuro-chemical and hormonal data were analyzed by one-way ANOVA. Post hoc comparisons were made by Tukey's test. *P* values <.05 were considered as statistically significant.

Results

Figure 1 shows the effect of restricted feeding on weekly body-weight and food-intake. Two-way ANOVA showed significant effect of restricted feeding ($F=72.79$, $df 2, 21$, $P<.01$), weeks ($F=46.97$, $df 5, 21$, $P<.01$), and restricted feeding × weeks interaction ($F=179.25$, $df 10, 21$, $P<.01$) on body-weight.

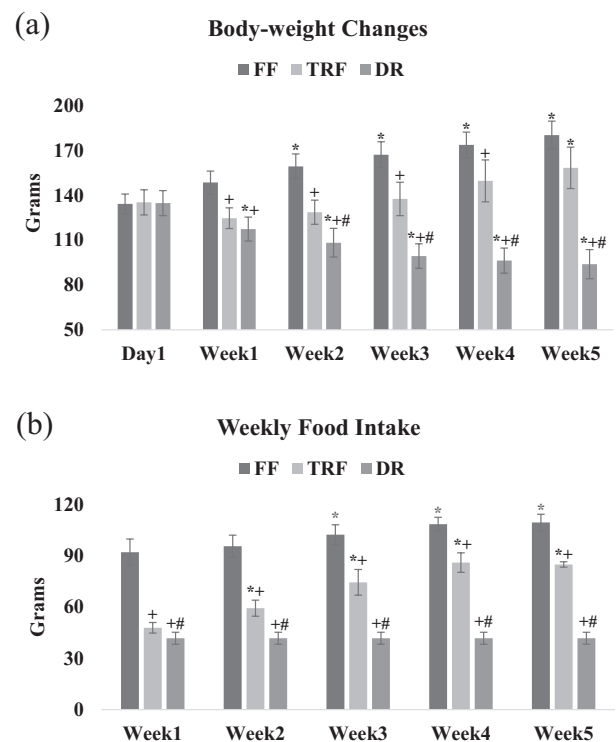


Figure 1. Effects of restricted feeding on weekly (a) body-weight and (b) food-intake. Values are mean ± SD (n=8). Significant difference by Tukey's test: **P* <.05 from respective day-1 (for body-weight) and week-1 (for food intake) values, +*P* <.05 from respective FF animals, and #*P* <.05 from respective TRF animals following two-way ANOVA.

Post-hoc analysis showed significant difference in the weekly body-weights of all starved rats compared to the FF animals. DR rats showed greater decreases in their body-weights compared to TRF rats. This decline was statistically significant during week-2 and onward. The TRF rats showed marginal decline in body-weight only during first week. After week-1, continuous increase was observed in their body-weight. After 5-weeks of TRF, there was a significant increase found in their body-weights as compare to the day-1 value. While the DR animals showed continuous decline in their body-weights throughout the study.

Two-way ANOVA performed on the data of effect of restricted feeding on weekly food-intake showed significant effect of restricted feeding ($F=426.67$, $df 2, 21$, $P<.01$), weeks ($F=770.7$, $df 4, 21$, $P<.01$), and restricted feeding × weeks interaction ($F=266.25$, $df 8, 21$, $P<.01$). Post-hoc analysis showed significant difference in the weekly food-intake of all starved rats compared to the FF animals. As mentioned, DR rats were given small calculated amount of food daily. This amount was significantly lower than the amount consumed weekly by FF and TRF rats which were allowed to eat ad libitum. TRF rats showed continuous increase in the food-intake from week-1 to week-4. It is due to the adaptation to the 2 hours feeding time. This increase in food-intake resulted in similar increases in their body-weight. The FF group also showed increased weekly food-intake and so in the body-weight.

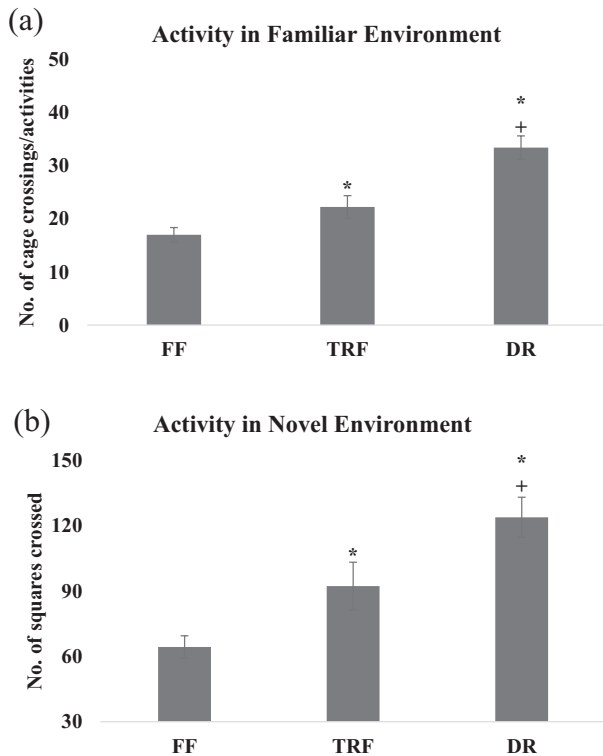


Figure 2. Effects of restricted feeding on activity in (a) familiar (activity-box test) and (b) non-familiar environment (open-field). Values are mean \pm SD (n=8). Significant difference by Tukey's test: * $P < .01$ from respective FF animals and + $P < .01$ from respective TRF animals following one-way ANOVA.

Figure 2 shows the effect of restricted feeding on activity in familiar and novel environment. One-way ANOVA showed significant effect of diet restriction on activity in both familiar ($F=148.98$, $df 2, 21$, $P < .01$) and novel environment ($F=92.27$, $df 2, 21$, $P < .01$). Post-hoc analysis showed significant increase in locomotor activity in starved rats as compared to FF group. The hyperactivity was significantly greater in DR animals as compared TRF group.

Figure 3 shows the effect of restricted feeding on the levels of 5-HIAA and 5-HT in the striatum. One-way ANOVA showed significant effect of diet restriction on the levels of both, 5-HT ($F=65.53$, $df 2, 21$, $P < .01$) and 5-HIAA ($F=186.65$, $df 2, 21$, $P < .01$) in the striatum. Post-hoc analysis showed significant decline in the striatal 5-HT and 5-HIAA in both the starved groups. The decline in these levels were significantly greater in DR animals as compared to TRF.

Figure 4 shows the effect of restricted feeding on the levels of Trp in the striatum and serum. One-way ANOVA showed significant effect of diet restriction on the levels of Trp in both striatum ($F=50.32$, $df 2, 21$, $P < .01$) and serum ($F=91.44$, $df 2, 21$, $P < .01$). Post-hoc analysis showed significant deficiency of Trp in the striatum and serum of both the starved groups. The decline in Trp levels was greater in DR animals.

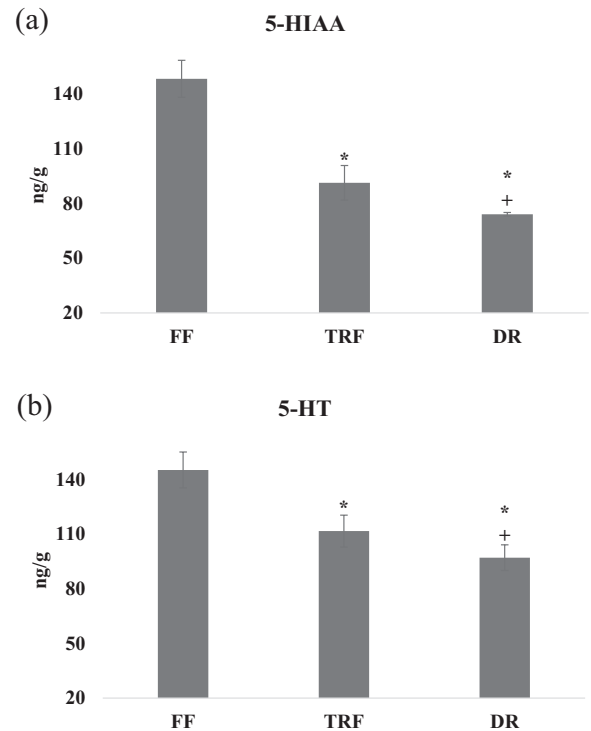


Figure 3. Effect of restricted feeding on the levels of (a) 5-HIAA and (b) 5-HT in the striatum. Values are mean \pm SD (n=8). Significant difference by Tukey's test: * $P < .01$ from respective FF animals and + $P < .01$ from respective TRF animals following one-way ANOVA.

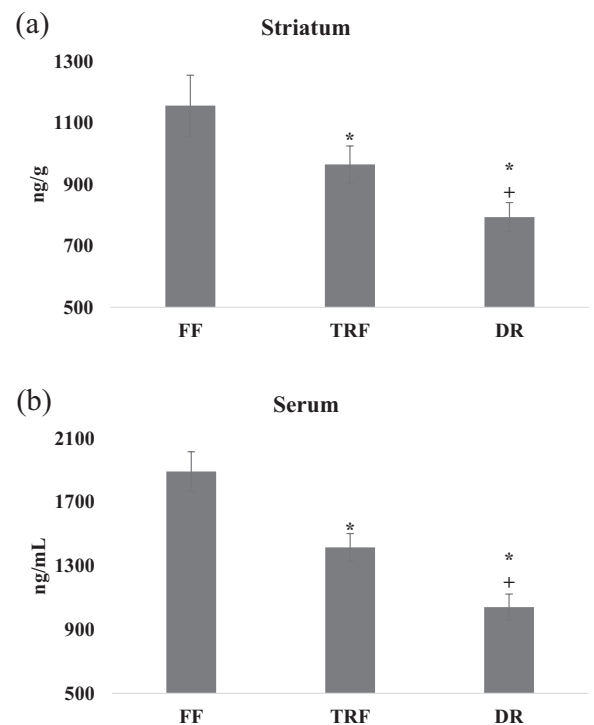


Figure 4. Effects of restricted feeding on the levels of tryptophan in (a) striatum and (b) circulation. Values are mean \pm SD (n=8). Significant difference by Tukey's test: * $P < .01$ from respective FF animals and + $P < .01$ from respective TRF animals following one-way ANOVA.

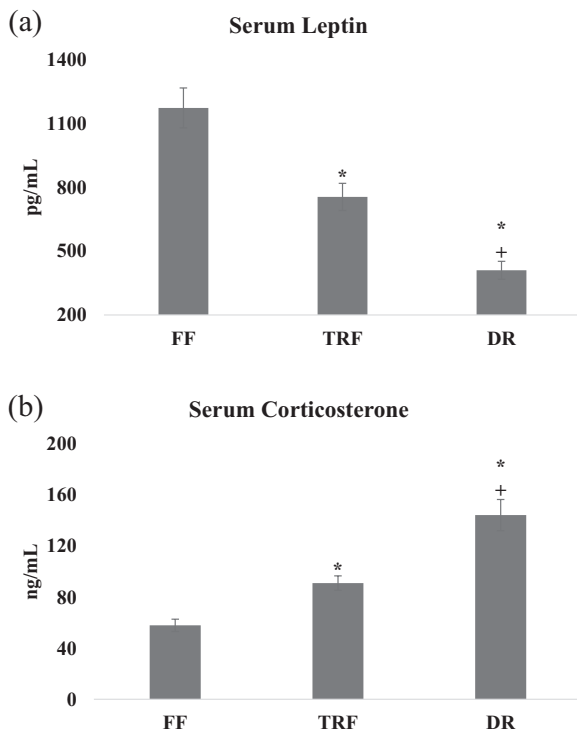


Figure 5. Effects of restricted feeding on the circulating levels of (a) leptin and (b) corticosterone. Values are mean \pm SD (n=8). Significant difference by Tukey's test: * $P < .01$ from respective FF animals and + $P < .01$ from respective TRF animals following one-way ANOVA.

Figure 5 shows the effect of diet restriction on the serum levels of leptin and corticosterone. One-way ANOVA showed significant effect of diet restriction ($F = 187.2$, $df 2, 21$, $P < .01$) on circulating levels of leptin. Post-hoc analysis showed significant hypoleptinemia in both the starved groups as compared to FF group. DR animals showed greater decline in serum leptin levels as compared to TRF group.

One-way ANOVA performed on the data of effect on serum corticosterone levels showed that the effect of diet restriction ($F = 222.99$, $df 2, 21$, $P < .01$) was statistically significant. Post-hoc analysis showed significant increase in corticosterone levels in both the starved groups. But, the hypersecretion of corticosterone found in DR animals was significantly higher as compared to TRF group.

Figure 6 shows the effect of diet restriction on mRNA expression of 5-HT_{1A} in the raphe nuclei. One-way ANOVA showed significant effect of diet restriction ($F = 293.38$, $df 2, 21$, $P < .01$). Post-hoc analysis showed significant decline in the relative abundance of 5-HT_{1A} auto-receptor in both the starved groups as compared to FF group. Also, the DR rats showed significant decline in the expression of 5-HT_{1A} auto-receptor compared to TRF group.

Discussion

The present study was designed to understand the effect of restricted feeding on 5-HT, leptin, and glucocorticoid in rat models. In view of a role of 5-HT in the modulation of motor

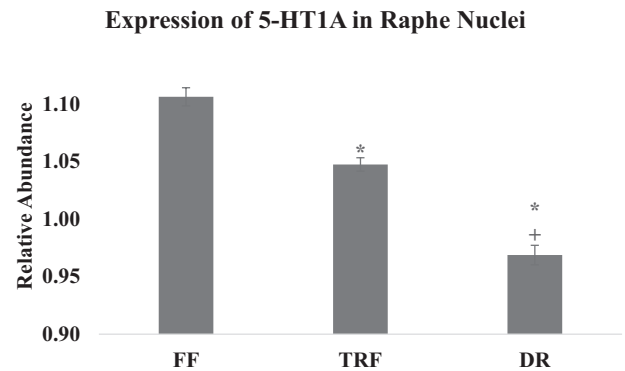


Figure 6. Effects of restricted feeding on the relative abundance of 5-HT_{1A} mRNA in the raphe nuclei. Values are mean \pm SD (n=8). Significant difference by Tukey's test: * $P < .01$ from respective FF animals and + $P < .01$ from respective TRF animals following one-way ANOVA.

behavior,¹⁷ effect of food restriction was monitored in the striatum. In view of the potential role of leptin in hyperactivity¹⁸ and altered eating behavior,¹⁹ circulating levels of leptin were determined. Also, due to a relation of chronic stress and anxiety with glucocorticoids,²⁰ circulating levels of corticosterone were monitored. To understand the role of 5-HT_{1A} auto-receptor in the modulation of striatal 5-HT metabolism and in the release of leptin and cortisol, expression of the auto-receptor in the raphe nuclei was also studied. All these estimations were done in 2 separate animal models which were assessed comparatively for their appropriateness as a rat model for food restriction induced hyperactivity.

It was found that, food restriction for 5 weeks significantly decreased the body-weights of DR animals. The percent difference in the body weights of these rats, from day-1 and that on 35th day was about 30%. As limited amount of food was given to DR animals, greater decrease was observed in their body weight as compared to TRF rats. The TRF animals adopted the 2 hours diet restriction model. Therefore, after week-1, an increase was observed in the food intake and body-weight of TRF animals.

Food restricted rats exhibited hyperactivity which was smaller in TRF animals. There was a decrease observed in the serum and striatal levels of Trp in both the starved groups. Similar decrease was observed in the striatal levels of 5-HT and 5-HIAA in these groups. But as compared to TRF group, greater decrease was found in the levels of Trp, 5-HT, and 5-HIAA in DR animals. Similarly, hypoleptinemia and the increased corticosterone levels found in both the groups was more profound in DR animals. Expression of 5-HT_{1A} auto-receptor was also decreased in the raphe nuclei of both the starved groups.

Hyperactivity, Trp deficiency, and striatal 5-HT metabolism

Food restriction alone is found to result in accumulation of anxiety and behavioral deficits.²¹ Few studies also indicate that

persistent anxiety or depression in adolescent females could result in disturbed eating habits and leads to eating disorder.²² Chronic food restriction has shown to result in accumulation of anxiety/depression like symptoms in lab animals.²³ Food restriction and malnutrition can cause serotonergic disorders²⁴ such as anxiety/depression,²⁵ psychosis,²⁶ and memory impairment.²⁷

Most of the behavioral symptoms associated with food restriction are the one found in conditions linked to decreased 5-HT metabolism in brain regions.²⁸ Patients undergoing excessive food restriction show hyperactivity, anxiety, loss of appetite, and mood disorder.²⁹ Studies suggest a relationship between behavioral disorders and deficiency in 5-HT neurotransmission.^{30,31} 5-HT levels in brain are dependent upon the dietary intake of Trp.^{32,33} which cannot be synthesized by the body. Trp deficiency is found in chronic food restriction and mal-nutrition.³⁴ Deficiency of Trp is associated with decreased 5-HT neurotransmission.^{35,36} Along with Trp, dietary deficiency of folate and vitamin B-12 is also known to influence 5-HT metabolism.³⁷ 5-HT is found to be responsible for the behavioral changes associated with anorexia nervosa, an eating disorder characterized by severe self-starvation.⁶

Previous studies show that chronic food restriction produces hyperactivity and anxiety in female rats.³⁸ Current study also shows similar behavioral deficits in female rats kept on food restriction. Hyperactivity was observed in both familiar and non-familiar environments. The DR rats were found to be more hyperactive than TRF group. This symptom could be related to the decreased dietary intake and reduction in the striatal 5-HT metabolism. Decrease in Trp is also related to increased corticosterone, which is known to increase the Trp metabolism by accelerating kynurenine pathway,³⁹ but this metabolism was not studied in the current experiment. Striatum, both dorsal and ventral, is known for its role in locomotor activities.⁴⁰ Present study suggests, that the decreased serotonergic metabolism in the striatum is responsible for this starvation induced hyperactivity.

5-HT1A auto-receptor in chronic food restriction

Somato-dendritic 5-HT1A auto-receptors located pre-synaptically on serotonergic neurons, act as natural brakes that control the release of 5-HT from these neurons. The auto-receptors are part of body's negative feed-back mechanism. When 5-HT binds to these auto-receptors, it inhibits the further release of 5-HT from serotonergic neurons into the synaptic cleft.⁴¹ While, the activation of 5-HT1A hetero-receptors, located post-synaptically in several brain regions, increases the serotonergic functions.

The current study focuses on the role of 5-HT1A auto-receptor in rat models of chronic food restriction induced hyperactivity. For this, mRNA expression of the auto-receptor was assessed in the raphe nuclei (both median and dorsal), because of the greater abundance of serotonergic neurons and the auto-receptors in this region. Studies suggest an important

role of the auto-receptor in 5-HT associated disorders.⁴² Its expression is impaired in patients of stress and depression.⁴³ An increase in the density of 5-HT1A auto-receptor is found in depressed suicide patients.⁴⁴ It is proposed that an increase in auto-receptor levels decreases the 5-HT neurotransmission.⁴⁵ But, the current study shows a decline in the relative abundance of 5-HT1A auto-receptor in the raphe nuclei of both the starved groups. The decrease was more profound in DR rats, compared to TRF group. Interestingly, these rats showed hyperactivity and a significant decrease in 5-HT metabolism. Suggesting a failure of the negative feedback mechanism in rats having declined 5-HT stores due to the dietary deficiency of Trp. The declined 5-HT1A auto-receptor is perhaps the body's response to cope-up with the reduced 5-HT neurotransmission. This demonstrates a potential role of 5-HT1A auto-receptor in food restriction induced stress and hyperactivity.

Hypoleptinemia and hypersecretion of corticosterone in chronic food restriction

Several studies suggest possible mechanisms behind the association between starvation and hyperactivity. Hypoleptinemia is found in starved rats.⁴⁶ This decrease in circulating leptin levels is due to a reduction in the number of leptin secreting adipose tissues, caused due to excessive food restriction. Studies suggest a relationship between hypoleptinemia and activity in the striatum, which is known for its role in locomotion.¹⁸ Improvement in leptin levels is observed when hypoleptinemic patients are provided high caloric meal.⁴⁷ This improvement also alleviates hyperactivity. Alleviation in hyper-activity is also observed when leptin is administered to starved lab rats.⁴⁸ Decreased leptin levels cause dysregulation in the neuro-endocrine functions performed by leptin.⁴⁹ This hypoleptinemia when sensed by brain, realizes that body is in a state of energy deprivation. Thus, necessary changes are made to manage this state.⁵⁰ These changes include decreased secretion of gonadotropin releasing hormone and thyroid stimulating hormone.⁵¹ While, release of ACTH is increased. This increase in cortisol accumulates the symptoms of hyperactivity and stress.⁵²

Increased levels of glucocorticoids are associated with chronic stress. Serum cortisol level is a classical biomarker for stress.⁵³ Cortisol levels are also elevated in underweight patients of anorexia nervosa.⁵⁴ Current study also affirms this. Increased circulating levels of corticosterone were found in both the starved groups. But, the hypersecretion of corticosterone found in DR animals was greater in comparison to the TRF group. Interestingly, similar pattern of hypoleptinemia was found in DR and TRF animals. Profound increase in corticosterone levels was associated with greater reduction in the circulating leptin levels.

Studies suggest that increase in brain 5-HT stimulates the release of corticosteroids from adrenal gland.⁵⁵ Current study shows a decrease in striatal 5-HT metabolism in starved rats

having increased corticosterone levels. Here the role of leptin cannot be ignored. Hypoleptinemia is associated with stress⁵⁶ and increased activity of HPA axis.⁵⁷ Starved rats showed hypoleptinemia due to decrease in 5-HT metabolism and leptin secreting adipose tissues. This decrease in circulating leptin levels activated the HPA axis to secrete ACTH.

Conclusion

In conclusion, the present study suggests that chronic food restriction decreases the body weight, dietary intake of Trp and subsequently the striatal 5-HT metabolism to produce hyperactivity. In order to manage this deficiency in 5-HT metabolism, expression of 5-HT_{1A} auto-receptor is also reduced. But due to reduced 5-HT metabolism, this declined expression of 5-HT_{1A} does not increase the post-synaptic availability of 5-HT. Declined post-synaptic availability of 5-HT in brain regions results in hypoleptinemia. Hypoleptinemia, along with chronic stress results in increased secretion of corticosterone. These changes and associated symptoms are more profound in rat model in which limited amount of food is given (DR model), compare to the model in which food is provided ad libitum for limited time (TRF model).

Author Contributions

Raheel Saeed performed all the experiments and wrote major part of the manuscript. Khalid Mahmood helped in animal dissection, brain isolation, neurochemical analysis and data interpretation. Sadia Basharat Ali helped in the behavioral studies and receptor expression. Darakhshan Jabeen Haleem supervised the entire research.

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