RESEARCH ARTICLE

High-fat Diet Increases Sprague-Dawley Corticosterone Blood Levels with Nominal Change in Adrenocorticotrophic hormone (ACTH) Level with Signs of Increased Mesenteric Adiposity

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Abstract

BACKGROUND: Corticosterone is a common hormone in research involving rodents as it is used to indicate and measure stress levels. It was widely reported that certain dietary habits and components induce Hypothalamic-Pituitary-Adrenal (HPA) axis activity, with corticosterone found in the bloodstream. Chronic corticosterone presence can portray signs and symptoms of certain endocrine. Certain food and chemicals were found to alter HPA axis activity leading to dysregulation of the HPA axis. Earlier studies have shown enhancement of the HPA axis to produce more glucocorticoids by an unbalanced diet. This study aims to shed more light on this subject.

METHODS: Sprague Dawley rats were divided into five groups of seven each and were fed five respective diets (control, high-fat, high-protein, high-sugar, and high-starch), with tap water as drinking water *ad libitum*. After eight weeks, the rats were euthanized, blood was collected, and serum harvested and kept for analysis. Mesenteric fat was identified, harvested, and stained with hematoxylin and eosin (H&E) and set for viewing under light microscope. The hormones of interest which is adrenocorticotropic hormone (ACTH) and corticosterone was extracted from the blood, to be processed accordingly and quantified using the High-Performance Liquid Chromatography (HPLC) with photodiode array (PDA) analysis technique.

RESULTS: The results showed an increase in Sprague-Dawley corticosterone blood levels with a nominal change in ACTH level. Advanced hypertrophy was observed in mesenteric adipose tissue in the high-fat diet group compared to the other diet groups.

CONCLUSION: This study confirms the negative effect of a high-fat diet on health from a hormonal and adipocyte perspective. A high-fat diet was found to instigate the HPA axis and influence blood corticosterone level.

KEYWORDS: adrenocorticotrophic hormone, ACTH, corticosterone, mesenteric fat, diet

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Introduction

In humans, problems related to the cardiovascular system, mental health and even autoimmune diseases was associated to metabolic dysfunction, with constant elevation of cortisol level as its hallmark.(1,2) Dietary habits were found in certain studies to have an impact on the Hypothalamic-Pituitary-Adrenal (HPA) axis. On a positive note, addition of omega-3 polyunsaturated fatty acid to a rat's diet was found to restrict HPA axis activity.(3) Probiotics and phytogenic substance from plants sources were also revealed to have

potency against cortisol blood concentrations.(4,5) More research has concluded that higher levels of glucocorticoids are produced as a result of repetitive HPA activation, and the cause is usually pre-empted by an unhealthy diet. (6) To counter these HPA activations, one must begin with a healthy habit of eating nutritious food and stop all unhealthy practices.(7) An unbalanced diet especially concerning a macronutrient will inevitably bring serious health complications if consumed for an extended period of time. This kind of eating pattern will stress the physiological systems in our body leading to metabolic diseases.(8)

Data from epidemiological studies over the past three decades have revealed that excess visceral fat tissue can impose a serious negative health impact and increases the risk for cardiovascular diseases, hypertension, diabetes mellitus and dyslipidaemia.(9) Previous studies have shown that visceral fat increase is found in many pathological environments and is characterized by an increase in the cellular size (hypertrophy) rather than the cell number count (hyperplasia).(10) Keeping in mind the effect of daily dietary habits on our good health, it is therefore important to investigate the effect of different diets on both the hormonal and cellular health homeostasis.

Methods

Rats Model

Sprague Dawley rats (selected at 8 weeks old) were grouped into five groups of seven each and stored in a room with controlled temperature (22°C) with alternating 12-hour daylight and night cycle. Each group was given a different rat feed formula for the duration of eight-weeks *ad libitum* and drinking water was supplied with tap water.

The rat feed for each group was prepared based on a previous published study.(11) Briefly, the groups were fed control diet (standard rat chow macronutrients), high-fat diet (vegetable-oil-based, 35% fat), high-protein diet (whey-based, 52% protein), high-sugar diet (table sugar, 96% glucose) and high-starch diet (rice-based, 83% carbohydrates), respectively. At the end of the eighth week of the controlled-feeding, the rats were euthanized in a glass chamber using carbon dioxide gas. To avoid post-prandial plasma glucocorticoid increase, the rats were fasted overnight before the euthanization day. After no signs of life was observed, blood was collected via cardiac puncture. This was followed by an abdominal dissection to harvest the mesenteric fat which was immediately fixed in a 10% formalin. All blood samples were centrifuged at 1000 rpm for 15 minutes to separate the serums, respectively. The respective serum was then collected into a 1.5 mL centrifuge tube and immediately stored at -20°C prior to further analysis.

The animal care and practices applied in this study were in accordance and in compliance with the specifications and recommendation stipulated by the FOM IACUC University of Malaya (Ref: 2019-21114/UNIKL/R/KAMJ).

Blood Extraction and High-Performance Liquid Chromatograph (HPLC)

The processing of blood samples and the HPLC settings for the quantification of corticosterone and adrenocorticotropic hormone (ACTH) were based on previous study.(12) Corticosterone and ACTH standards were used as positive controls and for the constructions of the respective hormone's standard curve. The corticosterone standard (≥98.5% HPLC grade, Sigma-Aldrich, Baden-Württemberg, Germany) and ACTH standard (≥95% HPLC grade, Sigma-Aldrich) were diluted in 20% acetonitrile (ACN). Both ACTH and corticosterone standards were stored at -20°C. The hormones were analysed by using HPLC in which two mobile phase solvents (1 L pH_2O and 500 mL of ACN), one purge solvent (250 mL ACN; 250ML MeOH; 500 mL pH_2O) and one washing solvent (500 mL of 10% ACN) were used. The sample run was programmed via the Empower software with the sample injection set at 50 µL and the running time set at 10 minutes. A photodiode array detector was utilized to monitor the eluent at a wavelength of 245 nm. The room temperature was controlled at 24±2°C.

Hematoxylin & Eosin (H&E) Staining of Mesenteric Fat

The fixed mesenteric fat was processed with a tissue processor machine (Leica, Weltzer, Germany) and stained according to H&E staining protocol. Briefly, after rehydration using xylene and a descending concentration of alcohol, each tissue sample was submerged into hematoxylin solution (Sigma-Aldrich) for 15 minutes, followed by acid alcohol 1% for three seconds and then eosin solution (Sigma-Aldrich) for three minutes. Each sample was then dehydrated using ascending concentrations of alcohol-xylene solution and fixed on glass slide using DPX mountant solution (Sigma-Aldrich). Afterward, all the samples were viewed using an electronic light microscope (Leica) for the presence of mesenteric fat.

Data Analysis

Analysis of data was done using the one sample t-test (available on *https://www.statology.org/one-sample-t-test-*

calculator/). The data from high-fat, high-protein, high-sugar and high-starch diet groups were calculated against the control group data. This analytical method was selected as there was only one sample obtained for each group. Only one sample was obtained due to the limited availability of ACTH and corticosterone standard solutions. The $p<0.01$ was considered statistically significant.

Results

Figure 1 showed the HPLC-PDA absorbance for ACTH in the control, high-fat, high-protein, high-sugar, and high-starch diet groups. Although there was not much difference between the groups, it was observed that high-sugar diet group registered the highest peak at 0.050022 AU. The control group registered at 0.034726 AU, high-fat at 0.034312 AU, high-protein at 0.029853 AU and high-starch 0.028739 at AU. This result presented a minimal response and thus proposes a nominal change in the ACTH production and secretion.

Meanwhile Figure 2 showed the HPLC-PDA absorbance for corticosterone in the control, high-fat, high-protein, high-sugar, and high-starch diet groups. The high-fat and high-sugar diet group recorded the highest peak at 0.997458 and at 0.95963 AU, respectively. The control group registered at 0.002022 AU, high-protein at 0.009857 AU and high-starch at 0.014517 AU. This result suggests that a high-fat and high-sugar diet may induce metabolic stress as evident by the high corticosterone levels.

Based on the electron micrographs after eight weeks of feeding, the mesenteric adipocytes of high-fat group is visibly larger than the rest of the group in which the adipocytes of the control , high-protein , high-sugar and high-starch groups exhibit similar morphological size and properties (Figure 3). This suggest that a high-fat diet promotes the enhancement of mesenteric fat hypertrophy.

Discussion

Earlier study have suggested that a high fat diet could lead to a decreased proopiomelanocortin (POMC) gene expression and a change in insulin signalling metabolism in the hypothalamus which led to metabolic disorders via neuroinflammatory action.(13) Glucocorticoids regulating the HPA axis is related to the hypothalamus Corticotrophin-Releasing-Hormone (CRH) inhibition and restriction of POMC expression and ACTH production.(14) The inhibition of ACTH production by corticosterone is almost instant and considered as nongenomic. A precursor molecule such as proopiomelanocortin (POMC) is expressed and kept in immature secretory granules. When a stressor starts the HPA axis, swift cleavage of POMC into ACTH is executed without involving POMC gene stimulation.(15) We can

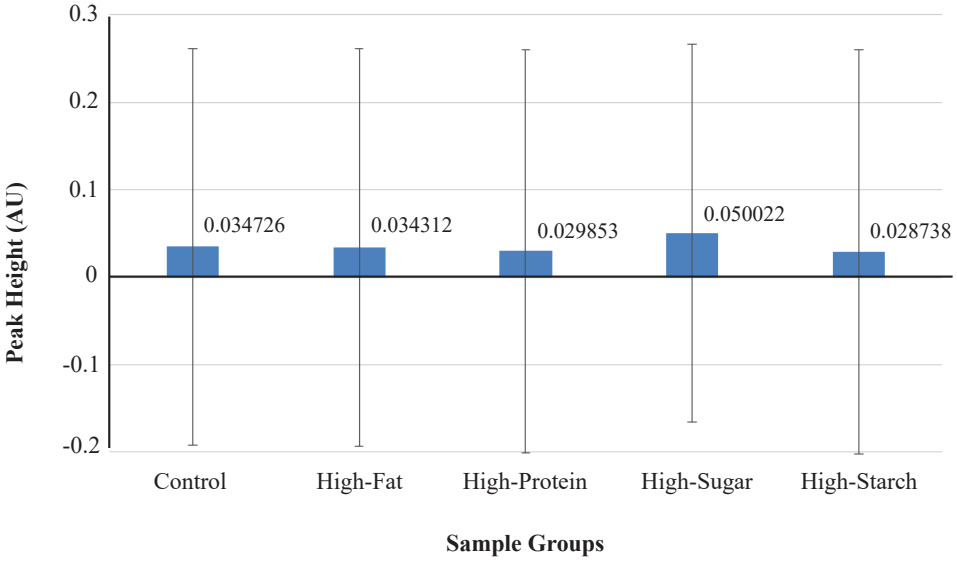

Figure 1. HPLC-PDA absorbance for ACTH in the control, high-fat, high-protein, high-sugar, and high-starch diet group blood extract sample. AU is the absorbance units.

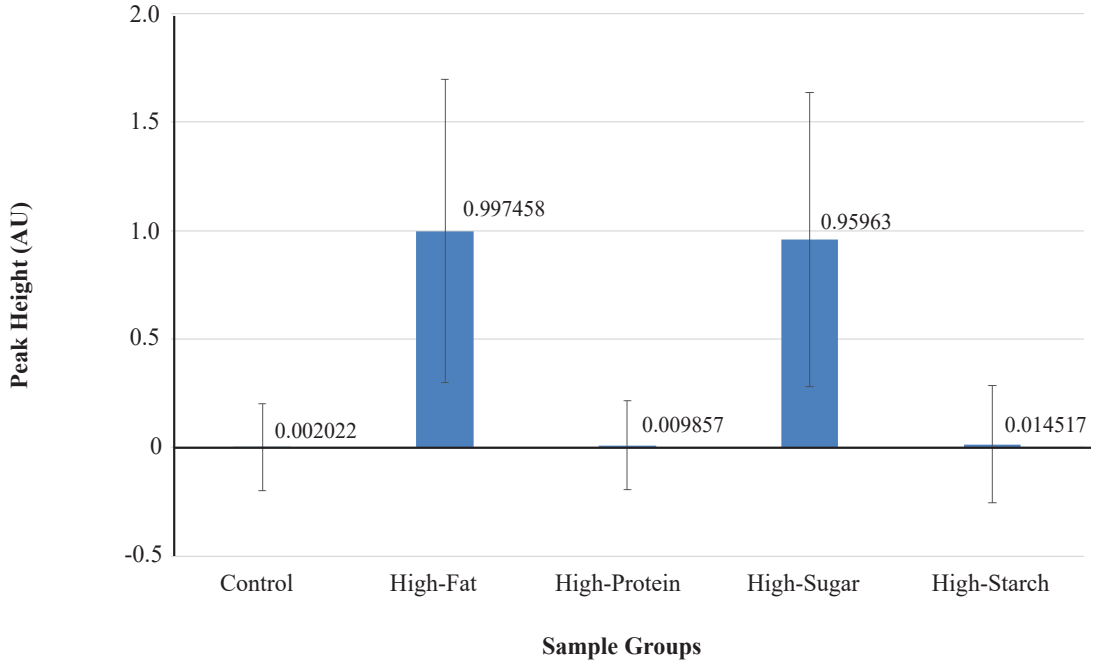

Figure 2. HPLC-PDA absorbance for corticosterone in the control, high-fat, high-protein, high-sugar, and high-starch diet group blood extract sample. AU is absorbance units.

correlate our findings in Figure 1 and Figure 2 with the findings in the literatures presented above. In our study, the highest blood level for corticosterone was achieved by the high-fat and high-sugar diet, respectively (Figure 2). As for our findings in Figure 1, the ACTH levels in the high-fat and high-sugar diet were most probably diminished by the quick negative feedback mechanism caused by the high corticosterone blood levels while the ACTH levels in the control, high-protein and high-starch groups were not increased due to lack of stress stimulation.

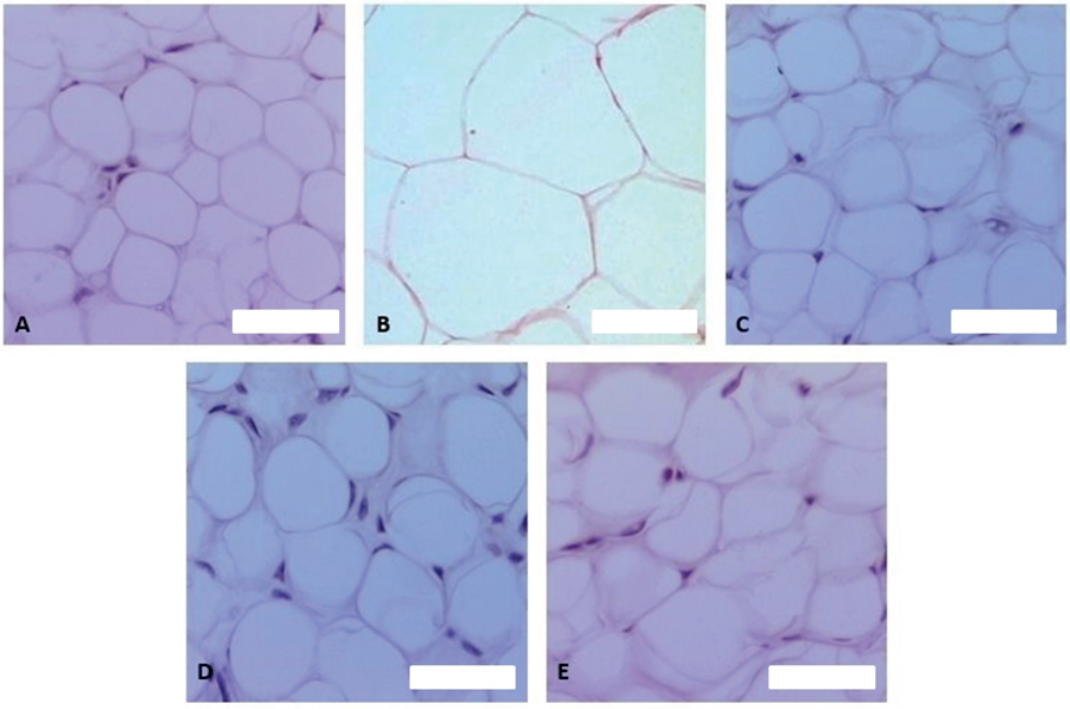

Figure 3. Electron micrograph of mesenteric adipocytes of all diet groups after eight weeks of feeding. A: control group, B: high-fat group, C: high-protein group, D: high-sugar group, E: high-starch group. White bar: 10 μm.

The higher corticosterone level in the high-fat and high-sugar groups agrees with earlier studies which suggested that a high-fat diet in both rodents and human increases glucocorticoid blood concentrations.(16,17) In rat fed with high-fat diet, high corticosterone levels were detected and inflammation was found in the limbic region of the brain where insulin and glucocorticoid receptors are found in large numbers.(18,19) High-fat diet was suggested to have the ability to change basal corticosterone blood level which indicates a dysregulation of the HPA axis homeostasis.(20) Similar effect has been suggested with a low carbohydrate diet.(21)

The constant production of ACTH and corticosterone as a result of chronic high-fat eating has been suggested to serve as the foundation of most metabolic diseases.(22) Furthermore, a study on the central nervous system gave evidence that a high-fat diet causes hippocampus function decline, adiposity and polyphagia, which disrupts the HPA axis.(23) In addition, a genetic study suggested that a high-fat diet not only affect the HPA axis but also alters all hormonal axis regulated by the pituitary gland which causes the augmentation of corticosterone production as a result of POMC gene transcription multiplication.(24)

Our results indicate that a high-protein diet has the benefit of keeping the blood corticosterone level low. Indeed, young males who undergo strenuous training reportedly achieved low blood cortisol levels after consuming whey protein for six weeks.(25) In addition, whey protein was found to better attenuate post-exercise cortisol levels when compared to plant-based protein source.(26) This is further attested in another study which reported reduced insulin and cortisol blood level after 14 consecutive days of whey protein consumption in gestational diabetic women with stress issues.(27) Indeed, whey protein consumption have been suggested to be beneficial in combatting metabolic syndrome parameters.(28) Consumption of whey protein by a group of pigs for 19 consecutive days were found to significantly reduce their cortisol level.(29)

Regarding complex carbohydrates, decreased blood cortisol level was observed on 16 adults engaging in stressful activity when carbohydrate rich food was consumed immediately before the activity.(30) On the contrary, a 16-day diet consisting of high-fructose corn syrup was found to increase cortisol concentrations when compared to a whole food diet.(31) Interestingly, a reduction in cortisol level was also observed in a 12-week high-carbohydrate low-fat diet involving a group of athletes.(32) Starch catabolism is slower than simple sugar.(33) Thus, it can be deduced that starch metabolism does not incite HPA axis activation as bad as simple sugar, and therefore the ACTH and corticosterone levels registered unremarkable in our study.

Previously, it was thought that a 12-week or 14-week high-fat feeding was needed for the accumulation and deposition of mesenteric fat tissue.(34-36) Our study, however, showed that eight weeks of high-fat diet not only causes fat tissue hypertrophy but that it is enough for the accumulation of mesenteric fat tissue. Involvement of both macrophages and adipocytes in lipid signalling metabolism which encourages HPA axis and inflammatory pathways activation have been indicated in the stimulation of low-grade chronic stress in obese individuals.(37) Also, earlier study has shown that obese individuals possessing severe metabolic disorders tend to have their visceral adipose tissue depots flourishing with hypertrophic adipocytes.(38) A continuous interaction was suggested to be present between the HPA axis and the adipose tissue proper in the stress system where the interaction is conducted by the HPA axis hormones, such as, ACTH, glucocorticoids and adipokines of the adipose tissue itself.(39) Indeed, obesity and the inflammation of adipose tissues have been suggested to be correlated to macrophage and proinflammatory cytokines activation.(40) Furthermore, based on the cellular and hormonal evidences in our study, a high-fat diet is potentially detrimental even at the early stage of obesity development.

Conclusion

This study indicates the negative effect of a high-fat diet on health. Specifically, continuous intake of high-fat diet for eight weeks is proposed to instigate physiologic and metabolic stress as evidenced by HPLC-PDA analysis of ACTH and corticosterone. Furthermore, enhanced hypertrophy of mesenteric adipose tissue was observed on diet high in fat.

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Authors Contribution

KA and MN were involved is in the conception, acquisition, analysis and interpretation of data. KA, RM, JF, DKS, PRD, and NM discussed the results and was involved in the manuscript preparation and revision.

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