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Research Article

Diets That Have Potential to Stimulate the Hypothalamic-Pituitary-Adrenal (HPA) Axis in Sprague Dawley Rats

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Abstract

Background: The pituitary gland is the master gland regulating body hormones. From the anterior pituitary's corticotrophs, adrenocorticotrophic hormone (ACTH) is secreted and influences corticosterone production in rodents via the hypothalamic–pituitary–adrenal (HPA) axis. ACTH and corticosterone are commonly used as hormonal stress markers. Disturbances in ACTH and corticosterone are seen in diseases like Addison's disease, Cushing's syndrome, and metabolic syndrome. **Objective:** To observe which diet induces the most and least stress on Sprague Dawley rats' physiology. **Methods:** Thirty-five Sprague Dawley rats were divided into five groups (n=7) and given different diets (control, high-fat, high-protein, high-sugar, and high-starch) with tap water ad libitum. After eight weeks, blood samples were collected and serum separated. ACTH and corticosterone were extracted, purified, identified, and quantified using High-Performance Liquid Chromatography (HPLC) with photodiode array (PDA) analysis. **Results:** High-sugar diet led to the highest ACTH blood level. Both high-fat and high-sugar diets showed the highest corticosterone peaks. **Conclusion:** Consumption of high-fat and high-sugar diets for eight weeks is suggested to induce physiological and metabolic stress in Sprague Dawley rats, as evidenced by HPA axis activation.

Keywords: adrenocorticotrophic hormone (ACTH), corticosterone, diet, high-fat diet, high-performance liquid chromatography (HPLC), high-sugar diet, hypothalamic-pituitary-adrenal (hpa) axis

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1. Introduction

The anterior part of the pituitary gland (or adenohypophysis) is a lobe that coordinates several important physiological functions namely lactation, stress, reproduction, and growth. Corticotrophs are a type of cell available in the anterior lobe of the pituitary which function is to produce and secrete adrenocorticotropin hormone (ACTH) [1]. ACTH is polypeptide hormone cleaved from the precursor proopiomelanocortin (POMC) protein inside corticotrophs and are released under the influence of hypothalamus [2]. ACTH main function is to stimulate production and release of glucocorticoids by the zona fasciculata of the suprarenal gland cortex, including influencing the circadian rhythm in mammals [3].

A recent article explains the dynamics of ACTH and cortisol secretion. Apparently, there is a short lag time between ACTH and cortisol secretion, with cortisol secretion closely succeeding each ACTH secretion.

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ACTH secretion was rapidly inhibited afterwards, and the site of inhibition was found to be in the anterior pituitary [4]. This negative feedback mechanism is a part of the hypothalamus-pituitary-adrenal (HPA) axis which is stimulated by stress and its end product is glucocorticoids [5]. The rapid nature of negative feedback on ACTH secretion agrees with a ligand- dependent nongenomic GR-mediated negative feedback process in the anterior pituitary [6]. In short, the presence of cortisol in the blood stream starts a negative feedback mechanism to inhibit the hypothalamus from stimulating ACTH production by the adenohypophysis [7].

Cortisol is the standard glucocorticoids found in mammals, including humans, while corticosterone is found in rodents and reptiles. Mice and rats are unable to synthetise cortisol due to the lack of enzyme 17-α hydroxylase (CYP17) in their adrenal cortex cells [8]. Corticosterone is very important in promoting normal physiological homeostasis. Among its functions are gluconeogenesis by the liver, aiding in fat and protein metabolism and suppressing the immune system [9]. Corticosterone is usually used in physiological and metabolic studies to detect and measure stress [10] Rodents which are continuously exposed to various kind stress was found to have high blood corticosterone levels.

Constant chronic high corticosterone level is proposed to cause a wide range of metabolic dysfunction which will lead to cardiovascular, autoimmune and mental problems [11]. Overtime, high stress levels provoke the HPA axis to produce more corticosterone which can bring disruption to important organs [12]. Certain food and chemicals were found to alter HPA axis activity. Ingestion of alcohol during pregnancy was found to render the individual exposed to prenatal alcohol consumption vulnerable to stress related disorders due to dysregulation of the HPA axis [13]. On the other hand, supplementation of omega-3 polyunsaturated fatty acid to mice revealed a decreased level of HPA axis action [14]. Also, there is early evidence that a probiotics supplementation can improve stress hormone levels [15]. Also, addition of plant phytogenics to a diet was reported to produce a significant markdown in cortisol blood concentrations [16].

Chronic and poor diet quality can be a powerful stressor and can implicate major health problems especially when coupled with other unhealthy lifestyle choices [17]. Role of certain dietary patterns and foods in the promotion of high-quality sleep, which is an important decelerator for stress, is currently being studied upon [18]. There is early evidence showing that an unbalanced diet can act as a stressor by enhancing the HPA axis to produce more glucocorticoids [19]. Whether the disturbance started with an unhealthy diet or after attaining obesity is anybody's guess. A healthy functional diet gives the body essential nutrition such as, macronutrients, micronutrients and sufficient calories and fluids [20].

Established health agencies advocate that the public keep a healthy lifestyle by minimizing red meat and processed food consumption, curbing intake of rich foods and high-sugar drinks and consuming more plant-based meals [21]. A diet which has a deficiency or an excess of one macronutrient can bring hazardous results if consumed long term. This habit can also constitute as harmful stress to the body as the body will have to adjust and counteract leading to metabolism related diseases [22, 23].

High-performance liquid chromatography coupled with photodiode array (HPLC-PDA) method of material analysis is widely used by scientists as this method is straightforward, cost-effective, gives a robust result, gives result in a short period of time and relatively accessible to labs without an extensive chemical-analytical knowledge foundation. By comparing retention time to commercially available chemical standards, samples are detected and quantified for precise analysis [23] Among the advantages of using PDA are simultaneous multiwavelength measurement, fast scan speed, high signal to noise ratio and acquisition of precise data at a specific wavelength by electrical scanning with minimal stray light effects. PDA is a rugged, solid-state device which makes it more secure, stable, and more reliable when compared to older conventional analytical Instruments [24]. High-performance liquid chromatography (HPLC) coupled to photodiode array (PDA) detection was chosen in this study as it is a developed, validated, reliable, and robust analytical method [25]. HPLC is proven to have the advantageous edge in molecular analysis as it is the widely favoured and preferred applied technique for quantification [26].

2. Methods

Thirty-five with 8 weeks old male Sprague Dawley rats were stored in a room with controlled temperature (22 degree Celsius) with alternating twelve hours daylight and night cycle. The rats were grouped into five groups of seven. Each group were given a different rat feed formula for the duration of eight weeks ad libitum and drinking water was supplied with tap water.

The different rat feed macronutrients formula are as follows: Control diet (standard rat chow macronutrients), high-fat, vegetable-oil-based diet (35% fat), high-protein, whey-based diet (52% protein), high-sugar diet using table sugar (96% glucose) and high-starch, rice-based diet (83% carbohydrates) [27]. To avoid post-prandial plasma glucocorticoid increase, the rats were fasted overnight before euthanization day. At the end of the eighth week, the rats were euthanized using carbon dioxide gas in a chamber. After no

signs of life was observed, blood was collected via cardiac puncture. All blood samples were centrifuged at 1000 rpm for 15 minutes to separate the serum. The serum was then collected into a 1.5 mL centrifuge tube and immediately stored at - 20°C for further analysis. Animal ethics practices are cleared in compliance with the guidelines approved by the FOM IACUC University of Malaya (Ref: 2019-21114/UNIKL/R/KAMJ).
Blood extraction & HPLC

In a 100 ml beaker, 40 ml of 356 g/L MeOH (25%) was mixed well with 10 ml of 89 g/L ZnSO4 and then 900 µL of the mixture was removed into 1.5 ml centrifuge tube before 450 µL sample (blood serum) was added into the same tube. The sample was vortexed to mix and centrifuged at 3250 rpm for 10 minutes. After centrifugation, 900 µL of supernatant was transferred into new 1.5 mL centrifuge tube followed by the addition of 270 µL of 4% orthophosphoric acid (H3PO4). The mixture was then loaded into a solid phase extraction (SPE) platform and the resulting eluate was discarded. The SPE was washed twice by 500 µL of 25% methanol and discarded eluate. The sample was eluate twice with 25 µL mixture of acetonitrile (ACN) and MeOH (9:1) into the 150 µL insert vial before 25 µL pure water was added. The standards for corticosterone and ACTH were used as positive controls and for standard curve construction for hormone level measurement in samples. The hormones standard (both from SIGMA-ALDRICH, Germany) for corticosterone and ACTH each of 0.10 MG standards were diluted in 20% ACN. Both ACTH and corticosterone standard was stored at -20°C temperature.

The hormones were analysed by using a HPLC machine. The HPLC machine (Waters) work with two mobile phase solvents (1 litre pH2O and 500 mL of ACN), 1 purge solvent (250 mL ACN; 250ML MeOH; 500 mL pH2O) and 1 washing solvent (500 mL of 10% ACN). The sample run was programmed via Empower software, set at sample injection of 50 µL. Using a photodiode array detector, a wavelength of 245 nm (Viljoen 2012) was utilized to monitor the eluent. Running time of the samples was 10 minutes each. Room temperature was controlled to be at 24°C ± 2°C [28].

3. Results

HPLC analysis for ACTH

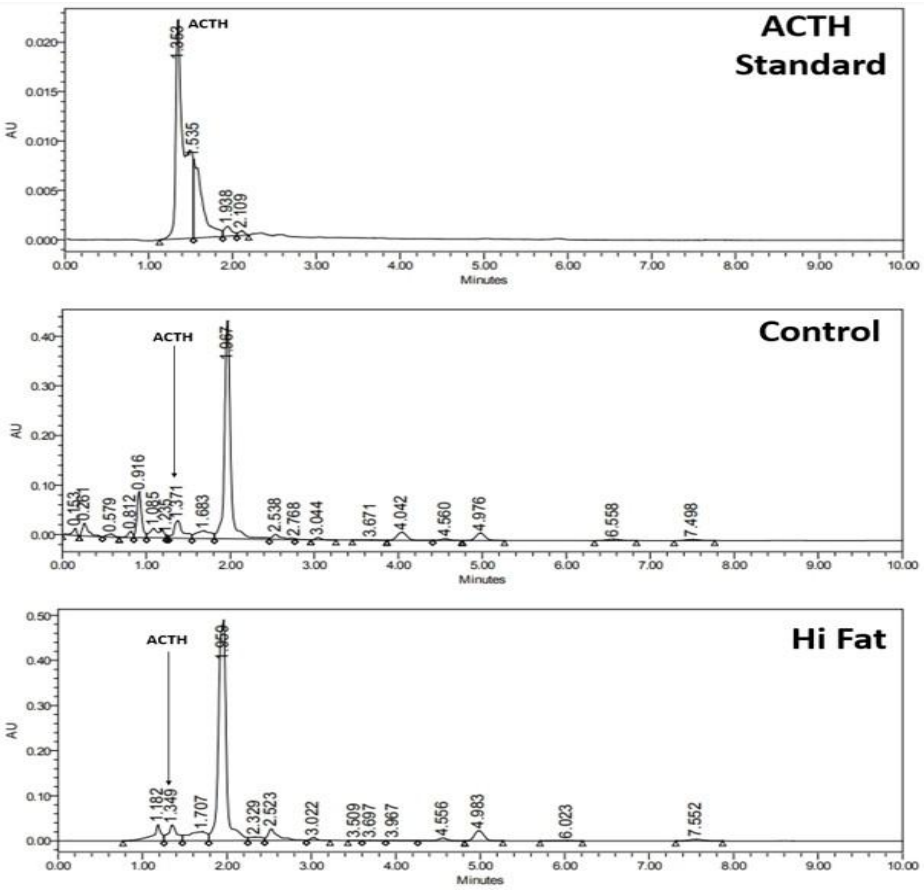

Figure 1. HPLC chromatograms of ACTH standards, ACTH in control and high-fat blood extraction samples.

The x-axis shows the retention time while the y-axis indicates absorbance units (AU) which is the indicator matching to the signal generated by the detector at 245 nm.

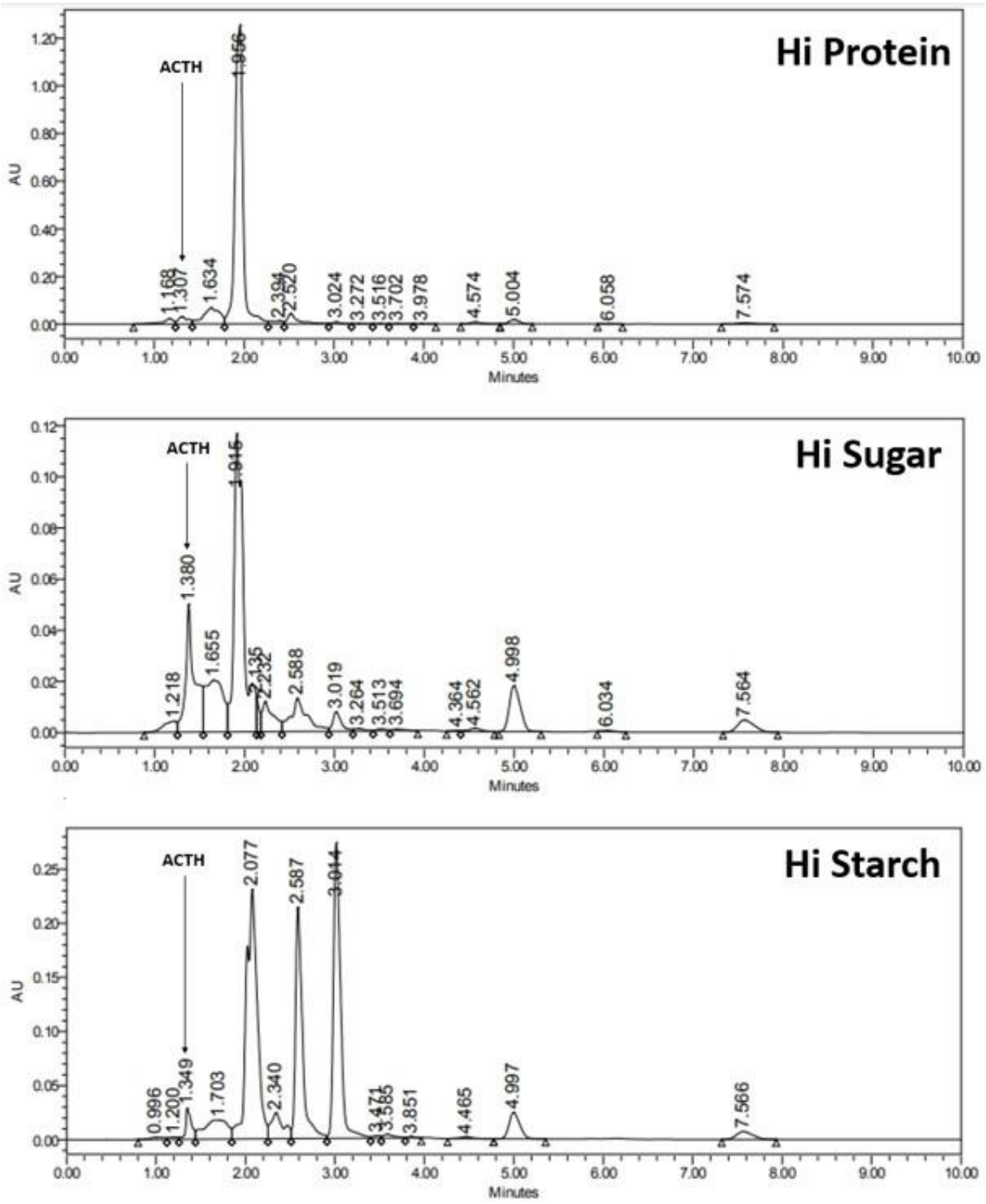

Figure 2. HPLC chromatograms of ACTH in high-protein, high-sugar and high-starch blood extraction samples

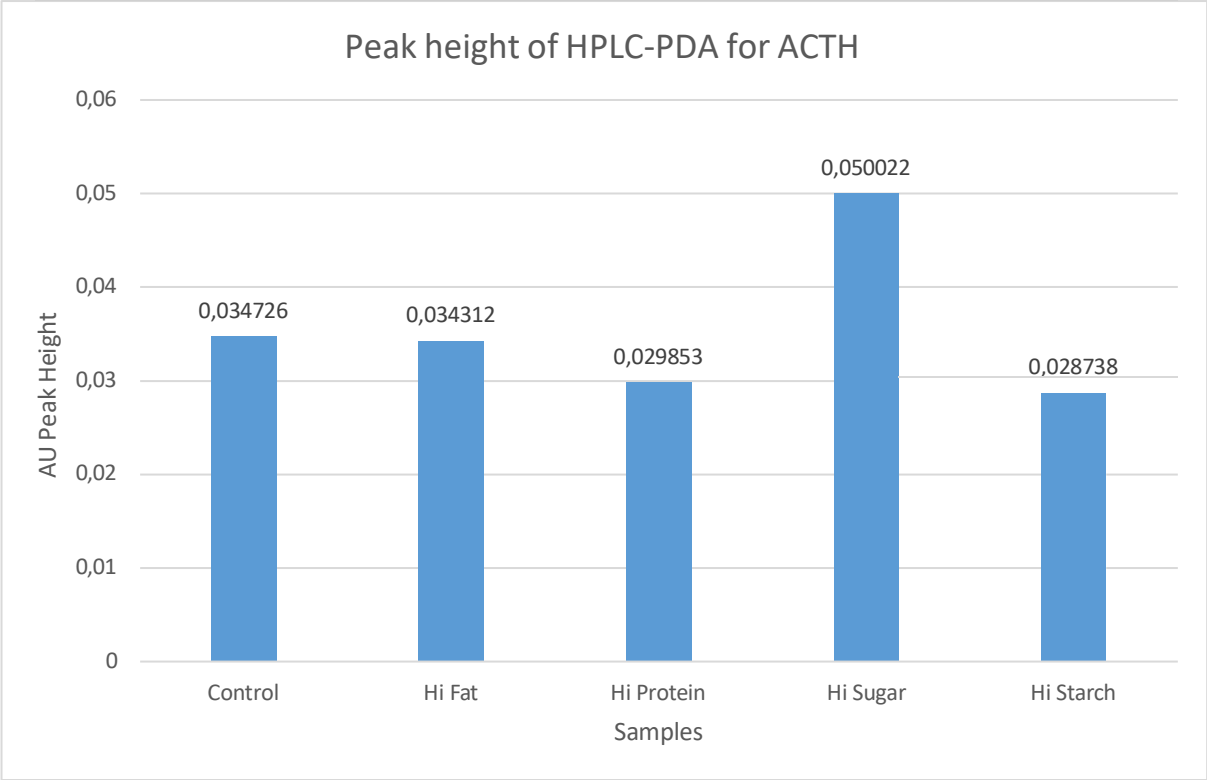

Figure 3. Showing peak height of HPLC-PDA for ACTH in absorbance unit (AU) at 245nm for control, high-fat, high-protein, high-sugar, and high-starch diet group blood extraction samples.

It was noted that the high-sugar diet group have the highest peak at 0.050022 AU. Control group registered 0.034726 AU, high-fat 0.034312 AU, high-protein 0.029853 AU and high-starch 0.028739 AU respectively.

HPLC analysis for Corticosterone

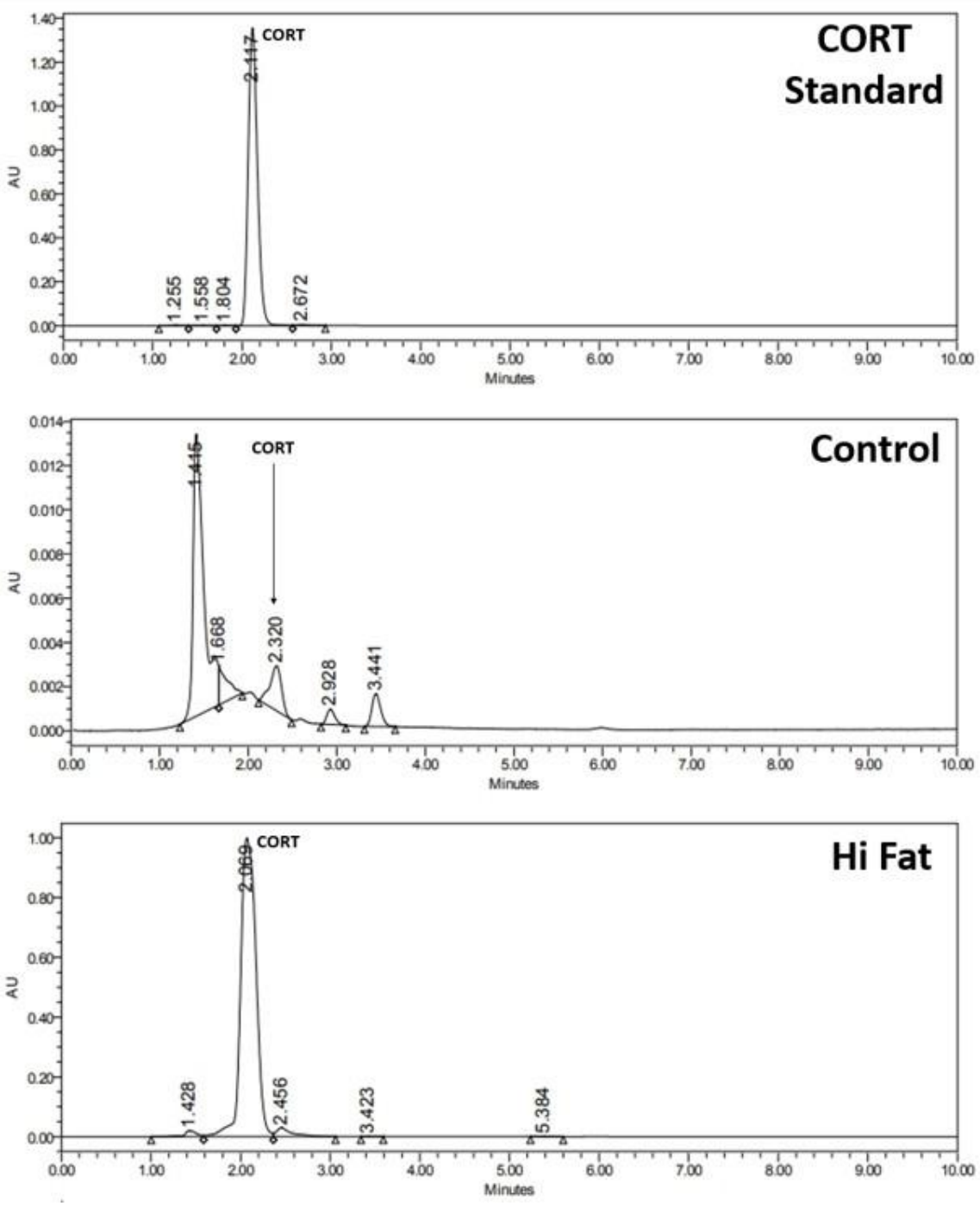

Figure 4. HPLC chromatograms of corticosterone standards, corticosterone in control and high- fat blood extraction samples.

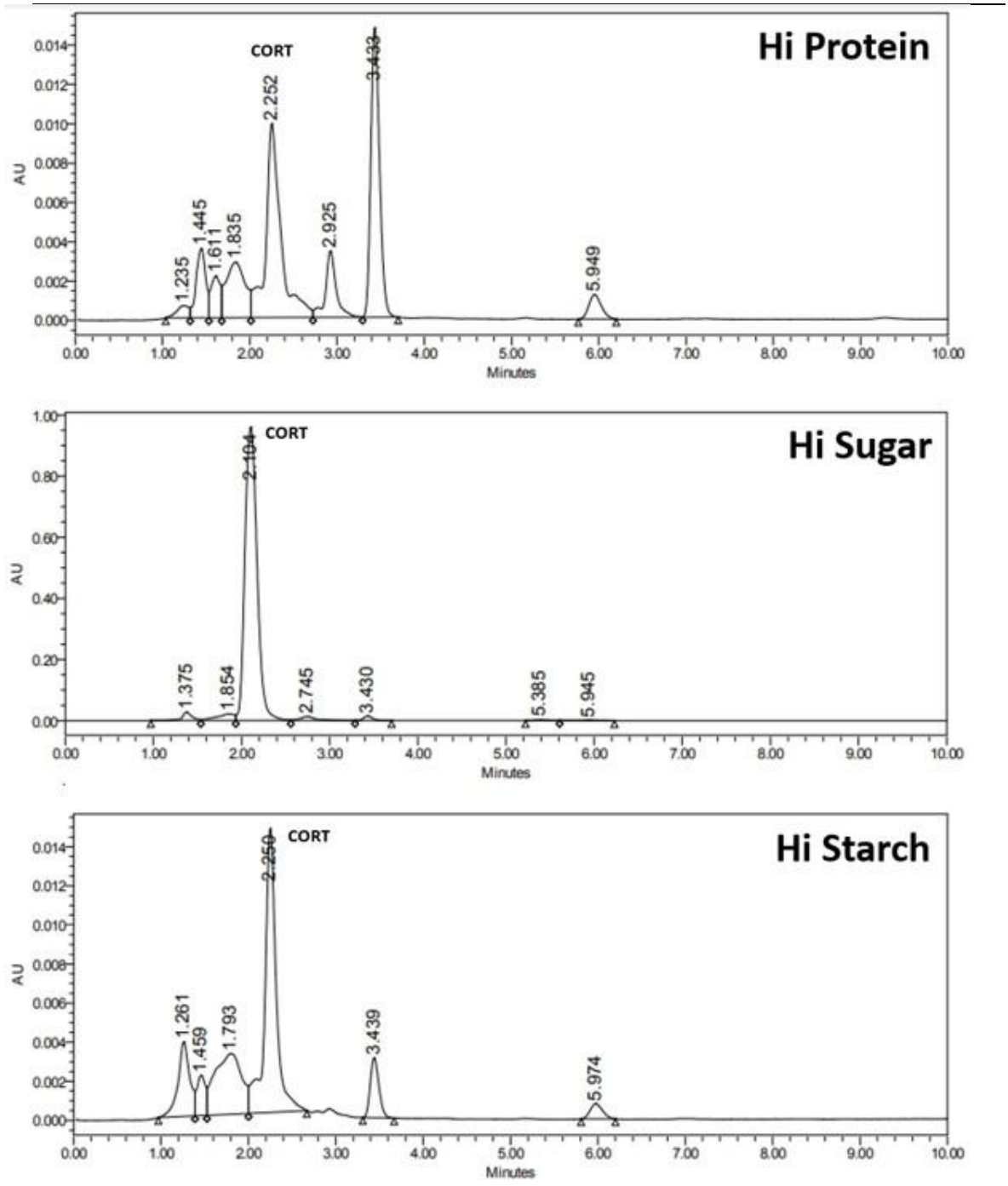

Figure 5. HPLC chromatograms of corticosterone in high-protein, high-sugar, and high-starch blood extraction samples.

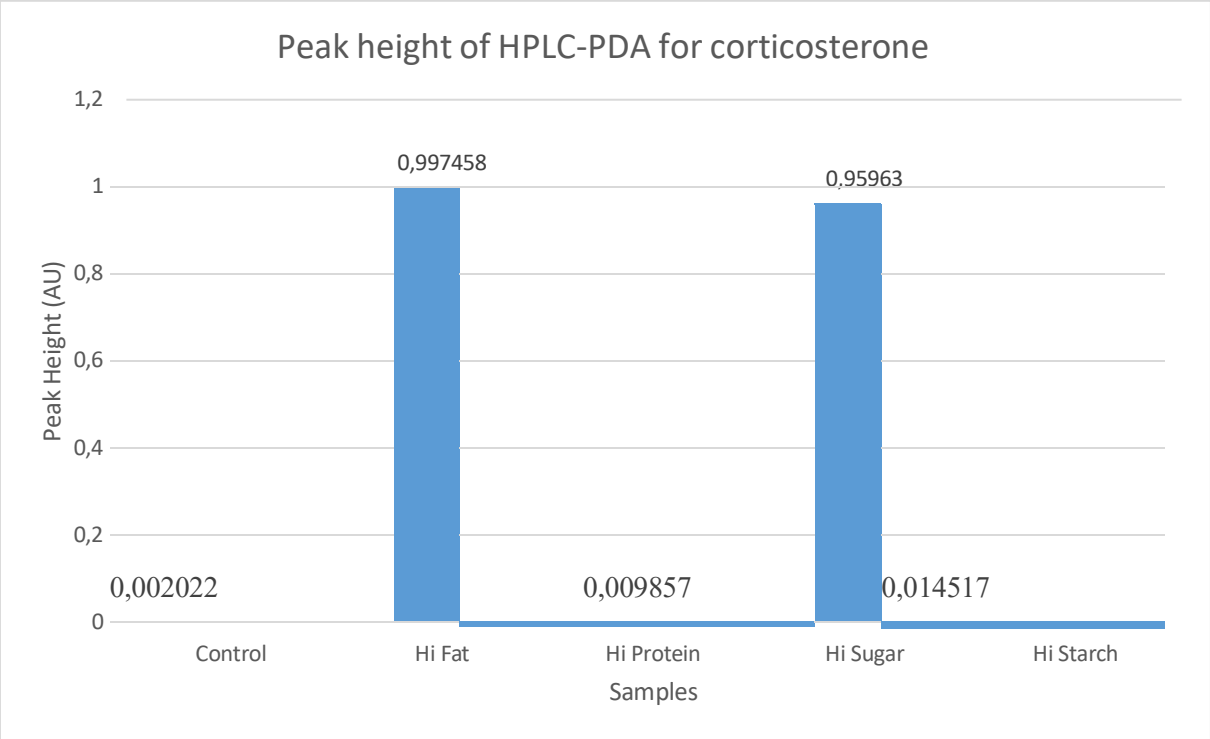

Figure 6. Showing peak height of HPLC-PDA for corticosterone in absorbance unit (AU) at 245nm for control, high-fat, high-protein, high-sugar, and high-starch diet group blood extraction samples.

It was noted that the high-fat and high-sugar diet group have the highest peak at 0.997458 and 0.95963 AU respectively. The control group registered 0.002022 AU, high-protein 0.009857 AU and high-starch 0.014517 AU respectively. This result suggests a high-fat and high-sugar diet may induce metabolic stress and it is translated by the high corticosterone levels.

4. Discussion

With a glycaemic index of 65, fully refined table sugar is 99.9% sucrose [28, 29]. Studies show that sucrose-rich diets fed to rats cause changes in the adrenal cortex morphology and function with elevated corticosterone concentrations which is correlated to development of insulin resistance [30]. The changes happening to the adrenals are associated to HPA axis increased activity. Oxidative stress formation within the pituitary gland and its inflammation increases POMC transcription and expression resulting in increased ACTH secretion in rats treated with sucrose-rich diets, which prompts increased secretion of corticosterone by the adrenal glands [31].

High-fat diet was found to reduce POMC expression and disturb insulin signalling pathway in the hypothalamus resulting in metabolic disorders via neuroinflammatory activity [32]. Glucocorticoids inhibiting the HPA axis is related to the hypothalamus corticotrophin-releasing-hormone (CRH) regulation and restriction of POMC expression and ACTH production [33]. The inhibition of ACTH release by corticosterone is rapid and considered as nongenomic. A precursor molecule such as POMC is produced and stored in immature secretory granule. When a stressor activates the HPA axis, rapid cleavage of POMC into ACTH is done without involving POMC gene stimulation [34]. Figure 3 shows that the high-sugar group sample have a slightly higher ACTH peak than the rest of the samples.

Based on explanation by Harno, our result propose that a high-sugar diet may have developed more POMC secretory granules than other diet groups because of a repetitive and chronic HPA axis stimulation by sucrose. However, this hypothesis must be confirmed by sub-cellular ultra- morphometry imagery or other suitable methods.

Figure 6 shows high-fat and high-sugar diet group registered the highest corticosterone sample peaks. Chronic treatment using high-fat diets in rats and mice were found to elevate blood corticosterone concentrations and develop inflammations plus deficiency of the limbic system of the brain where receptors for glucocorticoid and insulin are rampant [35, 36]. Consistent with previous studies showing elevated HPA axis activity in men and women as well as in rodents our results show that feeding of high-fat diet increases serum corticosterone levels. Elevation of corticosterone can be an index of HPA axis activation [37-39].

Dysregulation of the HPA axis may be an example of how copious amount of high-fat food will result in disruption of homeostasis. These results point to an altered basal plasma corticosterone concentration which produces a metabolic disturbance due to a high-fat diet [40]. The results of another study indicated that a diet low in carbohydrates may induce stress-like effects such as elevated cortisol levels [41].

A study suggest that a high-fat diet could trigger the foundation of metabolic diseases by constant initiation of the stress axis, therefore agitating the generation and secretion of associated hormones [42]. More evidence shows that hippocampus deterioration, polyphagia and adipose tissue proliferation is caused by elevated blood corticosterone, which can be correlated to HPA alteration caused by a high-fat diet [43]. In a genetic study, it was announced that numerous pituitary hormone axes alteration was initiated by a high-fat diet. This study also specified that a high-fat diet readily increases ACTH precursor (POMC) gene transcriptions and as a result, promptly augments the secretion of corticosterone manifolds [44].

Catabolism of sucrose in mammals generates two types of simple sugar which is glucose and fructose [45]. Castrejón-Téllez et al. reported that the exposure of young rats to high-sucrose feed for 28 days incites high blood pressure, and a chronic consumption can activate signs and symptoms of metabolic syndrome [46]. When consumed in surplus, sucrose may be responsible to the establishment of metabolic syndrome, not to mention heightened chances to acquire diabetes mellitus, insulin resistance, adiposity and obesity in children and adults [47].

Keeping in mind the relationship of corticosterone and inflammation, a recent study regarding dietary sucrose reveals a high-sucrose diet was the cause of a low-grade central and systemic inflammation. These inflammations were characterized by a significant increase in circulating white blood cells, even in rats which were not obese [48]. Particularly consumed in the west, high-fructose corn syrup (HFCS), a sweetener made from processed corn starch, is produced to resemble the original proportion of fructose and glucose, which are the monosaccharide building blocks of sucrose [49]. The current scientific community hypothesize the reasoning behind metabolic syndrome is due to high-fructose corn syrup and sucrose consumption, as their usage increased exponentially in the last couple of decades [50]. A study even put forward the idea that fructose induces psychological stress via inflammatory activities [51].

Hypothalamus AMP-activated protein kinase (AMPK) activation stimulated by fructose molecules increases gluconeogenesis in the liver by elevating the level of blood corticosterone, exacerbating insulin resistance in general [52]. Adjustments to glucocorticoid signalling by a high- fructose-diet is proposed take place in the hypothalamus at the central level and inside the adipose tissue at the local tissue level [53]. Cells react to high fructose influx by metabolizing them rapidly and this causes inflammation. Cortisol production is stimulated in response to the inflammation.

Moreover, increased cortisol production was observed when fructose reaches the brain across the blood-brain barrier [54]. A recent study even suggested that a centrally controlled stress response increases cortisol level when high concentration of fructose was able to cross the blood-brain barrier [55]. A finding suggests that a high-sugar diet could cause oxidative damage to the hypothalamus, and this might influence the regulation of HPA axis [56]. A radical change in the hypothalamic mRNA transcripts, as well as the HPA axis operation was reported to take place in male rats fed with a high fructose diet [57]. Constant elevated cortisol levels in low protein diets may be explained by the physiological need to bolster protein catabolism for tissue normal function and repair.

Protein ingestion was reported to significantly lower cortisol blood levels [58]. Protein-rich foods is abundant in phospholipids called phosphatidylserine, which was demonstrated to decrease cortisol production and secretion [59]. A recent randomized, double-blinded study pointed out that amino acids such as arginine and valine could decrease both ACTH and cortisol blood levels by preserving a stable blood glucose level thus withholding glucocorticoid production [60, 61].

Another amino acid called tryptophan was reported to decrease cortisol level and alter mood for the better during stressful conditions [62]. Whey protein contains 17% α-Lactalbumin which is a natural reservoir of opioid peptides [63]. These peptides increase tryptophan level which was revealed to reduce basal glucocorticoid plasma concentrations [64, 65]. Natural source of opioid peptides includes animal products (milk and cheese) and proteins originating from plants such as barley, wheat, and soy [66]. α-Lactalbumin was also reported to enhance general mood condition and drops cortisol blood level in post-stress experiments [67].

One more protein which was noted to significantly reduce blood corticosterone level is the bovine serum albumin, another component of whey [68]. Ingestion of whey protein concentrate for 6 continuous weeks was proven to decrease blood cortisol level following a resistance training session by trained young males [69]. When comparing between plant based and animal-based protein, it was reported that secretion of post-exercise cortisol levels was attenuated better by whey protein [70]. Consuming whey protein for 14 days

suggested that whey was able to decrease cortisol and insulin level in women suffering from gestational diabetes and stress [71].

No wonder whey protein utilisation is currently viewed by most researchers today to have a gaining advantage in regulating or improving some parameters of metabolic syndrome [72]. Cortisol plasma concentrations were found to be significantly lowered in research done on pigs which consumed whey protein concentrate for 19 days [73]. Our result in this current study concurs with the scientific literature in showing a high-protein diet consisting of whey tend to mitigate corticosterone blood levels.

A study has established that a low carbohydrate, ketosis-inducing diet boosts HPA axis activity significantly resulting in an elevated blood ACTH and glucocorticoid levels. At the same time, the study also reiterated that plasma glucocorticoids levels in animals and human was observed to be diminished when consuming food high in dietary carbohydrates [74]. Starch is a type of polysaccharide which acts as storage carbohydrate in plants and is a main source of energy in the human diet throughout history [75]. A randomized controlled trial proclaimed that cortisol blood concentration caused by psychological mental stress was lowered when a whole food, high carbohydrate consumption was boosted. Also, elevated serotogenic action in the brain was thought to be the reason behind the effects of starchy whole food diet in attenuating stress and blood cortisol levels by increasing the negative feedback in the HPA axis activity [76]. In a recent study, low carbohydrate diets were reported to stimulate cortisol release in athletes and lead to a higher blood cortisol level when training [77].

A clinical study involving 16 men getting involved in stressful physical and mental stress suggested that blood cortisol would be decreased if carbohydrate rich food was consumed just before the stress-trigger [78]. Another study was done on healthy adults for 18 days, comparing the effects between a diet consisting of a high-starch, whole food source and another diet containing high-fructose corn syrup. It was observed that the diet containing high-fructose syrup increased cortisol levels compared to the whole food diet [79]. Another clinical study reported that a low fat, high-carbohydrate diet consumed by strength-training men for 12 weeks significantly decreased cortisol blood levels [80]. As starch is broken down to maltose, and maltose is a disaccharide formed from two units of glucose, this could explain the relatively low levels of ACTH and corticosterone reflected in our results as the digestion and metabolism of starch is a slow and complex process in addition to requiring numerous steps thus producing a more nuanced HPA axis and glycaemic response [81].

5. Conclusion

Consumption of high-fat and high-sugar diet for eight weeks is suggested to induce physiologic and metabolic stress as evidenced by the HPLC-PDA analysis of ACTH and corticosterone blood level. Further investigations of cells or organelles at a cellular or sub-cellular level should be fulfilled to elucidate any anatomical and morphological correlation. A longer or shorter period of similar experiments is best done to verify the metabolic effects of these diets.

6. Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available due to privacy and ethical considerations but are available from the corresponding author upon reasonable request.

7. Ethical Statement

All animal procedures were carried out in accordance with ethical guidelines and were approved by the Institutional Animal Care and Use Committee, Faculty of Medicine, University of Malaya (Ref: 2019-21114/UNIKL/R/KAMJ).

8. Author Contributions

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10. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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