

# Diets that have Potential to Stimulate the Hypothalamic-Pituitary-Adrenal (HPA) Axis in Sprague Dawley Rats

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## Abstract.

**Introduction:** The pituitary gland is the master gland which regulates the body hormones. From the corticotrophs of the anterior pituitary gland, adrenocorticotrophic hormone (ACTH) is secreted and influences corticosterone production in rodents via the hypothalamic-pituitary-adrenal axis (HPA axis). ACTH and corticosterone are frequently used as a hormonal marker of stress level. Disturbance in ACTH and corticosterone were observed in many diseases especially Addison's disease, Cushing's syndrome, and metabolic syndrome. **Objective:** Our objective is to observe which diet produces the most and least stress to the Sprague Dawley rat's physiology. **Methods:** Thirty-five Sprague Dawley rats were grouped into five groups (n=7) and were given five different types of diets (control, high-fat, high-protein, high-sugar, and high-starch) with tap water supplied as drinking water ad libitum. By the end of eight weeks, the rat's blood was collected, and serum separated. The individual components of ACTH and corticosterone from the blood was extracted, purified, identified, and quantified using the High-Performance Liquid Chromatography (HPLC) with photodiode array (PDA) analysis method. **Results:** The analytical studies revealed a high -sugar diet registered highest ACTH blood level. It was also revealed that the high-fat and high-sugar diet group presented with the highest peak for corticosterone. **Conclusion:** Consumption of high-fat and high-sugar diet for eight weeks is suggested to induce physiologic and metabolic stress in Sprague Dawley rats as evidenced by the HPA activation.

**Keywords:** Adrenocorticotrophic Hormone (ACTH); Corticosterone; Diet; High-Sugar Diet; High-Fat Diet; High-Performance Liquid Chromatography (HPLC); Hypothalamic-Pituitary-Adrenal (HPA) Axis

Received: March 28, 2023 | Revised: May 3, 2023 | Accepted: May 12, 2023

## 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).<sup>1</sup> ACTH is polypeptide hormone

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cleaved from the precursor proopiomelanocortin (POMC) protein inside corticotrophs and are released under the influence of hypothalamus.<sup>2</sup> 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.<sup>3</sup>

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. ACTH secretion was rapidly inhibited afterwards, and the site of inhibition was found to be in the anterior pituitary.<sup>4</sup> 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.<sup>5</sup> The rapid nature of negative feedback on ACTH secretion agrees with a ligand-dependent nongenomic GR-mediated negative feedback process in the anterior pituitary.<sup>[6]</sup> 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.<sup>7</sup>

Cortisol is the standard glucocorticoids found in mammals, including humans, while corticosterone is found in rodents and reptiles. Mice and rats are unable to synthesise cortisol due to the lack of enzyme 17- $\alpha$  hydroxylase (CYP17) in their adrenal cortex cells.<sup>8</sup> 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.<sup>9</sup> Corticosterone is usually used in physiological and metabolic studies to detect and measure stress.<sup>10</sup> 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.<sup>11</sup> Overtime, high stress levels provoke the HPA axis to produce more corticosterone which can bring disruption to important organs.<sup>12</sup> 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.<sup>13</sup> On the other hand, supplementation of omega-3 polyunsaturated fatty acid to mice revealed a decreased level of HPA axis action.<sup>14</sup> Also, there is early evidence that a probiotics supplementation can improve stress hormone levels.<sup>15</sup> Also, addition of plant phytonutrients to a diet was reported to produce a significant markdown in cortisol blood concentrations.<sup>16</sup>

Chronic and poor diet quality can be a powerful stressor and can implicate major health problems especially when coupled with other unhealthy lifestyle choices.<sup>17</sup> 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.<sup>18</sup> There is early evidence showing that an unbalanced diet can act as a stressor by enhancing the HPA axis to produce more glucocorticoids.<sup>19</sup> 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.<sup>20</sup>

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.<sup>21</sup> 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.<sup>22</sup>

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.<sup>23</sup> 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.<sup>24</sup> 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.<sup>25</sup> HPLC is proven to have the advantageous edge in molecular analysis as it is the widely favoured and preferred applied technique for quantification.<sup>26</sup>

## 2. Methods

Thirty-five, 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).<sup>27</sup> 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

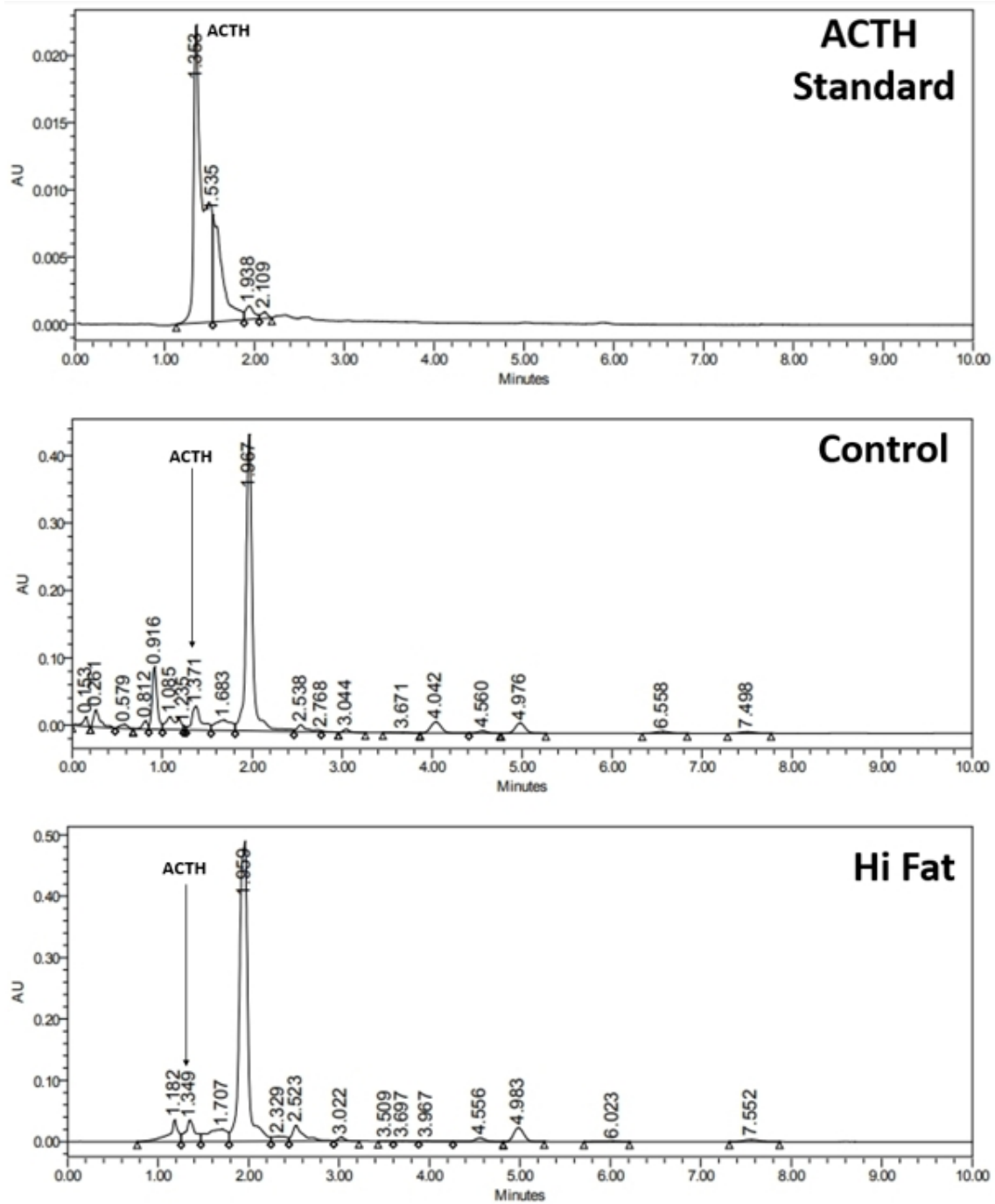
In a 100 ml beaker, 40 ml of 356 g/L MeOH (25%) was mixed well with 10 ml of 89 g/L ZnSO<sub>4</sub> and then 900 µL of the mixture was removed into 1.5 ml centrifuge tube before 450 µL sample (blood serum) was added into the same tube. The sample was vortexed to mix and centrifuged at 3250 rpm for 10 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 (H<sub>3</sub>PO<sub>4</sub>). 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.

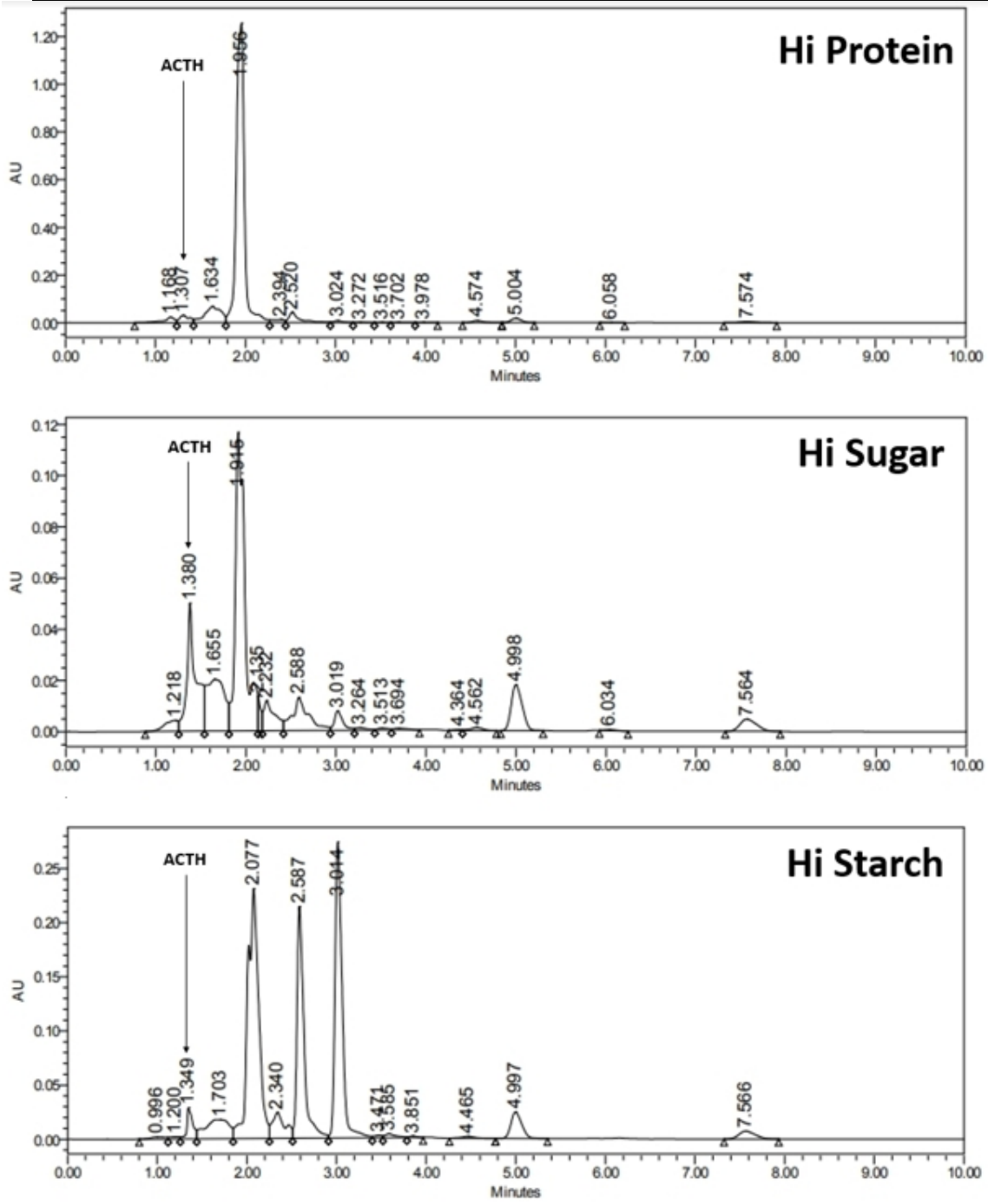
The hormones were analysed by using a HPLC machine. The HPLC machine (Waters) work with two mobile phase solvents (1 litre pH<sub>2</sub>O and 500 mL of ACN), 1 purge solvent (250 mL ACN; 250ML MeOH; 500 mL pH<sub>2</sub>O) and 1 washing solvent (500 mL of 10% ACN). The sample run was programmed via 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.<sup>28</sup>

### 3. Results

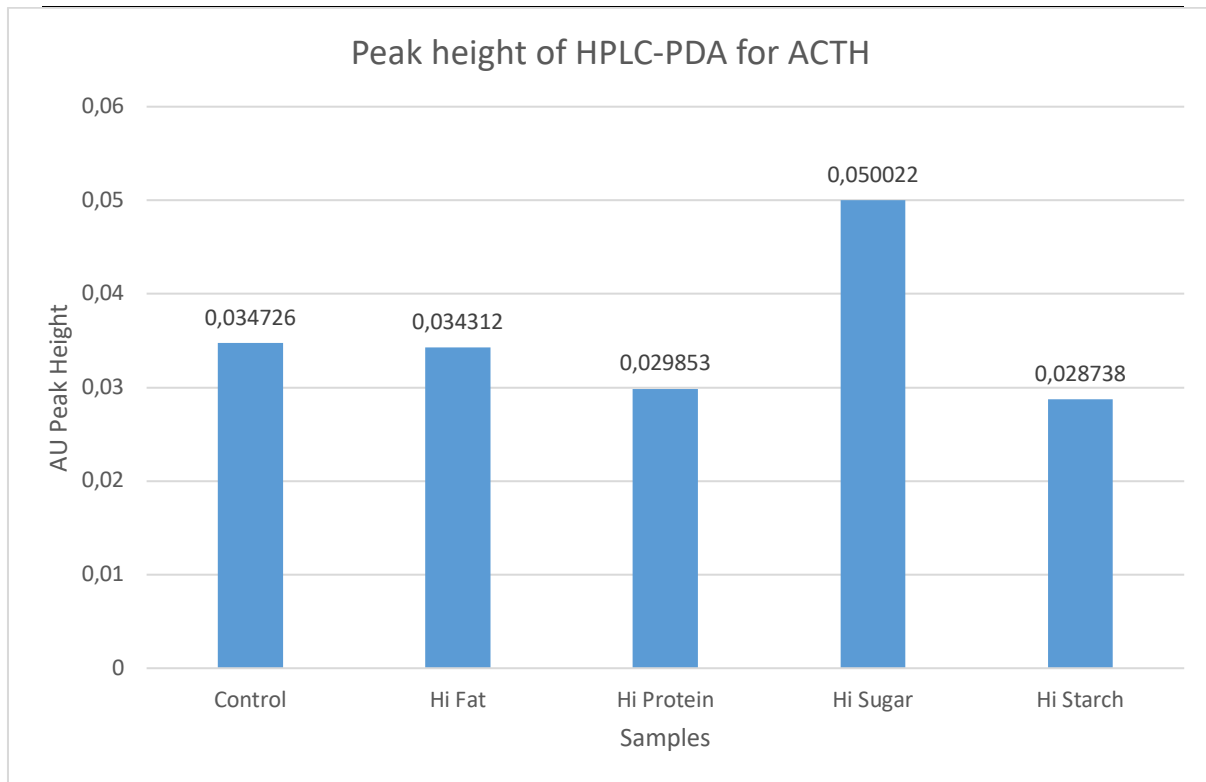
#### HPLC analysis for ACTH



**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

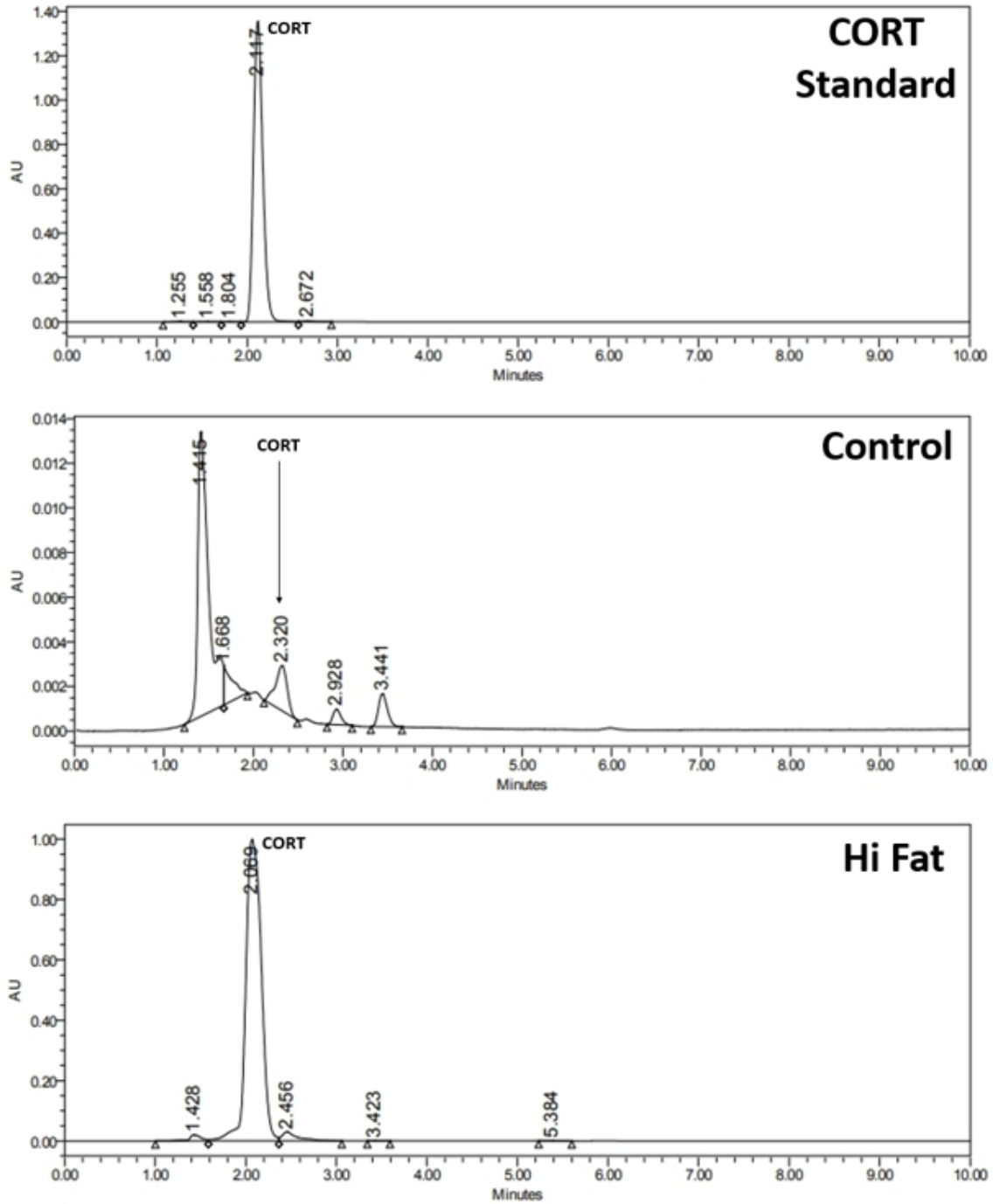
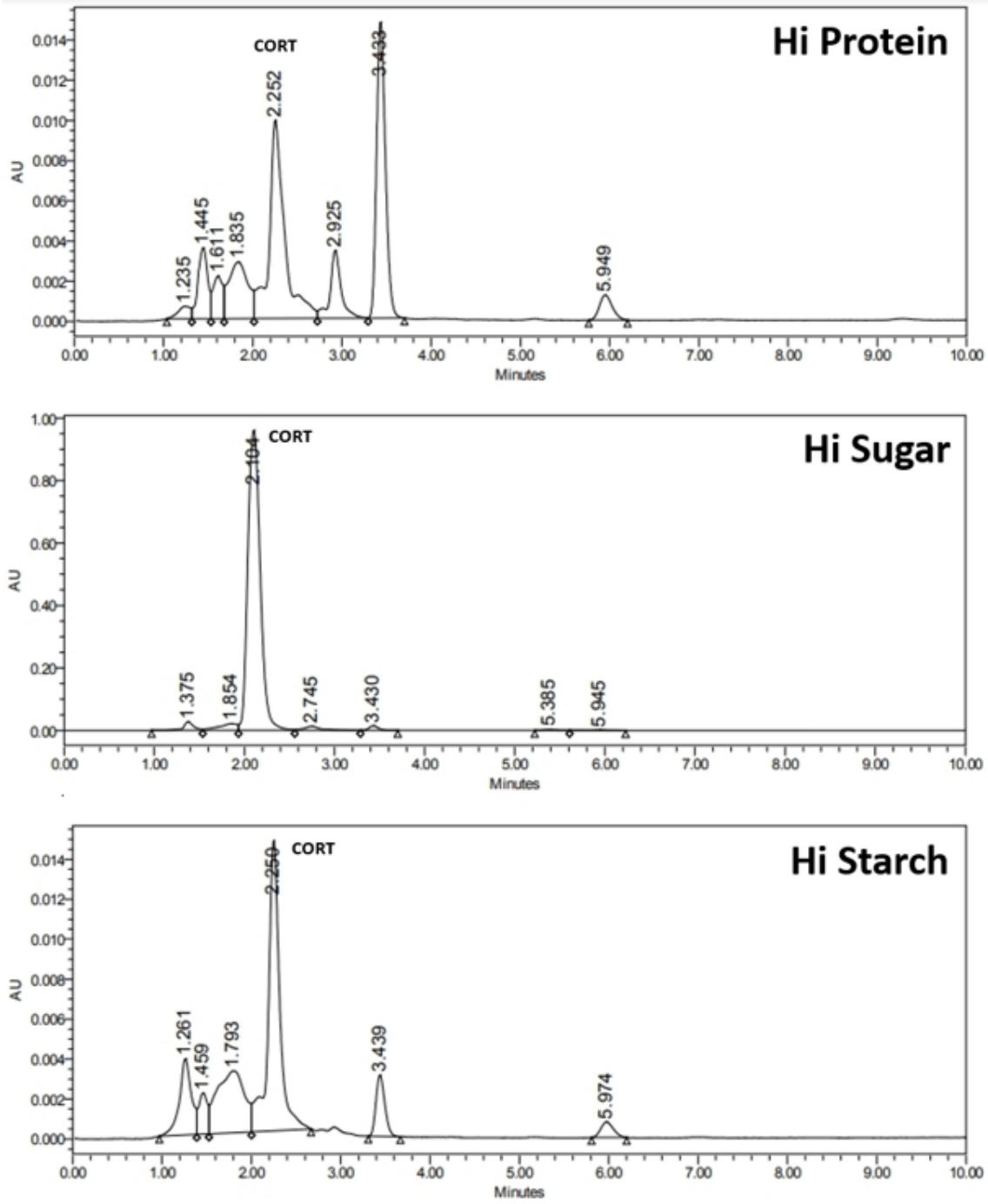
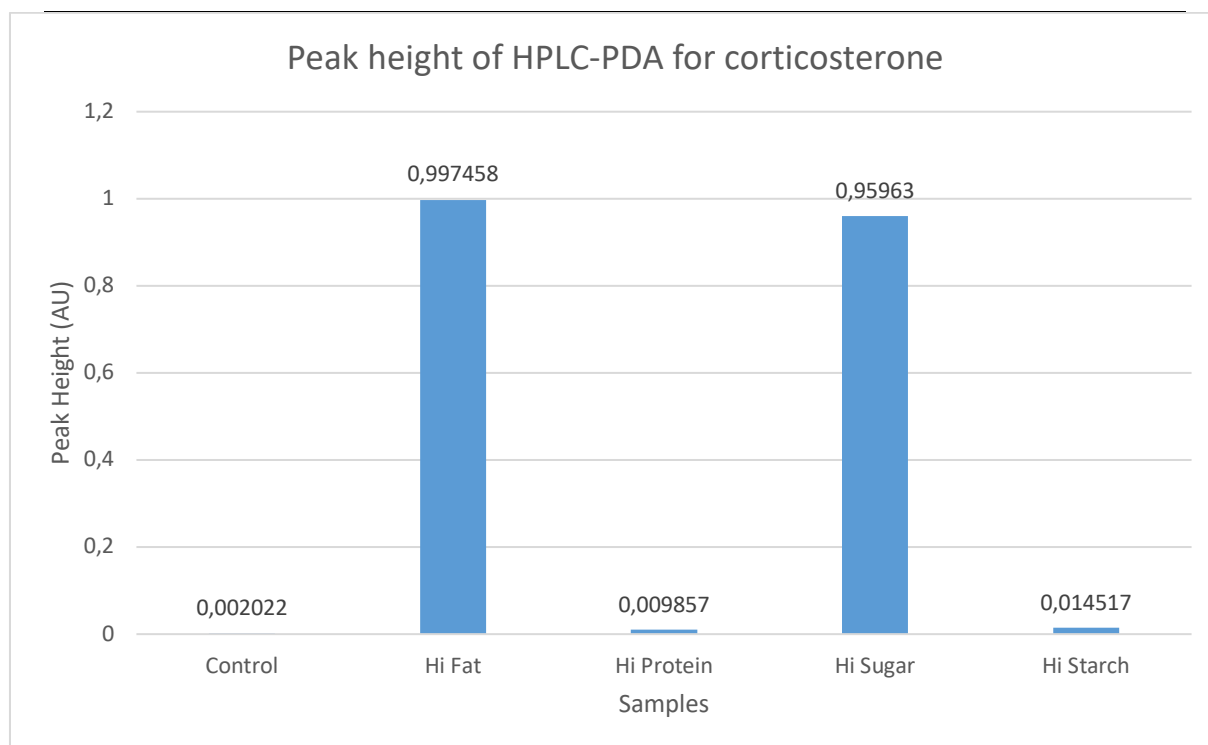


Figure 4. HPLC chromatograms of corticosterone standards, corticosterone in control and high-fat blood extraction samples.



**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.<sup>29</sup> 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.<sup>30</sup> 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.<sup>31</sup>

High-fat diet was found to reduce POMC expression and disturb insulin signalling pathway in the hypothalamus resulting in metabolic disorders via neuroinflammatory activity.<sup>32</sup> Glucocorticoids inhibiting the HPA axis is related to the hypothalamus corticotrophin-releasing-hormone (CRH) regulation and restriction of POMC expression and ACTH production.<sup>33</sup> 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.<sup>34</sup> 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.<sup>35,36</sup> 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.<sup>37,39</sup> 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.<sup>40</sup> The results of another study indicated that a diet low in carbohydrates may induce stress-like effects such as elevated cortisol levels.<sup>41</sup>

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.<sup>42</sup> 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.<sup>43</sup> 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.<sup>44</sup>

Catabolism of sucrose in mammals generates two types of simple sugar which is glucose and fructose.<sup>45</sup> 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.<sup>46</sup> 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.<sup>47</sup>

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.<sup>48</sup>

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.<sup>49</sup> 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.<sup>50</sup> A study even put forward the idea that fructose induces psychological stress via inflammatory activities.<sup>51</sup>

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.<sup>52</sup> 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.<sup>53</sup> 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.<sup>54</sup> 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.<sup>55</sup> A finding suggests that a high-sugar diet could cause oxidative damage to the hypothalamus, and this might influence the regulation of HPA axis.<sup>56</sup> 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.<sup>57</sup> 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.<sup>58</sup> Protein-rich foods is abundant in phospholipids called phosphatidylserine, which was demonstrated to decrease cortisol production and secretion.<sup>59</sup> 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.<sup>60,61</sup>

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

One more protein which was noted to significantly reduce blood corticosterone level is the bovine serum albumin, another component of whey.<sup>68</sup> 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.<sup>69</sup> 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.<sup>70</sup> 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.<sup>71</sup>

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.<sup>72</sup> Cortisol plasma concentrations were found to be significantly lowered in research done on pigs which consumed whey protein concentrate for 19 days.<sup>73</sup> 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.<sup>74</sup> 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.<sup>75</sup> 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.<sup>76</sup> 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.<sup>77</sup>

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.<sup>78</sup> 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.<sup>79</sup> Another clinical study reported that a low fat, high-carbohydrate diet consumed by strength-training men for 12 weeks significantly decreased cortisol blood levels.<sup>80</sup> 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.<sup>81</sup>

## 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.

**Ethics approval:** Sumatera Medical Journal (SUMEJ) is a peer-reviewed electronic international journal. This statement below clarifies ethical behavior of all parties involved in the act of publishing an article in Sumatera Medical Journal (SUMEJ), including the authors, the chief editor, the Editorial Board, the peer-reviewer and the publisher (TALENTA Publisher Universitas Sumatera Utara). This statement is based on COPE's Best Practice Guidelines for Journal Editors.

**Authors contributions:** The authors would like to extend their whole-hearted appreciation to the Ministry of Higher Education (MOHE) Malaysia. Also, a warm thank you to Amal Hayati of UniKL-RCMP for assisting us with the HPLC procedures.

**Funding:** Funds via the Fundamental Research Grant Scheme (FRGS) - grant number (FRGS/1/2018/SKK08/UNIKL/03/1) from Ministry of Higher Education (MOHE) Malaysia.

**Disclosure:** Authors declares no conflict of interest.

## References

- [1] Nakakura T, Nemoto T, Suzuki T, Asano-Hoshino A, Tanaka H, Arisawa K, et al. Adrenalectomy facilitates ATAT1 expression and  $\alpha$ -tubulin acetylation in ACTH-producing corticotrophs. *Cell Tissue Res* 2016;366(2):363-70.
- [2] Yang Y, Harmon CM. Molecular determinants of ACTH receptor for ligand selectivity. *Mol Cell Endocrinol* 2020;503:110688.
- [3] Clark AJL, Chan L. Stability and Turnover of the ACTH Receptor Complex. *Front Endocrinol (Lausanne)* 2019;10:491.
- [4] Lightman SL, Birnie MT, Conway-Campbell BL. Dynamics of ACTH and Cortisol Secretion and Implications for Disease. *Endocrine Reviews* 2020;41(3):bnaa002.
- [5] Deng Q, Riquelme D, Trinh L, Low MJ, Tomić M, Stojilkovic S, Aguilera G. Rapid Glucocorticoid Feedback Inhibition of ACTH Secretion Involves Ligand-Dependent Membrane Association of Glucocorticoid Receptors. *Endocrinology* 2015;156(9):3215–27.
- [6] Dunlavy CJ. Introduction to the Hypothalamic-Pituitary-Adrenal Axis: Healthy and Dysregulated Stress Responses, Developmental Stress and Neurodegeneration. *J Undergrad Neurosci Educ* 2018;16(2):59–60.
- [7] Allen MJ, Sharma S. Physiology, Adrenocorticotrophic Hormone (ACTH). 2022 August 15. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023.
- [8] Aragao-Santiago L, Gomez-Sanchez CE, Mulatero P, Spyroglou A, Reincke M, Williams TA. Mouse Models of Primary Aldosteronism: From Physiology to Pathophysiology. *Endocrinology* 2017;158(12):4129-38.
- [9] Jimeno B, Hau M, Verhulst S. Corticosterone levels reflect variation in metabolic rate, independent of 'stress'. *Scientific Reports* 2018;8(1):13020.
- [10] Neuman-Lee LA, Hudson SB, Webb AC, French SS. Investigating the relationship between corticosterone and glucose in a reptile. *Journal of Experimental Biology* 2020;223(Pt 2):jeb203885.
- [11] Jiang Y, Botchway BOA, Hu Z, Fang M. Overexpression of SIRT1 Inhibits Corticosterone-Induced Autophagy. *Neuroscience* 2019;411:11–22.
- [12] Kinlein SA, Phillips DJ, Keller CR, Karatsoreos IN. Role of corticosterone in altered neurobehavioral responses to acute stress in a model of compromised hypothalamic-pituitary-adrenal axis function. *Psychoneuroendocrinology* 2019;102:248–55.
- [13] Lam VYY, Raineki C, Wang LY, Chiu M, Lee G, Ellis L, et al. Role of corticosterone in anxiety and depressive-like behavior and HPA regulation following prenatal alcohol exposure. *Prog Neuropsychopharmacol Biol Psychiatry* 2019;90:1–15.
- [14] Robertson RC, Seira Oriach C, Murphy K, Moloney GM, Cryan JF, Dinan TG, et al. Omega-3 polyunsaturated fatty acids critically regulate behaviour and gut microbiota development in adolescence and adulthood. *Brain Behav Immun* 2017;59:21–37.
- [15] Foroozan P, Koushkie Jahromi M, Nemati J, Sepehri H, Safari MA, Brand S. Probiotic Supplementation and High-Intensity Interval Training Modify Anxiety-Like Behaviors and Corticosterone in High-Fat Diet-Induced Obesity Mice. *Nutrients* 2021;13(6):1762.

- [16] Serradell A, Torrecillas S, Makol A, Valdenegro V, Fernández-Montero A, Acosta F, et al. Prebiotics and phytochemicals functional additives in low fish meal and fish oil based diets for European sea bass (*Dicentrarchus labrax*): Effects on stress and immune responses. *Fish Shellfish Immunol* 2020;100:219–29.
- [17] Lechner K, von Schacky C, McKenzie AL, Worm N, Nixdorff U, Lechner B, Kränkel N, Halle M, Krauss RM, Scherr J. Lifestyle factors and high-risk atherosclerosis: Pathways and mechanisms beyond traditional risk factors. *Eur J Prev Cardiol* 2020;27(4):394–406.
- [18] St-Onge MP, Mikic A, Pietrolungo CE. Effects of Diet on Sleep Quality. *Adv Nutr* 2016;7(5):938–49.
- [19] Shin AC, Balasubramanian P, Suryadevara P, Zyskowski J, Herdt TH, MohanKumar SMJ, MohanKumar PS. Metformin effectively restores the HPA axis function in diet-induced obese rats. *Int J Obes (Lond)* 2021(2):383–395.
- [20] Carreiro AL, Dhillion J, Gordon S, Higgins KA, Jacobs AG, McArthur BM, Redan BW, Rivera RL, Schmidt LR, Mattes RD. The Macronutrients, Appetite, and Energy Intake. *Annu Rev Nutr* 2016;36:73–103.
- [21] Leroy F, Cofnas N. Should dietary guidelines recommend low red meat intake? *Crit Rev Food Sci Nutr* 2020;60(16):2763–72.
- [22] Biobaku F, Ghanim H, Batra M, Dandona P. Macronutrient-Mediated Inflammation and Oxidative Stress: Relevance to Insulin Resistance, Obesity, and Atherogenesis. *J Clin Endocrinol Metab* 2019;104(12):6118–28.
- [23] Grosser K, van Dam NM. A Straightforward Method for Glucosinolate Extraction and Analysis with High-pressure Liquid Chromatography (HPLC). *J Vis Exp* 2017;121:55425.
- [24] Nishizaki Y, Sato-Masumoto N, Yokota A, Mikawa T, Nakashima K, Yamazaki T, Kuroe M, Numata M, Ihara T, Ito Y, Sugimoto N, Sato K. HPLC/PDA determination of carminic acid and 4-aminocarminic acid using relative molar sensitivities with respect to caffeine. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess.* 2018;35(5):838–47.
- [25] Kumar S, Singh R, Gajbhiye N, Dhanani T. Extraction Optimization for Phenolic- and Withanolide-Rich Fractions from *Withania somnifera* Roots: Identification and Quantification of Withaferin A, 12-Deoxywithastromonolide, and Withanolide A in Plant Materials and Marketed Formulations Using a Reversed-Phase HPLC-Photodiode Array Detection Method. *J AOAC Int* 2018;101(6):1773–180.
- [26] Liu J, Hefni ME, Witthöft CM. Characterization of Flavonoid Compounds in Common Swedish Berry Species. *Foods* 2020;9(3):358.
- [27] Azwan K, Mona R, Firdous J, David PR, Muhamad N. A Whey-Based, High-Protein Diet Promotes the Best Body Weight and Blood Sugar Control When Compared with Other Types of Diet in Male Sprague Dawley Rats. *RSU International Research Conference 2021 on Science and Technology*, 68–74. <https://rsucon.rsu.ac.th/proceeding/article/2765>
- [28] Viljoen FP, Brand L, Smit EJ. An optimized method for the analysis of corticosterone in rat plasma by UV-HPLC. *Medical Technology SA* 2012;26(2):39–42. <https://repository.nwu.ac.za/handle/10394/14872>
- [29] Tan WS, Tan SY, Henry CJ. Ethnic Variability in Glycemic Response to Sucrose and Isomaltulose. *Nutrients* 2017;9(4):347.
- [30] Mercau ME, Repetto EM, Perez MN, Martinez Calejman C, Sanchez Puch S, Finkielstein CV, et al. Moderate Exercise Prevents Functional Remodeling of the Anterior Pituitary Gland in Diet-Induced Insulin Resistance in Rats: Role of Oxidative Stress and Autophagy. *Endocrinology* 2016;157(3):1135–45.
- [31] Mercau ME, Calanni JS, Aranda ML, Caldarelli LJ, Rosenstein RE, Repetto EM, Cymeryng CB. Melatonin prevents early pituitary dysfunction induced by sucrose-rich diets. *J Pineal Res* 2019;66(2):e12545. doi: 10.1111/jpi.12545. Epub 2019 Jan 21. PMID: 30586198.
- [32] Qian Y, Lei G, Wen L. Brain-specific deletion of TRIM13 promotes metabolic stress-triggered insulin resistance, glucose intolerance, and neuroinflammation. *Biochem Biophys Res Commun* 2020;527(1):138–145.
- [33] Deng Q, Riquelme D, Trinh L, Low MJ, Tomić M, Stojilkovic S, et al. Rapid Glucocorticoid Feedback Inhibition of ACTH Secretion Involves Ligand-Dependent Membrane Association of Glucocorticoid Receptors. *Endocrinology*. 2015 Sep;156(9):3215–27. doi: 10.1210/EN.2015-1265. Epub 2015 Jun 29. PMID: 26121342; PMCID: PMC4541620.

- [34] Harno E, Gali Ramamoorthy T, Coll AP, White A. POMC: The Physiological Power of Hormone Processing. *Physiol Rev* 2018;98(4):2381–2430.
- [35] Tsai SF, Wu HT, Chen PC, Chen YW, Yu M, Tzeng SF, et al. Stress Aggravates High-Fat-Diet-Induced Insulin Resistance via a Mechanism That Involves the Amygdala and Is Associated with Changes in Neuroplasticity. *Neuroendocrinology* 2018;107(2):147–157.
- [36] Lizarbe B, Soares AF, Larsson S, Duarte JMN. Neurochemical Modifications in the Hippocampus, Cortex and Hypothalamus of Mice Exposed to Long-Term High-Fat Diet. *Front Neurosci* 2019;12:985.
- [37] Hryhorczuk C, Décarie-Spain L, Sharma S, Daneault C, Rosiers CD, Alquier T, Fulton S. Saturated high-fat feeding independent of obesity alters hypothalamus-pituitary-adrenal axis function but not anxiety-like behaviour. *Psychoneuroendocrinology* 2017;83:142–149.
- [38] Shin AC, MohanKumar SMJ, Balasubramanian P, Sirivelu MP, Linning K, Woolcock A, et al. Responsiveness of hypothalamo-pituitary-adrenal axis to leptin is impaired in diet-induced obese rats. *Nutr Diabetes* 2019;9(1):10.
- [39] Boersma GJ, Tamashiro KL, Moran TH, Liang NC. Corticosterone administration in drinking water decreases high-fat diet intake but not preference in male rats. *Am J Physiol Regul Integr Comp Physiol* 2016;310(8):R733-43.
- [40] Shen Y, Huang G, McCormick BP, Song T, Xu X. Effects of high-intensity interval versus mild-intensity endurance training on metabolic phenotype and corticosterone response in rats fed a high-fat or control diet. *PLoS One* 2017;12(7):e0181684.
- [41] Soltani H, Keim NL, Laugero KD. Increasing Dietary Carbohydrate as Part of a Healthy Whole Food Diet Intervention Dampens Eight Week Changes in Salivary Cortisol and Cortisol Responsiveness. *Nutrients* 2019;11(11):2563.
- [42] Zhou X, Fouda S, Li D, Zhang K, Ye JM. Involvement of the Autophagy-ER Stress Axis in High Fat/Carbohydrate Diet-Induced Nonalcoholic Fatty Liver Disease. *Nutrients* 2020;12(9):2626.
- [43] Tomiyama AJ. Stress and Obesity. *Annu Rev Psychol* 2019:703–18.
- [44] Shaikh SR, Shaver PR, Shewchuk BM. High Fat Diet Dysregulates Hypothalamic-Pituitary Axis Gene Expression Levels which are Differentially Rescued by EPA and DHA Ethyl Esters. *Mol Nutr Food Res* 2018;62(13):e1800219.
- [45] Kendig MD, Martire SI, Boakes RA, Rooney KB. Comparable metabolic effects of isocaloric sucrose and glucose solutions in rats. *Physiol Behav* 2021;229:113239.
- [46] Castrejón-Téllez V, Villegas-Romero M, Rubio-Ruiz ME, Pérez-Torres I, Carreón-Torres E, Díaz-Díaz E, et al. Effect of a Resveratrol/Quercetin Mixture on the Reversion of Hypertension Induced by a Short-Term Exposure to High Sucrose Levels Near Weaning and a Long-Term Exposure That Leads to Metabolic Syndrome in Rats. *Int J Mol Sci* 2020;21(6):2231.
- [47] Rippe JM, Angelopoulos TJ. Sugars, obesity, and cardiovascular disease: results from recent randomized control trials. *Eur J Nutr* 2016;55(Suppl 2):45-53.
- [48] Patkar OL, Mohamed AZ, Narayanan A, Mardon K, Cowin G, Bhalla R, Stimson DHR, Kassiou M, Beecher K, Belmer A, Alvarez Cooper I, Morgan M, Hume DA, Irvine KM, Bartlett SE, Nasrallah F, Cumming P. A binge high sucrose diet provokes systemic and cerebral inflammation in rats without inducing obesity. *Sci Rep.* 2021 May 27;11(1):11252.
- [49] Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C, et al. Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. *J Hepatol* 2018;68(5):1063–75.
- [50] Pereira RM, Botezelli JD, da Cruz Rodrigues KC, Mekary RA, Cintra DE, Pauli JR, et al. Fructose Consumption in the Development of Obesity and the Effects of Different Protocols of Physical Exercise on the Hepatic Metabolism. *Nutrients* 2017;9(4):405.
- [51] de Sousa Rodrigues ME, Bekhbat M, Houser MC, Chang J, Walker DI, Jones DP, Oller do Nascimento CMP, Barnum CJ, Tansey MG. Chronic psychological stress and high-fat high-fructose diet disrupt metabolic and inflammatory gene networks in the brain, liver, and gut and promote behavioral deficits in mice. *Brain Behav Immun* 2017;59:158-172.
- [52] Woods A, Williams JR, Muckett PJ, Mayer FV, Liljevald M, Bohlooly-Y M, Carling D. Liver-Specific Activation of AMPK Prevents Steatosis on a High-Fructose Diet. *Cell Rep* 2017;18(13):3043–51.

- [53] Teofilović A, Brkljačić J, Djordjevic A, VojnovićMilutinović D, Tappy L, Matić G, et al. Impact of insulin and glucocorticoid signalling on hepatic glucose homeostasis in the rat exposed to high-fructose diet and chronic stress. *Int J Food Sci Nutr* 2020;71(7):815-825.
- [54] DiNicolantonio JJ, Mehta V, Onkaramurthy N, O'Keefe JH. Fructose-induced inflammation and increased cortisol: A new mechanism for how sugar induces visceral adiposity. *Prog Cardiovasc Dis* 2018;61(1):3-9.
- [55] Yu R, Wen S, Wang Q, Wang C, Zhang L, Wu X, et al. Mulberroside A repairs high fructose diet-induced damage of intestinal epithelial and blood-brain barriers in mice: A potential for preventing hippocampal neuroinflammatory injury. *J Neurochem* 2021;157(6):1979-91.
- [56] Żebrowska E, Chabowski A, Zalewska A, Maciejczyk M. High-Sugar Diet Disrupts Hypothalamic but Not Cerebral Cortex Redox Homeostasis. *Nutrients* 2020;12(10):3181.
- [57] Harrell CS, Gillespie CF, Neigh GN. Energetic stress: The reciprocal relationship between energy availability and the stress response. *Physiol Behav* 2016;166:43-55.
- [58] Drummen M, Tischmann L, Gatta-Cherifi B, Adam T, Westerterp-Plantenga M. Dietary Protein and Energy Balance in Relation to Obesity and Co-morbidities. *Front Endocrinol (Lausanne)* 2018;9:443.
- [59] Stachowicz M, Lebidzińska A. The effect of diet components on the level of cortisol. *European Food Research and Technology* 2016;242:12.
- [60] Tsuda Y, Iwasawa K, Yamaguchi M. Acute supplementation of valine reduces fatigue during swimming exercise in rats. *Biosci Biotechnol Biochem* 2018;82(5):856-61.
- [61] Tsuda Y, Murakami R, Yamaguchi M, Seki T. Acute supplementation with an amino acid mixture suppressed the exercise-induced cortisol response in recreationally active healthy volunteers: a randomized, double-blinded, placebo-controlled crossover study. *J Int Soc Sports Nutr* 2020;17(1):39.
- [62] Gibson EL. Tryptophan supplementation and serotonin function: genetic variations in behavioural effects. *Proc Nutr Soc* 2018;77(2):174-88.
- [63] Layman DK, Lönnerdal B, Fernstrom JD. Applications for  $\alpha$ -lactalbumin in human nutrition. *Nutr Rev* 2018;76(6):444-60.
- [64] Kazimierska K, Kalinowska-Lis U. Milk Proteins-Their Biological Activities and Use in Cosmetics and Dermatology. *Molecules* 2021;26(11):3253.
- [65] Höglund E, Øverli Ø, Winberg S. Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review. *Front Endocrinol (Lausanne)* 2019;10:158.
- [66] Tyagi A, Daliri EB, Kwami Ofofuo F, Yeon SJ, Oh DH. Food-Derived Opioid Peptides in Human Health: A Review. *Int J Mol Sci* 2020;21(22):8825.
- [67] Qin L, Wong SHS, Sun FH, Huang Y, Sheridan S, Sit CHP. Effects of Alpha-Lactalbumin or Whey Protein Isolate on Muscle Damage, Muscle Pain, and Mood States Following Prolonged Strenuous Endurance Exercise. *Front Physiol* 2017;8:754.
- [68] McManus BL, Korpela R, Speakman JR, Cryan JF, Cotter PD, Nilaweera KN. Bovine serum albumin as the dominant form of dietary protein reduces subcutaneous fat mass, plasma leptin and plasma corticosterone in high fat-fed C57/BL6J mice. *Br J Nutr* 2015;114(4):654-62.
- [69] Pourabbas M, Bagheri R, Hooshmand Moghadam B, Willoughby DS, Candow DG, Elliott BT, Forbes SC, Ashtary-Larky D, Eskandari M, Wong A, Dutheil F. Strategic Ingestion of High-Protein Dairy Milk during a Resistance Training Program Increases Lean Mass, Strength, and Power in Trained Young Males. *Nutrients* 2021;13(3):948.
- [70] Kerksick CM, Jagim A, Hagele A, Jäger R. Plant Proteins and Exercise: What Role Can Plant Proteins Have in Promoting Adaptations to Exercise? *Nutrients* 2021;13(6):1962.
- [71] Feng Y, Wang Y, Feng Q, Song X, Wang L, Sun L. Whey protein preloading can alleviate stress adaptation disorder and improve hyperglycemia in women with gestational diabetes mellitus. *Gynecol Endocrinol* 2021;37(8):753-7.
- [72] Sousa RML, Ribeiro NLX, Pinto BAS, Sanches JR, da Silva MU, Coêlho CFF, et al. Long-term high-protein diet intake reverts weight gain and attenuates metabolic dysfunction on high-sucrose-fed adult rats. *Nutr Metab (Lond)* 2018;15:53.
- [73] Nielsen CH, Hui Y, Nguyen DN, Ahnfeldt AM, Burrin DG, Hartmann B, et al. Alpha-Lactalbumin Enriched Whey Protein Concentrate to Improve Gut, Immunity and Brain Development in Preterm Pigs. *Nutrients* 2020;12(1):245.

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- [74] Ryan KK, Packard AEB, Larson KR, Stout J, Fourman SM, Thompson AMK, et al. Dietary Manipulations That Induce Ketosis Activate the HPA Axis in Male Rats and Mice: A Potential Role for Fibroblast Growth Factor-21. *Endocrinology* 2018;159(1):400–13.
- [75] Lovegrove A, Edwards CH, De Noni I, Patel H, El SN, Grassby T, et al. Role of polysaccharides in food, digestion, and health. *Crit Rev Food Sci Nutr* 2017;57(2):237–53.
- [76] Soltani H, Keim NL, Laugero KD. Increasing Dietary Carbohydrate as Part of a Healthy Whole Food Diet Intervention Dampens Eight Week Changes in Salivary Cortisol and Cortisol Responsiveness. *Nutrients* 2019;11(11):2563.
- [77] Terink R, Witkamp RF, Hopman MTE, Siebelink E, Savelkoul HFJ, Mensink M. A 2 Week Cross-over Intervention with a Low Carbohydrate, High Fat Diet Compared to a High Carbohydrate Diet Attenuates Exercise-Induced Cortisol Response, but Not the Reduction of Exercise Capacity, in Recreational Athletes. *Nutrients* 2021;13(1):157.
- [78] McAllister MJ, Webb HE, Tidwell DK, Smith JW, Fountain BJ, Schilling MW, et al. Exogenous Carbohydrate Reduces Cortisol Response from Combined Mental and Physical Stress. *Int J Sports Med* 2016;37(14):1159-1165.
- [79] Ibrahim M, Bonfiglio S, Schlögl M, Vinales KL, Piaggi P, Venti C, et al. Energy Expenditure and Hormone Responses in Humans After Overeating High-Fructose Corn Syrup Versus Whole-Wheat Foods. *Obesity (Silver Spring)* 2018;26(1):141–9.
- [80] Wrzosek M, Woźniak J, Włodarek D. The Combination of a Diversified Intake of Carbohydrates and Fats and Supplementation of Vitamin D in a Diet Does Not Affect the Levels of Hormones (Testosterone, Estradiol, and Cortisol) in Men Practicing Strength Training for the Duration of 12 Weeks. *Int J Environ Res Public Health* 2020;17(21):8057.
- [81] Miao M, Jiang B, Cui SW, Zhang T, Jin Z. Slowly digestible starch--a review. *Crit Rev Food Sci Nutr* 2015;55(12):1642-57.