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Overexpression of Heat Shock Cognate Protein 71 kDa and Pyruvate Dehydrogenase in the Brain Tissue at the Early Stage of High Fat Diet Consumption

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ABSTRACT

High-fat diet (HFD) increases the risk of obese, while obesity increases the prevalence of metabolic syndrome and non-communicable diseases. Therefore, it will be interesting to evaluate the changes in metabolic parameters and brain profile upon the early consumption of HFD. In this study, a total of 12 Sprague-Dawley male rats were divided into two groups (n = 6), each group was fed with normal diet and HFD (39% of total calories from fats), respectively, for 6 weeks consecutively. The body weight, blood glucose, cholesterol and triglyceride values were measured. Furthermore, the brain and visceral adipose tissues were harvested at the end of the experiment. Protein was extracted from the brain tissue, and the protein extracts were separated by using two-dimensional gel electrophoresis and analyzed by liquid chromatography tandem mass spectrometric analysis (LC/MS/MS). In terms of food calorie, the rats fed with HFD consumed more energy than the rats fed with normal diet. Nevertheless, the blood triglyceride and cholesterol, and the visceral adipose tissue of both the HFD and normal diet fed rats were indifferent. At the molecular level, overexpression of stress proteins, namely heat shock cognate protein 71 kDa (Hsc70) and pyruvate dehydrogenase were detected in brain tissue of HFD group. These results suggest that HFD intake causing significant change in brain proteins profile at the early phase of its consumption when no clear metabolic changes were observed. This showed that the brain was affected by HFD.

1. Introduction

The prevalence of obesity and overweight is increasing worldwide. Death associated to obesity accounted for approximately 2.8 million yearly (Roberto *et al.* 2015). Obesity is recognised as a chronic disease that increases the global burden of non-communicable diseases such as cardiovascular disease, diabetes and cancer (Engin 2017).

Body weight will be increased if the energy intake exceeds the energy expenditure over a given period of time (Hill *et al.* 2012). Dietary fat is one of the major factors contributing to energy intake related to obesity, and adiposity increases with increasing fat content up to 60% (Hu *et al.* 2018). The acceptable macronutrient distribution range for total fat is 20–35% of total daily calories, diets that fall beyond this range are considered as HFD (Bothclett and Wu 2018). Donahoo *et al.* (2008) suggested that HFD increases the likelihood of excessive energy intake as fats are stored more efficiently than excess carbohydrates. Chronic HFD consumption could result in weight gain/obesity, increased systemic inflammation, oxidative stress and disruption of glucose homeostasis (Botchlett and Wu 2018b).

The brain is enriched with fat; lipids account for ~50% of brain's dry weight, possibly because brain uses more energy than other organs (Chianese *et al.* 2017). Dietary fats transfer energy to the brain and therefore calories intake has an impact on brain health. Over-consumption of calories could cause the formation of free-radicals that surpass the buffering capacity of cellular anti-oxidant responses, which in turn reduce synaptic plasticity and lead to cell damage (Gomez-Pinilla 2008). Typically, diet contains mixture of different types of fatty acid including saturated and mono/polyunsaturated

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fatty acids. Dietary fats impact brain's processes by regulating the synaptic transmission, membrane fluidity and signal-transduction pathways (Chianese *et al.* 2017; Melo *et al.* 2019).

Saturated fatty acids (SFAs) were the main contributors of obesity (Figueiredo *et al.* 2017). Furthermore, an SFA, namely palmitic acid can induce the activation of Toll-like receptor 4 in hypothalamic microglia of the brain (Valdearcos *et al.* 2014), that potentially lead to brain inflammation. Oil palm based cooking oil used in this study was made up of 100% high grade pure palm olein, consisting of ≈ 40% of palmitic acid and ≈ 40% of oleic acid (Malaysia Palm Oil Board). Animals fed with high saturated fat diet was shown to have higher concentrations of protein amyloid-ß, a marker for Alzheimer's disease (Galloway *et al.* 2007), and compromised blood-brain barrier integrity (Farrall and Wardlaw 2009; Takechi *et al.* 2013).

Diet consisting of HFD is the leading cause of obesity, while obesity is one of the major causes of non-communicable diseases. Although the impact of HFD on health was well documented, the bodily change due to early consumption of HFD is not well reported. Therefore, we aimed to study the impact of HFD on the metabolic parameters and brain protein profile of rats that were fed with HFD for six-week consecutively. The information may be useful for the intervention of obesity-related diseases.

2. Materials and Methods

2.1. Materials

Urea, trizma base, thiourea, CHAPS, acrylamide, *N,N'*-methylenebisacrylamide, sodium dodecyl sulfate (SDS), glycine, sodium chloride, Brilliant Blue R, Bromophenol blue, *N,N,N',N'*-tetramethylethylenediamine (TEMED), DL-dithiothreitol (DTT) and iodoacetamide were obtained from Sigma-Aldrich (Missouri, USA). The diet powder (#1820) was customized from Altromin (Lage, Germany). Sodium phosphate dibasic dihydrate ($Na_2HPO_4{\cdot}2H_2O$), sodium phosphate monobasic dihydrate ($NaH_2PO_4{\cdot}2H_2O$) and acetic acid (glacial 100%) were purchased from Merck (Massachusetts, USA). A few solvents have been used including methanol (analytical grade, R and M chemical), MS grade acetonitrile and MS grade water (Fisher scientific, Hampton USA), and acetone (QRëC, New Zealand). Trichloroacetic acid was procured from Merck (New Jersey, USA). The RC DCTM protein assay kit, protein assay standard I (lyophilized bovine γ-globulin), ampholytes 3/10 and low melting temperature agarose were purchased from BioRad (California, USA). Mineral oil (light, white) and glycerol were obtained from Ameresco (Ohio, USA). The applied anaesthetic agents were ketamine (TROY Laboratories, Glendenning, Australia) and xylazine (Indian Immunilogicals, Jubilee Hills, India). Formic acid was purchased from Fisher scientific (Hampton, USA). Palm olein cooking oil was obtained from Buruh (Lamsoon, Malaysia).

2.2. Procedure for Animal Study

2.2.1. Animals Preparation

A total of 12 Sprague-Dawley male rats, aged 12 weeks and weighed between 280–300 g were obtained from the Animal Research and Service Centre (ARASC), Universiti Sains Malaysia. The procedures of housing and handling used in this study conformed to the animal ethics guidelines of the USM Institutional Animal Care and Use Committee (USM IACUC, USM/Animal Ethics Approval/2016(717)). The rats were acclimatized individually (one rat in a cage) for 1 weeks prior to the experiment under controlled environmental conditions (12 h light/dark cycles, 22-24°C) to allow them habitually familiar to new environment. Finally, only the rats weighed between 300-350 g were used in this study.

2.2.2. Animal Feeding Program

After acclimatization, the rats were divided into two groups, each group consisted 6 animals (n = 6), and one rat is placed in a cage. The rats were further monitored for 4 weeks. After 4 weeks, one group was fed with normal diet food pellets while the other group was fed with HFD. The Calorie for normal diet was 3.61 kcal/g while for HFD, it was 4.56 kcal/g. Both types of food pellet were daily supplied to the rats at 25±1 g weight, together with water (ad libitum) for 6 weeks. Both normal diet and HFD pellets were self-prepared by mixing the custom-made diet powder with water at a ratio of 1:1 and baked in an oven at 42°C, overnight. The HFD pellets were made to contain extra oil by adding palm olein cooking oil at 22.4% (w/v). On each day, the uneaten food pellet that left was weighed and was replaced with fresh food pellets.

2.3. Procedure of Biochemical Analysis

2.3.1. Harvesting of Animal Specimens

At the end of experiment, all rats were euthanized using a cocktail of ketamine (75 mg/kg) and xylazine (8 mg/kg) followed by cardiac puncture using 27G×½" needle to collect blood into clean tubes (MiniCollect®, Greiner Bio-One, Austria). The blood was spun at 3000 × g, 4°C for 10 mins. After the centrifugation, the supernatant (plasma) was immediately transferred into the clean polypropylene tubes and stored at -80°C until further analysis. After cardiac puncture, the brain was harvested and rinsed 3 times with phosphate buffered saline (pH 7.4, 140

mM sodium chloride, 10 mM phosphate buffer and 3 mM potassium chloride). The brain tissue was cut into two parts (left and right brain). The brain tissues were then ground into fine powder forms in liquid nitrogen and stored at -80°C.

2.3.2. Measuring of Blood Biochemical Parameters and Key Health Factors

The body weight and fasting blood glucose (FBG) level of each rat were monitored and recorded weekly. The triglycerides and cholesterol analysis were carried out according to the manufacturer protocol (Thermo scientific, Netherlands). The plasma was thawed to room temperature. A volume of 300 µl InfinityTM Cholesterol Liquid Stable Reagent or InfinityTM Triglycerides Liquid Stable Reagent, respectively was pipetted into 96 well plates. Then, 3 µl of plasma was added to the wells (Sample: Reagent ratio is 1:100). The plate was left at room temperature for 15 minutes and readings was measured using a microplate reader at 500 nm wavelength. Chemistry Calibrator (Pointe Scientific, USA) was used as the calibrators. The values of cholesterol and triglycerides were calculated by comparing the absorbance values to the calibrators absorbance values. The mean values from each treatment groups were presented.

2.4. Procedure for Protein Analysis

2.4.1. 2D-gel Electrophoresis and In-gel Digestion

Prior to 2D gel electrophoresis, the proteins were extracted from ground brain powder, purified using trichloroacetic acid (TCA)/acetone precipitation, then the concentration was measured using the RC DCTM protein assay kit. A 2D gel electrophoresis using IPG strip (pH 4–7) was employed. The IPG strips were rehydrated with 600 µg of protein for at least 15 hours at 20°C before the isoelectric focusing. The PROTEAN®i12™ system (Bio-Rad, USA) was used to perform the focusing at 20°C. The applied voltage was from 0–150 V (rapid climb) for 1 h, 150–200 V (linear, 1 h), 200–500 V (linear, 1 hr), 500–4,000 V (linear, 2 h) and the exposure of 10,000 Vhr. The focused IPG strips were then equilibrated twice in equilibrium buffer [6M urea, 0.375M Tris-HCl (pH 8.8), 2% (w/v) SDS, 20% (v/v) glycerol], first with 2% (w/v) DTT and then with 2.5% (w/v) iodoacetamide in dark for 30 minutes each. Subsequently, the protein loaded strips were subjected to SDS-PAGE (10% polyacrylamide) using a Mini-PROTEAN tetra cell (Bio-Rad, USA). The IPG strips were laid on top of the polyacrylamide gel and fixed with agarose gel [1% (w/v) agarose, 25 mM trizma base, 192 mM glycine, 0.1% (w/v) SDS, trace amount of bromophenol blue and run with a constant voltage supply of 120 V until the dye-front reached the bottom of the gel. The gels were then stained with Coomassie blue and the image was captured. The detected protein spots were analysed using PDQuest software (Ver. 7.3). Gel images were cropped, and the protein spots were matched and compared statistically. A representative image containing the common spots from every image within the same group was displayed. The protein spots of interest were defined as differentially expressed when $p < 0.05$ and/or $\geq$2-fold expression change in spot intensity was identified between two groups. Differentially expressed spots were excised from the gels and subjected to trypsin in-gel digestion.

2.4.2. In Gel Digestion

In brief, trypsin in-gel digestion involved gel distaining, followed by reduction, alkylation and digestion of protein in the gel using trypsin. The targeted spots in the gel was cut and sliced into gel pieces. The gel pieces was distained and dehydrated using ammonium bicarbonate (NH_4HCO_3) (100 mM) and 100% acetonitrile respectively. The distained gel spots were then vacuum dried. The protein in the gel pieces was reduced in NH_4HCO_3 (100 mM) containing DTT (10 mM) for 1 hour at 56°C. The DTT solution was then discarded and replaced by iodoacetamide (55 mM) in NH_4HCO_3 (100 mM) and incubated in dark at room temperature for 45 minutes for alkylation. Next, iodoacetamide residues was removed. The gel pieces was washed by repeating the hydration and dehydration steps, vacuum dried and then proceeded to trypsin digestion. The gel pieces were immersed in digestion buffer (5 mM calcium bicarbonate, 50 mM NH_4HCO_3) containing trypsin (15 ng/µl) for 1 hour on ice. After that, the trypsin digestion buffer was discarded, replaced with fresh digestion buffer without trypsin and incubated overnight at 37°C. On next day, the digestion buffer was collected into a clean polypropylene tube. The digested peptides were further extracted from the gel pieces by using 20 mM NH_4HCO_3 and 70% acetonitrile with 5% formic acid. The obtained solutions were pooled together, dried under nitrogen stream and stored at -80°C prior to LC-MS/MS analysis.

2.4.3. Identification of Protein Identity using LC-MS/MS Analysis

Protein analysis was performed using Easy-nLC II nano liquid chromatography system (Thermo Scientific, USA) coupled with Finnigan LTQ linear ion trap mass spectrometer (Thermo Fisher, USA). The pre-column, Easy-column C18 (20 × 0.10 mm i.d., 5 µm, Thermo Scientific, USA) was equilibrated with 15 µl at 3 µl/min while the analytical column.

Meanwhile, Easy-Column C18 (100 × 0.75 mm i.d., 3 µm, Thermo Scientific, USA) was equilibrated with 4 µl at 0.3 µl/min. A gradient mode was set from 5% to 100% of buffer B in 80 mins: Buffer (A) 0.1% (v/v) formic acid in MS grade water and (B) 0.1% formic acid in 100% acetonitrile. The parameters of mass spectrometry were set at full scan mass analysis from m/z 300-2,000 at a resolving power (FWHM) of 60,000 at m/z and acquisition time of 1 sec at 220°C with a source voltage of 2.1 kV. The data-dependent MS/MS analyses triggered by the 8 most abundant ions from the parent mass list. Singly or unassigned charged peptides were rejected. The fragmentation was done by collision induced dissociation (CID) with collision energy of 35 V. The obtained mass spectra were internally calibrated with trypsin peptides. The masses were then used to search in PEAK® studio version 7.0 software (Bioinformatic solution, Canada) against the SwissProt2019 database for identified proteins. Carbamidomethylation and oxidation (M) were set as variable post-translational modifications (PTM) with maximum missed cleavage per peptide at 3. The maximum variable PTM per peptide was set at 3.

2.4.4. Statistical Analysis

The statistical analysis including student t-test and Two-way ANOVA of the collected data was performed using GraphPad Prism ver.8. All the statistic results were presented in mean ± standard deviation.

3. Results

3.1. Effect of HFD Intake on Key Health Markers

The daily intake of HFD pellets (17.5±1.1 g) by rats was significantly less than those fed with normal diet pellets (19.6±1.1 g) although the same amount of food pellets (25±1 g) were supplied to the rats daily. The calorie intake for the rats fed with HFD (70.8±7.3 kcal/day) was insignificantly higher than for those of normal diet (61.5±3.3 kcal/day). The metabolic efficiency, which was defined as calorie per gram of weight gain of the normal diet fed rats (249.8±91.6 kcal/g) were insignificantly higher than the HFD fed rats (143.9±69.3 kcal/g) (Table 1). After the 6 weeks feeding period, the body weight of both normal diet and HFD fed rats was relatively similar. All the rats in both groups have significantly gained weight when compared their initial body weight to their end body weight. The FBG level of both HFD and normal diet-fed rats was maintained within a normal range (≤6 mmol/l) throughout the feeding course (Figure 1B). Table 1 also shows that the serum total cholesterol concentration of rats of HFD and normal diet was within the normal range. Additionally, feeding of HFD did not cause significant difference in the accumulation of adipose tissue either as an absolute measurement nor as a percentage of body weight (data not shown).

Table 1. The food intake, biochemical parameters of rats fed normal diet and high fat diet (HFD)

	Normal diet	HFD	T-test p value
Food intake (g/day)	19.6±1.1	17.5±1.1	0.015
Calories intake (kcal/day)	61.5±3.3	70.8±7.3	0.078
Serum cholesterol (mmol/l)	1.9±0.2	2.1±0.3	0.296
Visceral adipose tissue (g)	6.1±1.7	8.2±2.7	0.138
Visceral adipose tissue (% of body weight)	1.5±0.4	2.2±0.6	0.058
Metabolic efficiency (kcal/g)	249.8±91.6	143.9±69.3	0.377

Data are presented as mean ± SD (n = 6 rats), p <0.05 is statistically significant

Metabolic efficiency is defined by calories intake divided by weight gain in 6 weeks

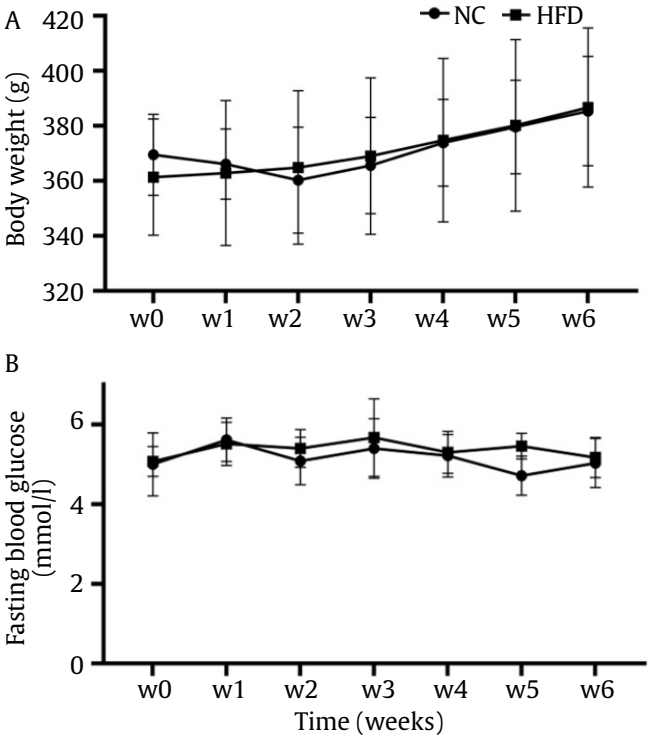


Figure 1. NC (normal diet), HFD (high fat diet). Effect of high fat diet on (A) body weight and (B) fasting blood glucose of rats over a 6-week time course. Data are presented as mean ± SD (n = 6-7 rats)

3.2. Effect of HFD on Brain Protein Expression

Mass spectrometry analysis was applied to identify the identity of the proteins of interest. The number of protein spots detected in HFD group was almost identical to normal diet group (≈145 spots) (Figure 2). Table 3 shows the protein spots' images, respectively from the HFD and normal diet fed groups. Of these protein spots, the protein spots with >2 fold difference in intensity between HFD and normal diet groups were subjected to mass spectrometry analysis. Two spots (spot 6702) and (spot 5201) were up-regulated at significant levels ($p < 0.05$), these protein spots were identified as heat shock cognate 71 kDa protein and pyruvate dehydrogenase by LCMS/MS analysis and protein database search (Table 2). The identified heat shock protein was characterized by oxidation of methionine residues.

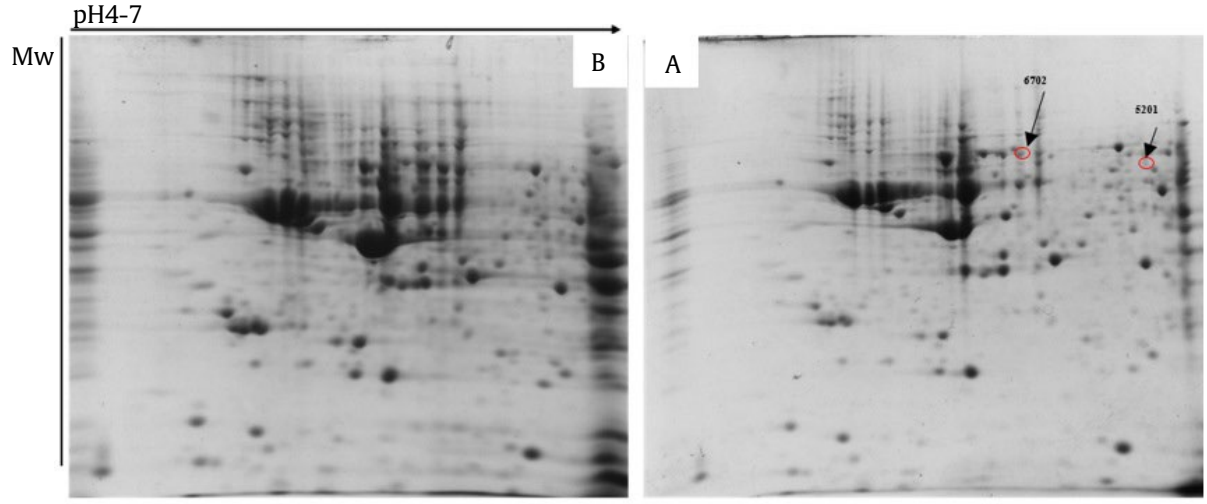
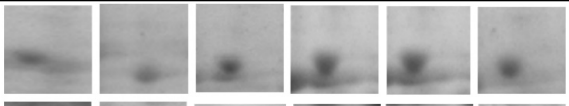
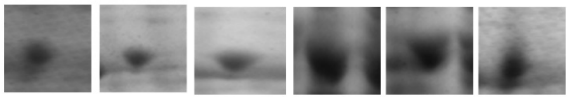
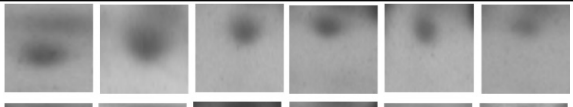
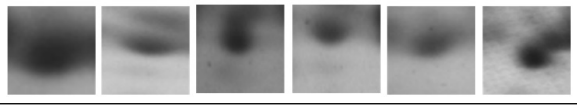


Figure 2. Representative images of 2D-gel electrophoresis of rat brain tissues. Proteins were separated in the first dimension by IPG strip with the pH ranging from 4 to 7 (left to right) and in the second dimension on a 10% SDS page gel. The protein spots of (A) rat fed NC, 146 spots (B) rat fed HFD, 142 spots. The identified proteins 71 kDa heat shock cognate protein (6702) and pyruvate dehydrogenase (5201) are labelled in (A)

Table 2. The identified proteins from LC-MS/MS

Spot number	Identified protein	Uniprot database accession no.	% coverage	Theoretical mW/pI	Matched peptides
6702	Heat Shock cognate protein (71 kDa)	P63018	28	70.739/5.37	18
5201	Pyruvate dehydrogenase	P49432	29	35.841/5.29	11

Table 3. The replicates gel images for spot 6702 and spot 5201 by normal diet and HFD fed rats

Type of feed		Fold-change in HFD group	P value
	Spot 6702		
Normal diet		≥2-fold	P <0.02
HFD			
	Spot 5201		
Normal diet		≥2-fold	P <0.05
HFD			

4. Discussion

Palm-oil based cooking oil has been used commonly in preparation of food. The health guideline for healthy adults recommended that daily calorie intake contributed by fat to consist ≤30% of total calories intake (Marshall and Bessesen 2002). In this study, we have carried out a comparison on two different values of fat contributing calories, namely HFD (39%) and normal diet (12%), respectively and evaluated the impact of these food on rats in a 6 weeks continual feeding program. Palm-oil based cooking oil has been used to enrich the fat content in HFD.

Metabolic efficiency is defined as the quantity of energy required to cause an increase in one gram of body weight (kcal/g). The higher value of metabolic efficiency by normal diet fed rats compared to HFD fed rats showed that more food (resulted in higher energy) was required by normal diet fed rats to gain a gram of body weight. This explained that although normal diet fed rats consumed significantly more quantity of food, the final body weight of the two groups of animals were insignificantly different after the feeding program (6 weeks). The visceral adipose tissue (VAT) of normal diet fed rats and HFD fed rats were 6.1 g and 8.2 g, respectively (Table 1). These values indicated that the calorie gained by HFD was stored as VAT, although it did not cause a significant VAT gain at this early stage of feeding program, on a long run, this accumulation of VAT by HFD can lead to obesity. Visceral adipose tissue (VAT) is known to be a pathogenic fat depot due to its association with various pathological conditions such as metabolic syndrome and cancers (Wagenknecht *et al.* 2003; Carr *et al.* 2004; Freedland 2004; Shuster *et al.* 2012) and causes ectopic lipid accumulation and lipotoxicity (Hardy *et al.* 2012). As for the six week feeding program, the deposition of VAT in rats between HFD and normal diet rats was not significantly different although a higher and more intense deposition of VAT was seen in HFD group (data not shown). Prolonged consumption of HFD is expected to increase VAT deposition.

Obesity or accumulation of excessive body fat is reported to increase the risk of developing insulin resistance and T2DM (Kahn *et al.* 2006). Mean total cholesterol and triglycerides concentrations are higher in obese persons in comparison to normal weight subjects, which increases the risk of coronary heart disease (Szczygielska *et al.* 2003). In conjunction with this, the serum cholesterol level of both feeding groups were within a normal range. The palm oil-based HFD in this study contained only traces amount of cholesterol. Usually, serum cholesterol could have resulted from the uptake dietary cholesterol via intestine adsorption and/or endogenously produced by the body (Arnold and Kwiterovich 2003). Hence, our data suggested the palm oil based HFD did not cause elevation in the serum cholesterol level and could possibly be due to the low amount of cholesterol in the diet.

Although there was no significant difference in the metabolic parameters and body weight of the rats after 6 weeks feeding program with HFD, at the molecular level, HFD was found to cause changes in the protein expression in brain tissues. The 2D gel electrophoresis analysis of rats fed HFD and normal diet revealed the number of protein spots detected was relatively similar (Figure 2). In this study, only the common protein spots that were found in all the rats within the same groups were considered for comparison study, this is to exclude protein spots that were expressed heterogeneously by individual animals and therefore cannot represent changes that due to diets treatment. HFD feed rats showed significant elevation of Hsc70 and pyruvate dehydrogenase expression by at least 2-fold in the brain tissue of rats fed on HFD compared to those fed on normal diet (Table 2). At the early stage of continually feeding of HFD may seems to produce tolerable effect to the physical body function of the rats as no significant difference in the metabolic parameters nor body weight increase were detected. However, the brain may experience the impact of HFD much earlier than the body. When the impact is prolonged, it can lead to mental health disorder (Lupien *et al.* 2018). Both the elevation of Hsc70 and pyruvate dehydrogenase expression could also correlate to HFD inducing oxidative stress (Freeman *et al.* 2013; Tan *et al.* 2018). This suggests that HFD possibly induced an early response to development of underlying health diseases.

Heat shock cognate protein 71 kDa is also known as Hsc70, Hspa8 and Hsp73. Hsc70 is a constitutively expressed member of the Hsp70 chaperone protein family that protects the proteome from stress and binds to nascent polypeptides to facilitate correct folding in an ADP/ATP-dependent manner (Stricher *et al.* 2013). Hsp70 is a common stress diagnostic marker for central nervous system (Fink *et al.* 1997; Beaucamp *et al.* 1998; Lu *et al.* 2014). The detected Hsc70 spot in rats fed HFD was characterised by post-translational oxidation of methionine residues. We have previously observed the upregulation of un-oxidised Hsc70 in diabetic rats on HFD treated with metformin. Oxidation of proteins can be used as a marker for oxidative damage and cellular stress (Shacter 2000). Hence, the up-regulated Hsc70 might

serve as an early indication of redox stress caused by HFD. Hsc70 is constitutively expressed and performs functions related to normal cellular processes, such as protein ubiquitylation and degradation (Goldfarb *et al.* 2006), which can lead to a wide impairment in the function of an entire repertoire of proteins. Overexpression of Hsc70 was reported in the Parkinson disease (Mandel *et al.* 2005).

Pyruvate dehydrogenase (E1) was also differentially expressed in rats on HFD compared to those fed a normal diet. Pyruvate dehydrogenase is involved in pyruvate oxidation, converting pyruvate into acetyl-CoA and linking glycolysis with the citric acid cycle. The three-enzyme pyruvate dehydrogenase complex, of which E1 is a component, an important target of oxidative stress. For example, hippocampal pyruvate dehydrogenase complex E1α subunit immunostaining is reduced 90% in animal models resuscitated on 100% O_2 (Martin *et al.* 2005). The differences in expression of pyruvate dehydrogenase suggest an alteration of glucose metabolism and possibly oxidative stress in the brain by HFD. Pyruvate dehydrogenase is an enzyme that catalyses the reaction of pyruvate, the overexpression of pyruvate dehydrogenase may also indicate the accumulation of pyruvate, which has been associated to the treatment of obesity (O'Mathuna 1999). Pyruvate contribute to weight loss by increasing the breakdown of fat (Stanko *et al.* 1992). Therefore, we believed that pyruvate has been induced as the feedback mechanism to counteract the increased calories due to fat intake, which implies in the weight indifference between the rats fed with HFD and NC despite of higher calories diet by HFD. The increased expression of pyruvate dehydrogenase may lead to the reduction in pyruvate quantity and causes the weight increase thereafter by continual consumption of HFD.

In conclusion, the fat content of a diet is an important determinant of energy intake. Upon six weeks of continual intake of HFD, no significant difference in the determined metabolic parameters and body weight of rats fed with HFD compared to those fed with normal diet. Nevertheless, significant overexpression of two chronic stress related proteins, namely Hsc70 and pyruvate dehydrogenase were detected. The upregulation of these two proteins indicate that the brain was experiencing oxidative stress and alteration of glucose metabolism as the result of HFD at its early stage of consumption. These data indicate that a short period of excessive fat intake has an impact on the brain at the molecular level much earlier than the onset of any standard clinical indications.

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References

Arnold, D.R., Kwiterovich, P.O., 2003. CHOLESTEROL | absorption, function, and metabolism. *Encyclopedia of Food Sciences and Nutrition*. 2003, 1226-1237. https://doi.org/10.1016/B0-12-227055-X/00225-X

Beaucamp, N., Harding, T.C., Geddes, B.J., Williams, J., Uney, J.B., 1998. Overexpression of Hsp70i facilitates reactivation of intracellular proteins in neurones and protects them from denaturing stress. *FEBS Letters*. 441, 215–219. https://doi.org/10.1016/S0014-5793(98)01553-1

Botchlett, R., Wu, C., 2018. Diet composition for the management of obesity and obesity-related disorders. *Journal of Diabetes Mellitus and Metabolic Syndrome*. 3, 10–25. https://doi.org/10.28967/jdmms.2018.01.18002

Carr, D.B., Utzschneider, K.M., Hull, R.L., Kodama, K., Retzlaff, B.M., Brunzell, J.D., Shofer, J.B., Fish, B.E., Knopp, R.H., Kahn, S.E., 2004. Intra-abdominal fat is a major determinant of the treatment panel III criteria for the metabolic syndrome. *Diabetes*, 53, 2087–2094. https://doi.org/10.2337/diabetes.53.8.2087

Chianese, R., Coccurello, R., Viggiano, A., Scafuro, M., Fiore, M., Coppola, G., Operto, F.F., Fasano, S., Laye, S., Pierantoni, R., Meccariello, R., 2017. Impact of dietary fats on brain functions. *Current Neuropharmacology*. 16, 1059–1085. https://doi.org/10.2174/1570159X15666171017102547

Donahoo, W., Wyatt, H.R., Kriehn, J., Stuht, J., Dong, F., Hosokawa, P., Grunwald, G.K., Johnson, S.L., Peters, J.C., Hill, J.O., 2008. Dietary fat increases energy intake across the range of typical consumption in the united states. *Obesity*. 16, 64-69. https://doi.org/10.1038/oby.2007.31

Engin, A., 2017. The definition and prevalence of obesity and metabolic syndrome. *Advances in Experimental Medicine and Biology*. 960, 1–17. https://doi.org/10.1007/978-3-319-48382-5_1

Farrall, A.J., Wardlaw, J.M., 2009. Blood-brain barrier: ageing and microvascular disease--systematic review and meta-analysis. *Neurobiology of aging*. 30, 337–352. https://doi.org/10.1016/j.neurobiolaging.2007.07.015

Figueiredo, P.S., Inada, A.C., Marcelino, G., Cardozo, C.M.L., Freitas, K.de C., Guimarães, R.de C.A., de Castro, A.P., do Nascimento, V.A., Hiane, P.A., 2017. Fatty acids consumption: the role metabolic aspects involved in obesity and its associated disorders. *Nutrients*. 9, 1–32. https://doi.org/10.3390/nu9101158

Fink, S.L., Chang, L.K., Ho, D.Y., Sapolsky, R.M., 1997. Defective herpes simplex virus vectors expressing the rat brain stress-inducible heat shock protein 72 protect cultured neurons from severe heat shock. *Journal of Neurochemistry*. 68, 961–969. https://doi.org/10.1046/j.1471-4159.1997.68030961.x

Freeman, L.R., Zhang, L., Nair, A., Dasuri, K., Francis, J., Fernandez-kim, S., Bruce-keller, A.J., Keller, J.N., 2013. Free radical biology and medicine obesity increases cerebrocortical reactive oxygen species and impairs brain function. *Elsevier*, 56, 226–233. https://doi.org/10.1016/j.freeradbiomed.2012.08.577

Freedland, Eric, S., 2004. Role of a Critical Visceral Adipose Tissue Threshold (CVATT) in metabolic ayndrome: implications for controlling dietary carbohydrates: a review. *Nutrition and Metabolism.* 1, 1–24. https://doi.org/10.1186/1743-7075-1-12

Galloway, S., Jian, L., Johnsen, R., Chew, S., Mamo, J.C.L., 2007. Beta-amyloid or its precursor protein is found in epithelial cells of the small intestine and is stimulated by high-fat feeding. *The Journal of nutritional biochemistry*. 18, 279–284. https://doi.org/10.1016/j.jnutbio.2006.07.003

Goldfarb, S.B., Kashlan, O.B., Watkins, J.N., Suaud, L., Yan, W., Kleyman, T.R., Rubenstein, R.C., 2006. Differential effects of Hsc70 and Hsp70 on the intracellular trafficking and functional expression of epithelial sodium channels. *PNAS.* 103, 5817–5822. https://doi.org/10.1073/pnas.0507903103

Gomez-Pinilla, F., 2008. Brain foods: the effects of nutrients on brain function. *Nature Reviews Neuroscience*. 9, 568–578. https://doi.org/10.1038/nrn2421

Hardy, Olga, T., Michael, P., Czech, Silvia Corvera,. 2012. What causes the insulin resistant underlying obesity?. *Current Opinion in Endocrinology Diabetes Obesity*. 19, 81–87. https://doi.org/10.1097/MED.0b013e3283514e13.What

Hill, J.O., Wyatt, H.R., Peters, J.C., 2012. Energy balance and obesity. *Circulation*. 126, 126–132. https://doi.org/10.1038/jid.2014.371

Hu, S., Wang, L., Yang, D., Wang, S., Douglas, A., Speakman, J.R., Hu, S., Wang, L., Yang, D., Li, L., Wu, Y., Liu, Q., Li, B., 2018. Dietary fat , but not protein or carbohydrate, regulates energy intake and causes adiposity in mice. *Cell Metabolism*. 28, 415–431. https://doi.org/10.1016/j.cmet.2018.06.010

Kahn, S.E., Hull, R.L., Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. 444, 840–846. https://doi.org/10.1038/nature05482

Lu, R.C., Tan, M.S., Wang, H., Xie, A.M., Yu, J.T., Tan, L., 2014. Heat shock protein 70 in alzheimer's disease. *BioMed Research International*. 2014, 8. https://doi.org/10.1155/2014/435203

Lupien, S.J., Juster, R., Raymond, C., Marin, M., 2018. The effects of chronic stress on the human brain: from neurotoxicity, to vulnerability, to opportunity. *Frontiers in Neuroendocrinology*. 49, 91-105. https://doi.org/10.1016/j.yfrne.2018.02.001

Mandel, S., Grunblatt, E., Riederer, P., Amariglio, N., Jacob-Hirsch, J., Rechavi, G., Youdim, M.B.H., 2005. Gene expression profiling of sporadic Parkinson's disease substantia nigra pars compacta reveals impairment of ubiquitin-proteasome subunits, SKP1A, aldehyde dehydrogenase, and chaperone HSC-70. *Annals of the New York Academy of Sciences.* 1053, 356-375. https://doi.org/10.1196/annals.1344.031

Martin, E., Rosenthal, R.E., Fiskum, G., 2005. Pyruvate dehydrogenase complex: metabolic link to ischemic brain injury and target of oxidative stress. *Journal of Neuroscience Research*. 79, 240–247. https://doi.org/10.1002/jnr.20293

Marshall, J.A., Bessesen, D.H., 2002. Dietary fat and the development of type 2 diabetes. *Diabetes Care.* 25, 620–622. https://doi.org/10.2337/diacare.25.3.620.

Melo, H.M., Santos, L.E., Ferreira, S.T., 2019. Diet-derived fatty acids , brain inflammation, and mental health. *Frontiers in Neuroscience*. 13, 1–12. https://doi.org/10.3389/fnins.2019.00265

O'Mathuna, D.P., 1999. Pyruvate for the treatment of obesity. *Integrative Medicine.* 2, 31–34. https://doi.org/10.1016/S1096-2190(99)00012-8

Roberto, C.A., Swinburn, B., Hawkes, C., Huang, T.T., Costa, S.A., Ashe, M., Zwicker, L., Cawley, J.H., Brownell, K.D., 2015. Patchy progress on obesity prevention : emerging examples, entrenched barriers, and new thinking. *The Lancet.* 385, 2400–2409. https://doi.org/10.1016/S0140-6736(14)61744-X

Szczygielska, A., Widomska, S., Jaraszkiewicz, M., Knera, P., Muc., K., 2003. Blood lipids profile in obese or overweight patients. *Ann. Univ. Mariae. Curie. Sklodowska. Med.* 58, 343-349.

Shacter, E., 2000. Quantification and significance of protein oxidation in biological samples. *Drug Metabolism Reviews* 32, 307-326. https://doi.org/10.1081/DMR-100102336

Shuster, A., Patlas, M., Pinthus, J.H., Mourtzakis, M., 2012.The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis. *The British Journal of Radiology*. 85, 1–10. https://doi.org/10.1259/bjr/38447238

Stanko, R.T., Tietze, L., Arch, J.E., 1992. Body composition, energy untilization, and nitrogen metabolism with a 4.25MJ/d low-energy diet supplmeneted with pyruvate. *The American Journal of Clinical NutritionJournal of Clinical Nutrition*. 56, 630–635. https://doi.org/10.1093/ajcn/56.4.630

Stricher, F., Macri, C., Ruff, M., Muller, S., 2013. HSPA8/HSC70 Chaperone Protein. *Autophagy*. 9, 1937-1954. https://doi.org/10.4161/auto.26448

Takechi, R., Pallebage-Gamarallage, M.M., Lam, V., Giles, C., Mamo, J.C., 2013. Nutraceutical agents with anti-inflammatory properties prevent dietary saturated-fat induced disturbances in blood-brain barrier function in wild-type mice. *Journal of Neuroinflammation*. 10, 1–12. https://doi.org/10.1186/1742-2094-10-73

Tan, B.L., Norhaizan, M.E., Liew, W., 2018. Nutrients and oxidative stress: friend or foe ?. *Oxidative Medicine and Cellular Longevity*. 2018, 24. https://doi.org/10.1155/2018/9719584

Valdearcos, M., Robblee, M.M., Benjamin, D.I., Nomura, D.K., Xu, A.W., Koliwad, S.K., 2014. Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. *Cell reports.* 9, 2124–2138. https://doi.org/10.1016/j.celrep.2014.11.018

Wagenknecht, L.E., Langefeld, C.D., Scherzinger, A.L., Norris, J.M., Haffner, S.M., Saad, M.F., Bergman, R.N., 2003. Insulin sensitivity, insulin secretion, and abdominal fat. *Diabetes*. 52, 2490–2496. https://doi.org/10.2337/diabetes.52.10.2490