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Whey supplementation alleviating brain damage of mother rats caused by a high fat diet

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Abstract: The research work was done to observe the therapeutic potential of bovine whey supplementation in improving the brain damage of mother rats fed on a high fat diet. Twenty –four mother rats (*Rattus norvegicus*) (200-220 g b.wt) were used in this study. They were categorized into four groups; control, whey supplemented group (*ad-libitum* every other day), a high fat diet and high fat diet in combination with whey supplementation. Also, whey supplementation at doses of 2 cubic mL^3 alone or in combination with a high fat diet every other day during the gestation and lactation period till 21 day-post-partum. Mother rats were sacrificed and their brain were dissected and subjected for biochemical investigations of DA,5-HT, TNF-α, Cas-3, TAC, SOD, MDA & GLUT-1. In brain of mother rats ingested a high fat diet, whey supplementation restored the decreased levels of SOD, TOC, DA, 5-HT and GLUT-1 and decreased the upregulation of MD, CAS-3 and TNF-α. These findings indicated that whey supplementation howed therapeutic potential against oxidative stress, inflammation and and apoptotic cell death as indicated by increased the level of caspase 3. The authors concluded that whey supplementation have therapeutic potential against maternal feeding on a high fat diet. They improve the brain from oxidative stress and inflammation while also increasing dopamine, serotonin and antioxidants levels which improve cognition

keywords: words: Brain, high fat diet, whey, antioxidant, inflammation and neurotransmitters

1.Introduction

Brain plays a crucial role in controlling the function of the body organs. Every minute, it requires 3.5 ml oxygen/100 g brain tissue due to its high metabolic rate [1,2]. Gray matter requires nearly twice as much oxygen as white matter [3]. As a result, oxygen deprivation has been associated to many kinds of brain injuries, including hypoxic–ischemic encephalopathy, stroke and metabolic abnormalities [4]. Through acute and subacute stages, the hypothalamus of HFD fed animal models showed an increase in the inflammatory cytokines (IL-1β, TNF-α, and IL-6) through acute and subacute phases [5]. Transmission electron microscopy revealed hypothalamic myelin degradation in oligodendrocytes of male C57BL/6 mice fed the HFD for 3–6 months. Chronic administration of a high fat diet (HFD) increases the loss of oligodendrocyte progenitors in the brain and spinal cord over time. Oligodendrocytes were more susceptible to oxidative stress and have a higher rate of apoptotic cell death [6].

Whey, is a protein-rich byproduct of yogurt and cheese, is made up of β-lactalbumin, α-lactoglobulin, immunoglobulin, bovine serum albumin, and other minor proteins [7]. Clinical research have also demonstrated that whey protein improves cognitive skills and mood. Supplementation of 20 g whey protein improved memory performance in stress-prone subjects aged 18 to 35 years old [8]. Glutathione (GSH) is an important molecule in every cell. It is made up of three amino acids: glycine, glutamate, and cysteine and is generated inside the cell. GSH is a powerful antioxidant produced by the cell, that shield it from “free radicals,” while also serving as a crucial detoxification agent [9]. In elderly rats,

whey protein reduced lipid hydroperoxide, protein carbonyl, reactive oxygen species, and NO levels. It also decreased the expression of inflammatory markers (tumour necrosis factor alpha, interleukin (IL)-1β, IL-6), while upregulated the expression of autophagy and neurodegenerative marker genes (neuron specific enolase, Synapsin-I, MBP-2) [10]. Elevated respiratory rates with increased levels of cytochromes a+a3 and c+c1 in brain mitochondria from whey protein isolate C57BL/6J mice indicated better mitochondrial activity [11].

The goal of this study is to demonstrate the therapeutic potential whey supplementation in preventing brain damage caused by a high fat diet.

2 Materials AND methods

Animal model and design of experiment:

During the experiment, twenty-four mother Wistar albino rats (*Rattus novergicus*) (8-M-old, weight 200-220gm body weight were employed. The trials were approved by the Faculty of Science's Experimental Animal Ethical Committee in Mansoura University, Egypt (decision statement No. MZ 170010). The National Institute of Health's guidelines for the use of laboratory animals were followed in this work (NIH Publication No, 8523, updated 1996). Animals were housed in an aerated environment with a light/dark cycle of roughly 12 hours and light intensity exposure at an of 180–200 lx. A typical diet and unlimited access to water were provided.

Preparation of a high fat diet:

A high fat diet was composed of 15% of lamb fat beside other standard dietary components was used in this research work. Rats were fed on it for four months before conception and during the time of gestation and lactation period.

Supplementation of whey:

The Dairy product Lab, Faculty of Agriculture, Mansoura University, Egypt provided fresh bovine whey on a regular basis. Each mother rats intragastrically administered twice doses of 2 cubic mL^3 once at 8 AM and second at 2 PM. every other day. Whey syrup was investigated according to the study carried out by [12].

Experimental Work:

Twenty four mother rats (*Rattus norvegicus*) (200-220g b.wt) were obtained from Ministry of Health, Egypt, acclimatized and used in this study. Half of them were fed on a high fat diet (20% fat) for 4 months before conception as well as during gestation and lactation period. The mother rats were categorized into four groups; control, whey supplemented group (ad-libitum every other days), a high fat diet and a combined high fat diet and whey supplementation. At 21 days-post-partum, the studied groups were fastened overnight, and euthanized using chloral hydrate (300 mg/kg body weight) and sacrificed in the next day. The mother rats of the studied groups were sacrificed and their brain were dissected. Homogenization was carried out in 10% ice-cold 2.5 mM-tris buffer (pH 7.5) and centrifuged at 14000 x g for 15 minutes at 4 °C and the supernatants were kept in a refrigerator. The following biochemical assays were measured:

1. Assay of brain dopamine and serotonin:

These were determined using a Rat ELISA Kit from CUSABIO TECHNOLOGY LLC, Houston, USA as directed by the manufacturer ELISA Kit catalogue Nu. . CSB-E08660r was uses t quantify dopamine (DA), whereas the Kit Cat Nu. E-El-0033 was used to measure serotonin (5-HT).

2. Assessments of caspase 3 and tumor necrosis factor--α. (TNF--α.):

Rat TNF-α. (Cat no., CSB-e11987r) was also measured using an ELISA kit from CUSABIO. TECHNOLOGY LLC, Houston, USA. The method used a competitive inhibitiory response involved labeling biotin and TNF-α or casp-3. The samples were incubated with avidin conjugated with horseradish peroxidase. To avoid fading, the amount of bounded HRP was proportional to the amount of the tested parameter, with the absorbance measured at 540 nm within 30 minutes. The assayed parameters were used to generate the standard curve.

3.Total antioxidant (TAC ((nmol/mg protein): This was carried out by adding 100 µl Cu 2+ working solution which led to conversion of it after incubation for 1.5 hours. The colour of the reduced Cu+ ion is measured

at 570 nm, proportional to the total antioxidant capacity. Total antioxidant capacity = $Sa/\ Sv\ /$ = nmol/mg protein.

4. Glucose transporter-1 (GLUT-1) (ng/mg protein) (of CUSABIO. TECHNOLOGY LLC,Houston, USA catalogue No. CSB-E13908r): The method is based on the competition interactions of GLUT-1 antibody-GLUT-1 antigen (immunosorbency) and an horse reddish peroxidase colorimetric detection system in samples. The colour reagent was measured at 540 nm.

5. Assays of superoxide dismutase and lipid peroxidation (MDA):

Super oxide dismutase activity (SOD), was measured using 100 µL of supernatant from brain tissue samples plus 100 µL xantine oxidase, 100 µL nitroblue tetrazolium and 3100 µL phosphate buffer solution (PBS) at 30 degrees Celsius for 30 minutes. At 500-600 nm, the produced colour. was measured spectrophotometrically [13]. The reaction of malondialdhyde with thiobarbituric acid produces thiobarbituric acid reactive substance (TBARS), which has a pink colour and may be measured calorimetrically at 532 nm [14].

11. Statistical analysis:

Between, the control and studied groups, statistical analysis was performed using SPSS one way ANOVA posthoc analysis (version 13). Data are presented as means ± standard error (SE). The t-test was calculated and considered statistically significant at $p<0.05$.

3. Results and Discussion

From table (1), mother rats ingest diet rich in fat for four months prior to conception as well as during pregnancy and lactation period exhibited significant reduction in brain dopamine, serotonin, total antioxidant and, superoxide dismutase and glucose transporter 1 reached to 4.58±0.29, , 117.61±1.6, 6.38±0.4, 11.9±0.8 and 7.09±0.4 respectively at $p < 0.05$. The percent of reduction of the mentioned parameters attained -26.95, -18.38, -31.41, -26.99 and -33.11 respectively. On the other hand whey supplementation to mother rats improved the brain levels and their percentages of improvement reached to -13.07, -9.56, -18.50, -15.33 and -15.66 respectively.

At the same time , Maternal consumption of a high fat diet led to significant increase of tumor necrosis factor –α,caspase 3 and malondialdhyde and reached to 141.5±2.5, 31.41 and 8.61±0.4 respectively at $p < 0.05$ with percent of increase of 94.74, 56.58 and 53.20 compared to control. Whey supplementation showed apparent improvement and their percentages reached to 45.44, 24.83 and 19.21 respectively

Table1. Biochemical markers of brain of mother rats ingested a high fat diet and or/ whey supplementation.

	DA (ng/mgP)	5-HT (ng/mgP)	TNF-α (pg/mgP)	CAS-3 (ng/mg P)	TAC (nmol/ mgP)	SOD (U/mgP)	MDA (nmol/ mgP)	GLUT1 (ng/mg)
Control	6.27±0.3	144.11±4.9	72.66±2.4	4.63±0.4	6.27±0.7	16.3±1.3	5.62±0.1	10.6±0.21
Whey	6.38±0.12	141.1±6.3	70.3±2.5	4.57±0.4	6.38±0.4	16.8±0.6	5.51±0.5	10.14±0.7
Fat diet	4.58±0.29*	117.61±1.6*	141.5±2.5*	7.25±0.7*	4.3±0.4*	11.9±0.8*	8.61±0.4*	7.09±0.4*
% of change from control	-26.95	-18.38	+94.74	+56.58	-31.41	-26.99	+53.20	-33.11
Fat diet +whey	5.45±0.12*	128.86±2.9	118.1±2.4*	5.78±0. 6*	5.41±0.8*	13.8±0.5*	6.7±0.3	8.94±0.1
% of change from fat diet	+18.99	+9.56	+16.53	-20.27	+25.81	+15.96	-22.18	+26.09
% of change of fat diet and whey from control	-13.07	-10.58	+45.44	+24.83	-18.50	-15.33	+19.21	-15.66
F-test	85.31	9.14	269.83	56.05	153.81	18.70	63.05	84.01

Each result represent mean±SD (n=6). Star means significant *Significant at $p < 0.05$ compared to G1. Abbreviations; Cas-3, caspase 3; DA, dopamine; GLUT-1, glucose transporter 1; MDA, malondialdhyde; 5-HT, serotonin; SOD, superoxide dismutase;TAC, total antioxidant ; TNF-α, tumor necrosis factor—α

Discussion

According to the present findings, consumption of diet rich in fat for four months before conception and during pregnancy and lactation period led to a significant decrease of maternal brain total antioxidant content and superoxide dismutase activity. The antioxidant defense is involved in maintaining cell structure and function [15] and leading to disrupt the cell homeostasis damaging the membranes and other macromolecules [16,17] The observed reduction of the antioxidant enzyme SOD and total antioxidant resulted in significant increase of malondialdehyde (MDA), the marker of lipid peroxidation in brain tissue of mother fed on a high fat diet. The present findings are in consistent with Nissankara Rao et al. [18] in rat fed on a high fat high sugar diet. It is known that MDA is a precursor of lipid peroxidation resulted from decomposition end products of α, β-unsaturated reactive aldehydes [19]. The lipid peroxidation resulted from the imbalance between the antioxidant and increase liberation of free radicals [20] which damaging the cell inclusions of lipids, proteins, and nucleic acids [21].

Whey supplementation to mother rats fed on a high fat diet led to moderately restoration of brain total antioxidant content and superoxide dismutase and decrease the increased level of malondialdhyde. The findings were in consitant with the studies carried out by [22] who mentioned that whey protein isolate increased accumulation of GSH and antioxidant defenses in the healthy and brain tissue of GFAP.HMOX1 transgenic mice. In elderly patients with ischemic stroke, whey protein also may reduce inflammation and boost antioxidant defenses [23]. Panda et al. [24] restored the serum levels of glutathione, superoxide dismutase, and catalase in rat fed on a high fat diet.

The observed increased lipid peroxidation resulted in significant increase of caspase 3 predicting damaging of the brain tissue. These findings were consistent with the study carried out by [25]. The authors mentioned that the hypothalamus astrocytes were highly susceptible to high fat diet and possessed cleaved caspase-3 leading to apoptosis. [26] found that a high fat diet caused neuronal cell death and a reduction of synaptic inputs in the arcuate nucleus and lateral hypothalamus.

Also, the upregulation of caspase 3 predicting neuronal damage was parallel increased inflammatory lesions assessed by increased level of TNF-α of neuronal tissues of a high fat diet.

The current findings corroborated the studies carried out by [26] who found that inflammation increased signal transduction resulting in the activation of signaling pathways of apoptosis.

Besides, the high fat diet associated lipid peroxidation and increased caspase 3 in brain tissues were markedly reduced post-whey supplementation. This facilitated improvement of the neuronal tissues. Whey supplementation was found to decrease brain MDA and inflammatory cytokines of aged mice [27] and serum of patients with malnutrition [28]. At the same time, Shertzer et al. [11] found that dietary whey protein decreases oxidative stress and improved the function of mouse brain mitochondria.

The findings of increased inflammation and apoptosis led to apparent reduction of dopamine and serotonin in mother rats fed on a high fat diet. In animal models fed on a high fat diet exerted a significant reduction of brain dopamine [18,29]) and serotonin level [30]. Reduction of the assayed transmitters reflected the increased pattern of neuronal cell damage as mentioned of increased brain caspase 3. The decrease of neurotransmitters reflected the loss of learning and memory process [31]. Dopaminergic neurons is present in the substantia nigra pars compacta and serves for voluntary movement and behavior function such as mood, reward, addiction, and stress [32]. The neurotransmitters Serotonin and dopamine (DA), have long been linked to adaptive behavior, including decision making and reinforcement learning. They serve a number of tasks, including emotional and functional activation [33]. Consumption of a high fat diet led to altering dopamine signaling, such as dopamine transporter (DAT) expression and dopamine reuptake [34]. 5-HT injection, on the other hand, causes an increase in the hepatic glycogen content, and the decrease of hepatic

triglyceride content [35]. After increasing energy expenditure, 5-HT reduces fat storage in the liver in an indirect way [36].

Dietary whey protein was found to increase large neutral amino acids followed by increase the serotonin level [8].

Consumption of fermented dairy products high in β-lactolin attenuated cognition loss and decreased synaptophysin, dopamine, brain-derived neurotrophic factor, and insulin-like growth factor 1 levels in the cortex in 5×FAD transgenic mice [37] .

The current findings revealed decreased levels of GLUT- 1 in brain tissues of a high fat diet mother rats. These findings were consistent with the study carried out by [38]. The author reported that three days consumption of a high fat diet led to impairment of glucose tolerance, an increase of plasma glucose and insulin and brain expression of the glucose transporter GLUT- 1 especially in the ischaemic hemisphere. The alterations in brain glucose homeostasis may result from reduction in vasodilatory and Enhancement of vasoconstriction which altered neuronal physiology and impair learning and memory in both humans and rodents [39,40]. The present findings revealed that mother rats fed on a high fat diet exhibited apparent reduction of brain GLUT-1.

It is documented that even hyperglycemia in diabetes, the lack of blood supply during ischemic stroke causes a glucose (and oxygen) shortage in the brain. The ischemic rat brain tissue required more energy and consequently glucose uptake [41]. The GLUT-1 found on cerebrovascular endothelial cells mediates uptake of glucose and vitamin C into the brain [42]. Reduced levels of GLUT-1 may decrease glucose availability in the brain resulting in ischemic tissue leading to increased cell death [43] and consequently cognitive impairment.

Whey, lactalbumin and lactoferrin improved glucose homeostasis through differential upregulation of glucoregulatory transcripts in the liver and skeletal muscle as well decreased a adiposity [45]. Whey protein also contains a lot amino acids, which can directly trigger beta cells to produce insulin, which help to lower postprandial glycaemia. Whey ingestion supresses apetite due to its effects on the gut-brain axis and the hypothalamus. These characteristics of whey protein imply that it could be useful in the treatment of type 2 diabetes [46,47].

The author concluded that whey supplementation have therapeutic potential against maternal feeding on a high fat diet. They improve the brain against oxidative stress, inflammation and improve cognition through increased level of dopamine and serotonin.

4.References

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