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Full Length Article

Margarine Consumption Induces Oxidative Stress in the Gut of Wistar Albino Rats

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Abstract

Diet has a great impact on human health and consumption of high caloric diets (rich in fat) may lead to metabolic disorders. High fat diets can trigger dysbiosis, cellular stress, intestinal epithelial permeability, onset of inflammation and ultimately CRC. The current research was designed to evaluate the effect of HFD on gut pathophysiology. For this purpose, HFD (15% and 30% Margarine: Blue Band®) was fed to Wistar rats for a period of 6 weeks (42 days). Blood serum samples were collected for evaluating liver, renal function markers, serum, Total Oxidative Stress (TOS) and Total Antioxidant Capacity (TAC). Tissue sampling from the gut (Ileum) of experimental animals was done to perform histopathology and cellular ROS (Reactive Oxygen Species) detection. The data were statistically analyzed by applying two-way ANOVA and DMR (Duncan's Multiple Range). Results revealed significant ($P \leq 0.05$) role of HFD in elevating body TOS (10.15±1.47 and 10.20±1.30) in comparison to the control group (3.32±0.72). The onset of inflammation was observed in response to HFD feeding even after a short time period (10^{th} day) in the intestinal epithelium. Apparently, the induction was triggered by HFD mediated stress response. Significantly higher ($P \leq 0.05$) cellular stress (cellular ROS production), was also observed in HFD fed groups compared to the control group. Taken together, HFD has a major impact on different aspects of Wistar rat intestinal physiology and it induces oxidative stress due to onset of cellular stress pathways.

Keywords: Margarine; Total oxidative stress; Total antioxidant capacity; Wistar Albino rats; Colorectal cancer

Introduction

Balanced diets contain all the essential nutrients that are necessary for survival of every living creature. Intake of nutrients has traditionally viewed within a context of homeostasis, behavioral contributor, maintain energy balance and body fat content (Friedman, 2008). According to recommendations from the American Diabetes Association (ADA), carbohydrate between 45% and 65% of total calories, a range of protein 10–20%, total fat contents ≤ 30%, saturated fatty acids (SFAs) <7%, unsaturated fatty acids (PUFAs) up to 10%, mono-unsaturated fatty acids (MUFAs) up to 20% of total calorie have been recommended in healthy human diet (Shadman *et al*., 2013).

A close association exists between persistent changes in food intake and change in energy homeostasis (Friedman, 2008). Imbalanced diets such as high-fat diets have been linked to the onset of metabolic syndrome. This syndrome is also known as deadly quarter, insulin resistance syndrome and syndrome X. The metabolic abnormalities that are associated with overconsumption of nutrients include; central obesity, glucose intolerance dyslipidemia, insulin resistance, hypertension and cardiovascular disease (Small *et al*., 2017).

Margarine is a commercially available vegetable origin fat and higher consumption of margarine has been linked to inflammatory changes by fat accumulation and eventually leading towards the onset of stress (Wang *et al*., 2017). Metabolic syndrome develops via chronic oxidative stress, leading to the onset of inflammation, tight junction disruption and changes in intestinal epithelium. Mammalian intestinal tissue is lined with a single layer of epithelial cells. Villi protruding into the intestinal lumen are responsible for the absorption of nutrients from the ingested food (Mao *et al*., 2013).

For a healthy gut environment, balanced diet intake is very important and crucial for gut homeostasis. However, appetite-related problems and changes in intestinal physiology can cause severe problems (Mao *et al*., 2013) such as cellular stress (intestinal epithelial secretory cells), diminished mucous layer (barrier) and a local immune response. An increase in inflammatory cytokines can cause amplification of inflammation (Gulhane *et al*., 2016) therefore, it is necessary to understand the mechanisms that how this ecosystem is regulated through diet and exogenous factors as well as how its manipulation is crucial during therapy and disease prevention.

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Materials and Methods

Experimental Design and Research Protocol

A total of ninety Wistar albino rats (6 weeks old) were used for the study, weighing about 150–180 g. These rats were housed in the animal facility (Institute of Pharmacy, Physiology and Pharmacology), University of Agriculture, Faisalabad and were maintained according to institutional committee (GSRB) guidelines on care and use of animal. During acclimatization (two week), rats were fed with a standard nutritionally balanced diet and water was provided *ad libitum* with a12:12 h light-darkness cycle. After the adaptation period, rats were divided randomly into three groups. The experimental groups and diets fed to rats during 6 weeks (42 days) of the study are as follows:

Group I (Control): Control group received routine rat chow diet + water *ad libitum*

Group II (15% HFD): Routine diet +15% Margarine + Water *ad libitum*

Group III (30% HFD): Routine diet +30% Margarine + Water *ad libitum*

Rats were decapitated at different days of the trial and blood serum samples were collected. Blood serum samples were used to evaluate the liver functioning test, renal function markers, TOS and TAC. Intestinal tissue samples (ileum) from the decapitated rats were also collected for histopathology and cellular ROS detection.

Liver Function Markers

Organ body weight ratio of liver was determined. Serum AST (Aspartate Transaminase) and ALT (Alanine Transaminase) were also measured by using commercially available kit method (Bioclin® Transaminase AST kinetic diagnostic kit; K048 and Bioclin® Transaminase ALT kinetic diagnostic kit; K049, respectively).

Renal Function Markers

Organ body weight ratio of kidney, serum creatinine, urea, uric acid were measured by using commercially available diagnostic kits.

Serum Proteins

Serum total protein, albumin and globulin concentrations were measured by using commercial available kits.

Oxidative Stress Marker

Serum TOS and TAC were also measured with automated calorimetric method.

Cellular ROS Activity

Reactive oxygen species (Superoxide dismutase) in intestine (ileum) were determined by using the commercially available Cellular ROS/Superoxide detection assay kit (Abcam). For conducting cellular ROS activity tissue samples from the ileum portion of intestine were fixed on glass slides and incubated at 37ºC with superoxide/ROS detection mix for 30 min. After washing, examination of oxidative stress (ROS; green) cells was done under confocal microscope.

Histopathalogical Analysis

Intestinal tissue samples (ileum) from the decapitated rats were collected and preserved in 10% buffered formalin solution and histopathology was performed.

Statistical Analysis

Obtained results were subjected to statistical analysis by applying variance (ANOVA), means were then compared by DMR (Steel *et al*., 1997) test and expressed as a mean ± SE. Statistical tests were performed by using Graph Pad prism 6 and CoStat computer softwares.

Results

Effect of High Fat Diet (Margarine) on Liver Function Markers

No significant effect of margarine rich diet has been seen on organ body weight ratio of liver and serum AST and ALT levels even after 6 weeks of margarine diet feeding (Fig. 1).

Effect of Margarine Diet on Renal Function Markers

We also observed non-significant effect of margarine consumption on organ body ratio of kidney, serum creatinine and urea levels compared to control group. However, a significant increase in serum uric acid level has been observed in both 15 and 30% margarine fed groups (Fig. 2).

Effect of Margarine Diet on Serum Protein

We also observed non-significant effect of margarine rich diet on serum total protein, albumin and globulin levels (Fig. 3).

Effect of Margarine Diet on Oxidative Stress Markers

Results revealed that addition of margarine in rat diet for 6 weeks significantly increased serum TOS in both 15 and 30% margarine diet fed groups compared to control group indicating increase production of reactive oxygen species (ROS) in serum of margarine diet fed rats (Fig. 4). However, non-significant effect was observed for serum TAC after margarine diet feeding among all groups (Fig. 5).

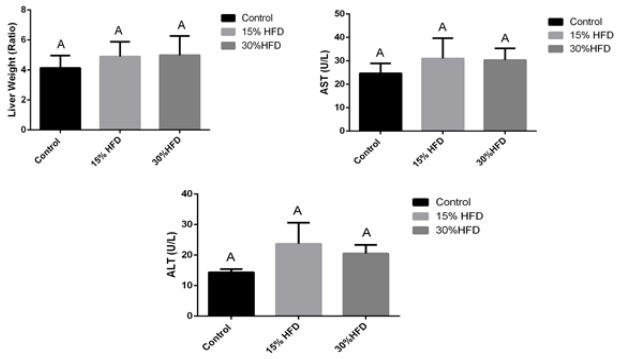

Fig. 1: Mean organ body weight ratio of liver, serum aspartate transaminase (U/L), alanine transaminase levels (U/L) in 15% HFD (margarine) and 30% HFD (margarine) treated groups in comparison to control group

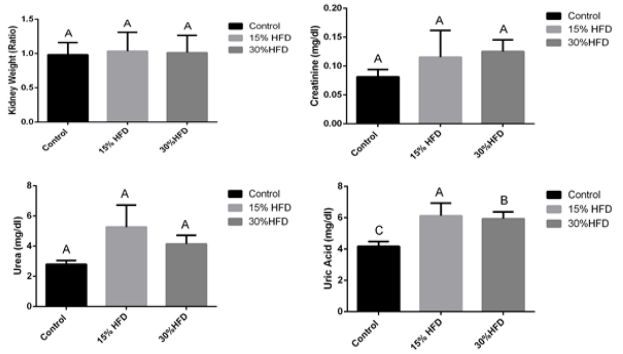

Fig. 2: Mean body weight ratio of kidney, serum creatinine (mg/dl), urea (mg/dl) and uric acid levels (mg/dl) in15% HFD (margarine) and 30% HFD (margarine) treated groups in comparison to control group

Effect of Margarine Diet on Cellular ROS Production

Cellular ROS/Superoxide dismutase detection assay kit was used to determine intracellular ROS production (Peng *et al*., 2017; Seto *et al*., 2017). Where NAC (N-acetyl-L-cysteine) was used as general ROS inhibitor and pyocyanin was used as ROS inducer. By using confocal microscope, superoxide dismutase production levels were monitored in green channels. Results showed (Fig. 6b) that there is marked damage mediated by the reactive oxygen species (Superoxide dismutase) generation at the mucosal surface in small intestine of rats fed with 30% margarine. Even as short as 10 days of margarine diet feeding has shown to increase cellular ROS production in a concentration dependent manner.

Effect of Margarine Diet on Histopathology of Gut

Photomicrographs (Fig. 7) showed histological changes in margarine rich diet supplemented groups especially marked destruction and disruption of the mucosal surface in intestine of those rats supplemented with 30% margarine

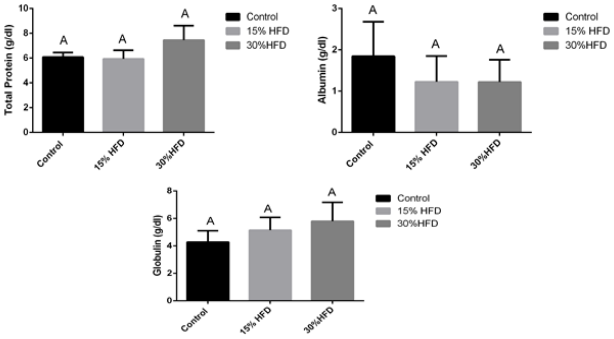

Fig. 3: Mean serum total protein (g/dl), albumin (g/dl), and globulin (g/dl) in 15% HFD (margarine) and 30% HFD (margarine) treated groups in comparison to control group

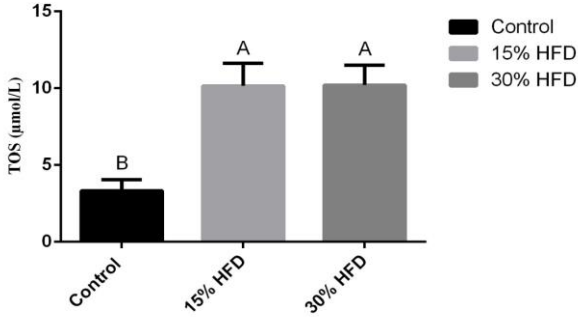

Fig. 4: Mean serum TOS levels (µmol/L) in 15% HFD (margarine) and 30% HFD (margarine) treated groups in comparison to control group

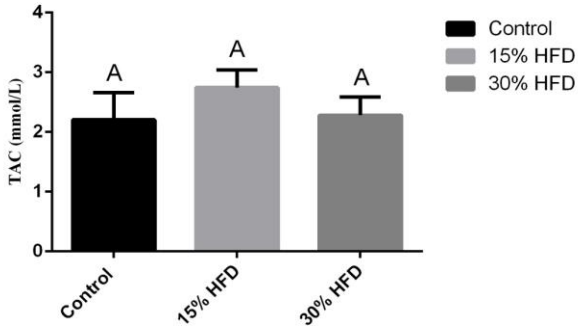

Fig. 5: Mean serum TAC (mmol/L) levels in 15% HFD (margarine) and 30% HFD (margarine) treated groups in comparison to control group

diet. In conclusion, as short as 10 days of HFD feeding (margarine) can damage the cellular tight junctions and cause the small intestine epithelium sloughing. However, control samples showed normal parenchyma (Fig. 7A). Morphological studies showed normal mucosal length, normal tight junction and normal parenchyma in control group. Among HFD fed groups mucosal surface is highly damaged in 30% HFD group (Fig. 7A) as compared to 15% HFD (Fig. 7A) and control group. A slight change in the morphology of intestine has been evident in 15% HFD group.

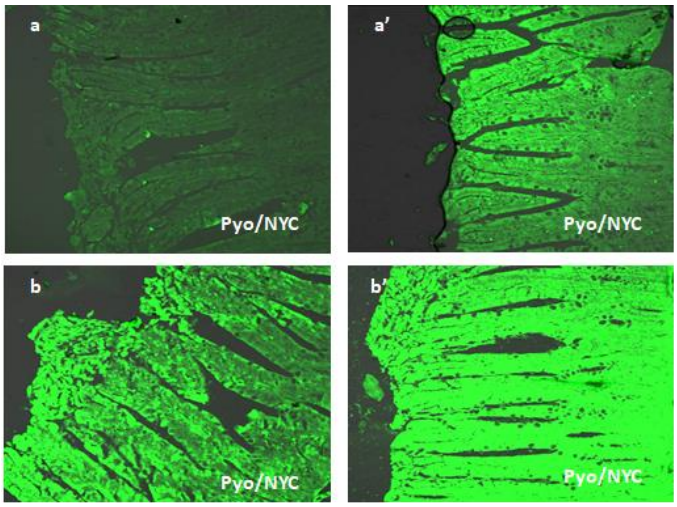

Fig. 6: Photomicrographs showing superoxide dismutase activity (at 10th day) in small intestine (Ileum) tissue of Wistar rats (Pyo/NAC; Green channel filter); **a:** Photomicrograph of rat Ileum tissue from negative control group; **a'**: Photomicrograph of rat Ileum tissue from Positive control group, **b:** Photomicrograph of rat Ileum tissue from 15% HFD (margarine) fed group; **b':** Photomicrograph of rat Ileum tissue from 30% HFD (margarine) fed group

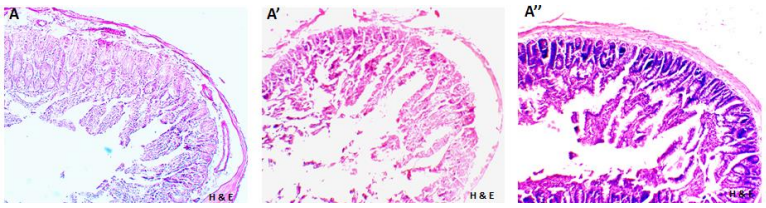

Fig. 7: Photomicrographs showing histopathology (at 10th day) of small intestine (Ileum) tissue of Wistar rats (H&E staining; 10X); **A:** Photomicrograph of rat Ileum tissue from control group; **A':** Photomicrograph of rat Ileum tissue from 15% HFD (margarine) fed group; **A'':** Photomicrograph of rat Ileum tissue from 30% HFD (margarine) fed group

Discussion

In developed countries, HFD intake has been associated with induction of metabolic disorders in humans. Besides this metabolic intervention in fat storage organs that is decisive for disease development as diabetes type 2, epithelium of intestine is the first most tissue that encounters the nutrients. Physiological and molecular changes in epithelial lining of intestine are triggered by HFD intake. Duodenal lipid causes intestinal cell tight junction impairment which may also contribute in metabolic syndrome development. Moreover, changes in gut microbiota may occur via direct dietary shift or indirectly through inflammatory response (Lee, 2013).

In rodent metabolic disorders and obesity after HFD feeding resemble with human metabolic syndrome, because rodent model is considered to be an appropriate model for HFD study. Along this, most effective induction of obesity has been seen in young animals with several weeks of HFD feeding (Buettner *et al*., 2007; Shang *et al*., 2017). In this study effect of margarine rich diet on different physiological aspects especially related to metabolic stress were evaluated.

We examined the relationship between diet-induced obesity and liver, kidney physiology. The results thus obtained revealed non-significant effect of margarine diet on liver, kidney function markers and weights. These findings are in agreement with a study of Anadón *et al*. (2010) as they observed non-significant difference in organ body weight to tissue ratio after HFD feeding. Mima *et al*. (2018) also found that obesity of 2 months can cause inflammation or oxidative stress but it may not be long enough to cause changes in renal pathology development. So, it may be attributed to the non-significant effect of HFD feeding (6 weeks) on serum creatinine, total protein, albumin and globulin levels in Wistar rats in present study. These findings are also supported by a study of high ketogenic diet intake in 37±12 years age adults (n=42) for 6 weeks that resulted in significant increase in urea and uric acid while non-significant effect has been observed on albumin and creatinine levels (Urbain *et al*., 2017).

Liver is a vital organ for metabolism. Metabolic disorders can lead to hepatocyte injury, thus releasing intracellular substances including different hepatic enzymes into the blood stream. Measurements of these enzymes provide a clue regarding hepatocellular damage (Han *et al*.,

2012). Current finding displayed non-significant effect of margarine diet feeding for 6 weeks on liver physiology, supported by the C57BL/6J mice study with HFD (40% fat) feeding for 6 weeks that also exhibited non-significant effect on liver weight and liver biomarkers (AST and ALT) but significant effect was observed after 24 weeks (Matsuzawa-Nagata *et al*., 2008; Toita *et al*., 2017).

Studies have also shown the negative impact of PUFA on oxidation status of lipids, including increased susceptibility towards oxidation, free radical formation and endogenous antioxidant depletion (Kraus, 2004), supporting increased serum TOS ($P \leq 0.05$) in rats and non-significant effect of margarine intake on TAC. Oxidative stress induced by dietary modulation at least partially may affect metabolic syndrome development (Miranda *et al*., 2014).

The current study demonstrated increase cellular ROS ($P \leq 0.05$) production in the intestine of rats fed with margarine rich diet for 6 weeks. This observation is consistent with findings that resulted in a discovery that HF feeding is responsible for the development of systemic insulin resistance (Long *et al*., 2017). Mitochondrial dysfunction and redox imbalance are two important determinants of insulin resistance (T2D) and other metabolic disorders (Wang *et al*., 2012). A major cause of insulin resistance could be insulin disruption via cellular ROS production by mitochondria (Barazzoni *et al*., 2012). Lipotoxicity, ROS generation, glucotoxicity and mitochondrial dysfunction are interconnected in metabolic abnormality context (Shah *et al*., 2013). A high-fat diet has been linked with the production of mitochondrial ROS, decreased mitochondrial membrane potential (MMP) and reduced oxygen consumption. Thus lead towards reducing mitochondrial function and intern causing insulin resistance (Long *et al*., 2017).

Histopathological examination of the small and large intestine has revealed inflammatory changes, sloughing of intestinal mucosa and changed gut barrier integrity. In consistent with previous findings where HF feeding has caused gut barrier deterioration, describing that these changes are associated with lipid metabolism, inflammation and immune response (De Wit *et al*., 2011). Nutrient overload and FFAs or high glucose in cultured cells of intestinal epithelium has been found to induce intestinal cell proliferation (Anagnostou and Shepherd, 2008). A high-fat diet is an important contributing factor to cause intestinal epithelium hypertrophy and increased capability of fat digestion (Brown *et al*., 1994).

Diets with high caloric contents are suggestive to cause "metabolic endotoxemia" and inflammatory changes to gut epithelium. Chronic HF feed consumption has an evident impact on the intestinal structure, especially on the enteroendocrine cells that are found at much higher amount following HFD, indicative of increased intestinal hormonal signaling load. Along with gut epithelium itself, indigenous microbiota is another trait that is affected by HFD feeding. The gut is the place where both commensal and pathogenic bacteria interact with the immune system (Ghanim *et al*., 2009). The first tissue, which comes in contact with these nutrients, is intestinal epithelium. Impairments in the tight junctions between the intestinal cells are activated by duodenal lipids. This may contribute to metabolic syndrome and reacts to LPS being transported into fat tissues via this route. Alteration of gut microbiota may occur which is either directly associated with this dietary shift or indirectly as a result of the inflammatory response. Moreover, HFD is the main source for causing physiological and molecular changes in the gut epithelium (Lee, 2013).

High fat diet plays important role in the functional decline in the intestinal epithelial tight junction and in the promotion of microbiota, resultantly plays a key role in translocation of PAMPs from GIT lumen to the circulation (Cani *et al*., 2008). High caloric diet thus leads to NF-κB activation via TLR4 inducing an inflammatory response (Deopurkar *et al*., 2010). After HFD feeding different levels of intestinal inflammation can change the microbiota composition being predictive for the development of obesity (Ding *et al*., 2010). Thus, high caloric diet can cause body weight gain, deposition of visceral fat, oxidative stress, reduced glucose tolerance and inflammation after gut microbiota modulation (Cani *et al*., 2008). Gut microbiota plays a very crucial role in the digestion and absorption of gut contents. Microbes present within the microbiota have been found to be responsible for utilization of fiber contents from the diet for absorption of energy after fermentation as well as for the production of short-chain fatty acids (Backhed, 2011). There is an alteration in the role of colonic gastrointestinal microbiota for the control of metabolic diseases and obesity following HFD feeding (Wanders *et al*., 2011).

Conclusion

Association of various adverse health effects with consumption of high fat diet has long been recognized as nutritional factors play vital role in the etiology of metabolic diseases. The results of current study on effects of margarine rich diet have revealed that high fat diet causes significant rise ($P \leq 0.05$) in serum TOS in HFD fed groups compared to control groups. Our results have also shown that high fat diet consumption is associated with oxidative stress generation in rats. Indicating that consumption of high fat (margarine) is the key causative agent of alteration in gut physiology; increased intestinal epithelium permeability and inflammation, which in turn lead to increased cellular ROS production.

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