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Dietary intake of parents affects antioxidant activity and inflammatory status in F2 offspring

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ABSTRACT

Background: Dietary intake is implicated in the pathogenesis of non-communicable diseases, especially those affecting metabolism. Many non-communicable diseases are mediated by alterations in antioxidant activity and chronic inflammation with its resultant effects. Developmental programming causes offspring of parents with particular metabolic phenotypes to adopt predisposition to these phenotypes during development.
Objective: This study investigated the effects of maternal macronutrient consumption in two generations of rats (F0 and F1) on programming of antioxidant activity and inflammatory status in F2 offspring.
Methods: The F0 and F1 animals were fed on different macronutrient diets (control, HCD, HFD, HPD) for nine weeks and mated, however F2 animals were fed on standard chow. Glutathione (GSH), Glutathione disulphide (GSSG), lipid peroxidation, Interleukin-6 (IL-6), and Transforming Growth Factor- β (TGF-β) were then determined in F0, F1 and F2 generations using standard methods.
Results: In all test groups, the F2 offspring reflected similar changes in measured variables as observed in F0 and F1 animals.
Conclusion: The results of the study suggest that dietary macronutrient intake in parent generations, could have an effect on developmental programming of antioxidant activity and inflammatory status in offspring.

1. Introduction

It is well-accepted that variations in environmental influences, especially in early life, increase the susceptibility of progeny to developing various conditions as they progress to adulthood. [1,2]. This was defined by Hales and Barker as 'programming', which is the "permanent or long term change in the structure or function of an organism resulting from a stimulus or insult acting at a critical period of early life" [1]. A major phenomenon which largely plays a part in programming is the "predictive adaptive response" which occurs when the fetus undergoes adaptations during the gestational period, or during the early years after birth, based on the predicted postnatal nutritional environment [3]. Basically, during the gestational period, poor nutrition (over-nutrition or undernutrition) in the maternal milieu may result in metabolic adaptations, which have the purpose of preparing the fetus for presumed poor nutritional conditions postnatally. These metabolic adaptations in utero become extremely disadvantageous if the postnatal environment has adequate and abundant nutrients for sustainability of the new-born. Programming thus results in changes in postnatal growth and development, different phenotypes characterised by changes in body composition, changes in metabolism with excess energy storage which might eventually lead to metabolic disorders [1,3].

Diet has been implicated in the pathogenesis of non-communicable diseases (NCDs) like morbid obesity, osteoporosis, non-alcoholic fatty liver disease (NAFLD), various disorders of the renal system, different types of cancer etc. [4]. These effects, attributable to different macronutrients found in diet, have been found to accumulate or reduce fatty acids [5]; elevate or depress liver enzyme function [6]; disrupt metabolism, secretion, and excretion of macronutrients by organs and systems of the body [7]; increase or decrease oxidative stress and activity of antioxidants [8]; and stimulate or depress inflammation in the body [9]. Nutrients are now implicated as causative factors of oxidative stress, because of their ability to cause redox imbalances, leading to pathological consequences due to accumulating reactive oxygen species (ROS) in vivo [10,11]. Inflammatory cytokines, like interleukin-6 (IL-6) and tissue growth factor – β (TGF-β), play important functions in tissue development at particular points in life [12,13], and trigger physiological reactions and several nonspecific immune responses, in response

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to environmental stimuli such as diet [14], but chronic inflammation, however, is linked to the pathogenesis of many NCDs [15–17]. Diet is a major environmental factor which apart from mitigating these effects, affects developmental programming and eventual outcomes in the fetus [18].

We have hypothesized that the macronutrient constituent of diet of parents during development may have long-term consequences on the phenotypic outcome of the offspring. We therefore designed this study to investigate the effects of maternal macronutrient consumption in two generations of rats (F0 and F1) on programming of antioxidant and inflammatory status in F2 offspring.

2. Methods

2.1. Animals

Male and Female Wistar rats from separate litters were obtained after weaning (PND 21) from the Central Animal House of the College of Medicine, University of Ibadan, Nigeria. The animals were housed singly in well-aerated experimental animal cages in the animal house of the Department of Physiology. During this period, they were fed on standard rat chow (Ladokun feeds, Nigeria Limited) and had free access to clean drinking water. They were acclimatised for 7 days prior to commencement of the dietary groupings. All females were nulliparous and the males used for the mating were proven breeders (fertility was certified by the isolated mating technique). This study followed all guidelines in accordance with the International Ethical Norms on Animal Care and Use as contained in NIH publication 80-23, revised in 1985.

2.2. Animal feed

The caloric composition of constituent macronutrients for the pelletised feeds in each dietary group was determined and is as follows:

- Group 1 (Control): Normal rats' chow (26.5% protein, 40% carbohydrates, 29% fat, and 4.5% crude fibre) amounting to 3.2 kCal/g
- Group 2: High Carbohydrate Diet (HCD) (20% protein, 58.5% carbohydrates, 17% fat, and 4.5% crude fibre) amounting to 4.4 kCal/g
- Group 3: High Fat diet (HFD) (22% protein, 13.5% carbohydrates, 60% fat, and 4.5% crude fibre) amounting to 5.2 kCal/g
- Group 4: High Protein diet (HPD) (55% protein, 25.5% carbohydrates, 15% fat, and 4.5% crude fibre) amounting to 3.3 kCal/g

The diets were designed so as to contain 20–25% total protein in order to provide the essential amino acids, in line with the recommendations of the American Institute of Nutrition [19].

2.3. Experimental protocol

For the F0 generation, a total of eighty (80) albino rats (40 males and 40 females), about three weeks old were used. All male animals were confirmed breeders, while virgin female rats with normal estrous cycles were used. The estrous cycle of the rats was assessed daily as described by Marcondes et al. [20]. Proestrous female animals were kept isolated for the duration of the feeding period of nine (9) weeks, but were mated with certified fertile male at a ratio of 1:1 overnight after the ninth week and the presence of sperm in the vagina, or a copulatory plug the next morning, marked GD 1. After confirmation of pregnancy, the female animals were separated. From their progeny (F1), sibling pairs (10 males and 10 females) were selected randomly (a male and female from each pair) as representative samples for each group. These were also fed for a period of nine (9) weeks on the experimental diets after a three-week weaning period. Animals in F0 and F1 generations were euthanised after each 12-week period and lier tissue samples collected for analysis (See Fig. 1). Glutathione content, lipid peroxidation, interleukin-6, and tissue growth factor – β were all assessed. In the final analysis, data from the male animals (F1) were utilised since a preliminary study in our laboratory had shown that female animals did not respond significantly to dietary changes within the experimental period [21].

2.4. Second filial generation (F2) offspring

Randomly selected F1 males from each dietary group were paired with randomly selected F1 females (both were 12 weeks old) from identical dietary groups at a ratio of 1:1 for 2 weeks. The female animals were checked daily. The day after positive confirmation of sperm in their vagina (positive vaginal smear) was taken as GD 1. The animals were continued on the individual diets and allowed to deliver without any other intervention. Glutathione content, lipid peroxidation, interleukin-6, and tissue growth factor – β were assessed in F2 offspring who were fed on the control diet for 9 weeks after weaning. Again, in the final analysis, data from the male animals (F2) were utilised since a preliminary study in our laboratory had shown that female animals did not respond significantly to dietary changes within the experimental period [21].

2.5. Measurement of GSH content

Glutathione content was determined according to the method of Chatuphonprasert et al. [22]. The sample homogenate was deproteinized with SSA and kept at 2–8 °C for 10 min before being centrifuged at 10,000 ×*g* at 4 °C for 10 min. For the determination of total glutathione, the supernatant was mixed with the reaction mixture, which consisted of EDTA, NADPH, DTNB, and glutathione reductase in PBS buffer (pH 7.0).The absorbance of the thiol anions at a wavelength of 405 nm was measured every 60 s for 5 min using a spectrophotometer. To determine the GSSG content, the homogenate was treated with 4-VP before the addition of the reaction mixture and then incubated at room temperature for 1 h. Total glutathione, GSH, and GSSG contents were determined by comparing the net slope of the samples with the slope of the standard curve of GSH or GSSG. The GSH/GSSG ratio was calculated as:

$$\frac{[\text{GSHtotal}]-2\times[\text{GSSG}]}{[\text{GSSG}]}$$

2.6. Estimation of lipid peroxidation

The thiobarbituric acid (TBA) assay was used to determine lipid peroxidation according to the method previously described by Ohkawa et al. [23], which depends on the formation of MDA as an end product of lipid peroxidation when it reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm, an MDA standard was used to construct a standard curve against which readings of the samples were plotted. The sample homogenates were incubated at 37 °C for 1 h before addition of trichloroacetic acid, TBA, and acetic acid. The reaction mixtures were then boiled for 15 min, and the TBA-reactive species (TBARS) were quantified using a spectrofluorometer at an emission wavelength of 551 nm and an excitation wavelength of 528 nm.

2.7. Enzyme-linked immunosorbent assay (ELISA)

For determination of total TGF-β in liver homogenate, TGF-β extracts were prepared according to the procedure described by Roberts et al. [24]. Commercially-available rat ELISA kits for Interleukin-6 (IL-6) and Tissue Growth Factor-β (TGF-β) were procured from Sigma-Aldrich® and used in accordance with manufacturer's protocols and instructions (R&D System, Inc., Minneapolis, USA).

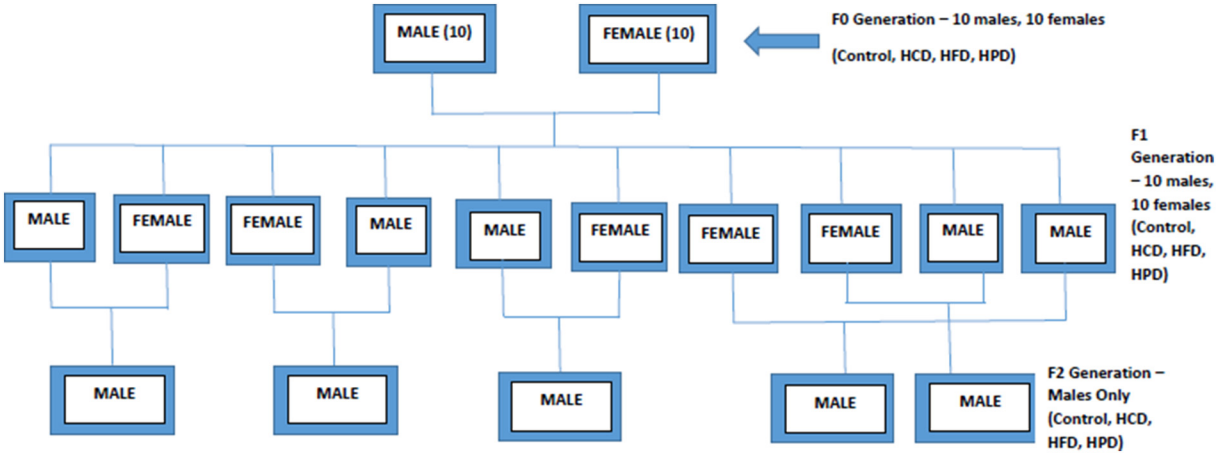

Fig. 1. Experimental design and dietary groupings of animals.

2.8. Statistical analysis

Data obtained were expressed as mean ± standard error of mean (Mean ± SEM). The significance of the results was evaluated using analysis of variance (ANOVA) and the means were compared using Tukey-Kramer Multiple comparison Test. $P < 0.05$ was regarded as statistically significant. Data were analyzed with the use of Graphpad Prism version 7.0 for Windows (GraphPad® Software, San Diego, CA, USA).

3. Results

3.1. Effect of different diets on GSH level in Wistar rats across generations

In the F0 generation, only HFD-fed animals showed a significant decrease ($P < 0.05$) in GSH concentration in comparison with control (Fig. 2). The HFD-fed animals also showed a decrease in GSH level in the F1 generation, while HPD-fed rats reflected a significant increase. The F2 generation (after weaning), however, showed a decrease in GSH level in offspring of HFD-fed rats and a significant increase in HPD offspring.

3.2. Effect of different diets on GSSG level in Wistar rats across generations

No significant differences were observed in GSSG level in all dietary groups in the F0 generation in comparison with control (Fig. 3). However, in the F1 (after experimental feeding) and F2 generations (after weaning), an increase in GSSG was observed in offspring of HPD-fed rats ($P < 0.05$).

3.3. Effect of different diets on GSH/GSSG ratio in Wistar rats across generations

No significant differences were observed in GSH/GSSG ratio in all dietary groups in the F0 generation in comparison with control (Fig. 4). However, in the F1 (after experimental feeding) and F2 generations (after weaning), a decrease in this ratio was observed in offspring of HFD-fed rats ($P < 0.05$).

3.4. Effect of different diets on lipid peroxidation in Wistar rats across generations

In both F0 and F1 generations, all experimental dietary groups had a

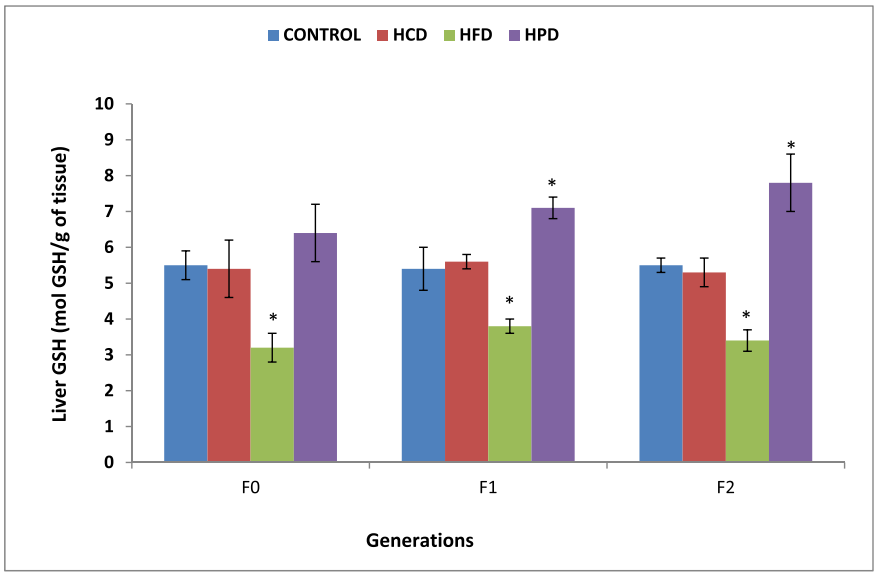

Fig. 2. Comparison of GSH concentration in F0, F1 and F2 generations.
Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

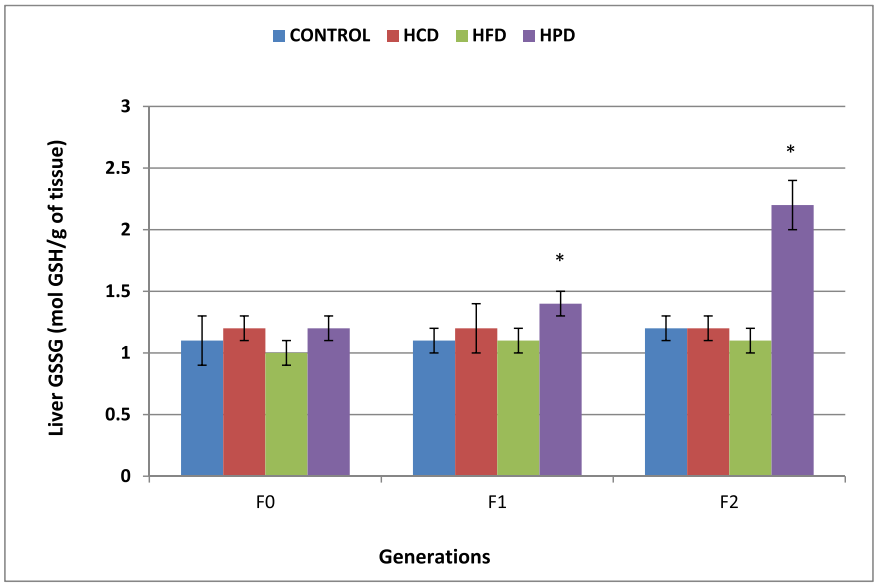

Fig. 3. Comparison of GSSG concentration in F0, F1 and F2 generations.
Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

significant increase ($P < 0.05$) in lipid peroxidation concentration in comparison with control (Fig. 4). The F2 generation (after weaning), also reflected this increase in all offspring.

3.5. Effect of different diets on interleukin-6 level in Wistar rats across generations

F1 and F2 generation animals fed on HFD and HPD showed a significant increase and decrease ($P < 0.05$) respectively on individual comparison with their respective generational controls. The F2 offspring of these two groups reflected a similar change in IL-6 concentration (Fig. 5).

3.6. Effect of different diets on tissue growth factor – β1 in Wistar rats across generations

No significant differences were observed in all experimental dietary groups in the F0 generation, however, the HFD-fed animals showed a significant increase ($P < 0.05$) in TGF-β1 concentration, while the HPD group reflected a decrease in comparison with control (Fig. 6). The F2 generation (after weaning), also showed an increase in TGF-β1 level in offspring of HFD-fed rats and a significant decrease in HPD offspring (Fig. 7).

4. Discussion

It is now a well-established fact that epigenetic factors, such as diet, influence development of particular phenotypes in individuals,

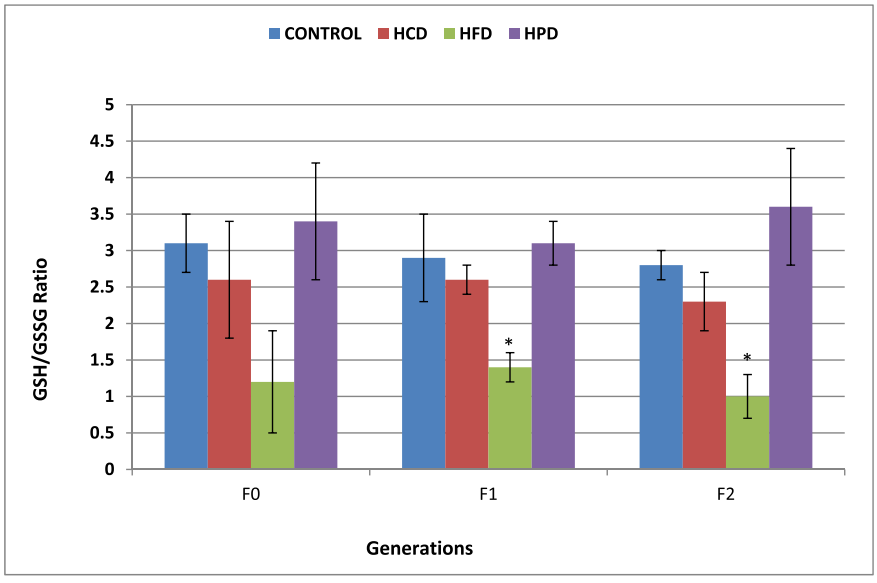

Fig. 4. Comparison of GSH/GSSH ratio in F0, F1 and F2 generations.
Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

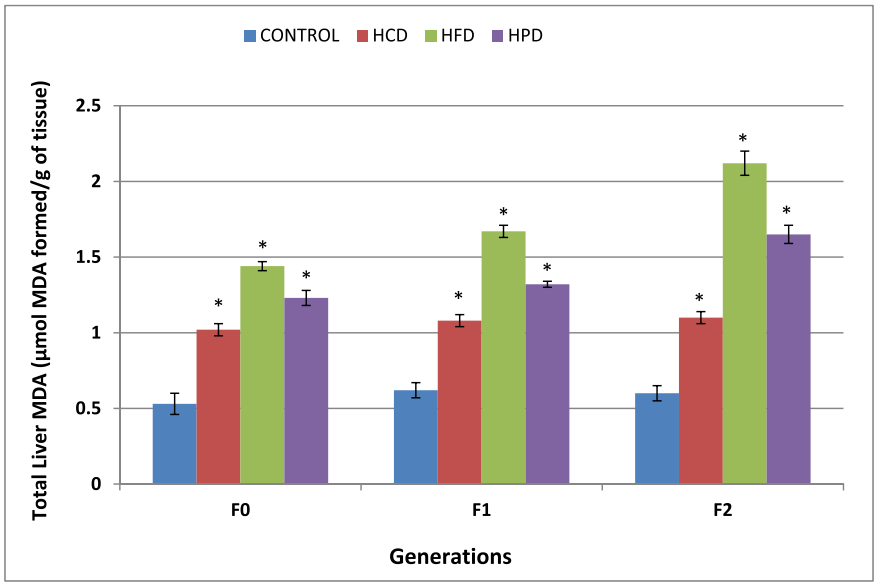

Fig. 5. Comparison of lipid peroxidation in F0, F1 and F2 generations.
Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

phenotypes which could affect development of offspring, affecting eventual phenotypic outcome of progeny [25]. This has been extensively elucidated in various high-fat diet models of obesity and metabolic syndrome, which show that offspring of obese parents are predisposed to certain phenotypes associated with obesity and metabolic syndrome [26]. Associated disorders like NAFLD, insulin resistance and type II diabetes mellitus are linked to increased activity of ROS, and reduction in antioxidant levels, especially GSH, and an elevation in lipid peroxidation in the liver [27]. These ROS are usually generated in the liver via several mechanisms involving mitochondria, peroxisomes, cytochrome P-450, reduced nicotinamide adenine dinucleotide oxidase, cyclooxygenase, and lipoxygenase and results in damage to proteins or unsaturated lipids in the cell membrane [28].

Oxidative stress is characterised by high formation of reactive oxygen species (ROS) as a result of inadequate antioxidant defence systems. This results in alterations in biological structures. Under high-fat conditions, reduction in reduced Glutathione (GSH) activity has been reported [29]. Glutathione is an essential and powerful intracellular and extracellular antioxidant, and the influence of high-fat diet induced obesity on it is confirmed by the findings of this study in both F0 and F1 generations (Fig. 2). It is however significant that offspring in the F2 generation also showed the same decrease in GSH as preceding generations, even though they were only weaned and not fed on the experimental diet. This was also observed in the F2 offspring of the HPD-fed animals who showed an elevation similar to that observed in both F0 and F1, even without consumption of the experimental diet. This suggests an inherent predisposition for this phenotypic reflection due to programming in the progeny from the parents.

When cells are exposed to increased levels of oxidative stress, the oxidised form of Glutathione (GSSG) accumulates, causing the ratio of GSH to GSSG to decrease. As such, GSH/GSSG ratio determination is a useful indicators of oxidative stress in cells and tissues. The GSH/GSSG ratio was decreased in the livers of animals fed on the HFD in the F1 generation. Again, we observed a reduction in this ratio in the F2 offspring in spite of the fact that they were not maintained on the HFD. Lipid peroxidation, as measured by MDA levels, was increased in all

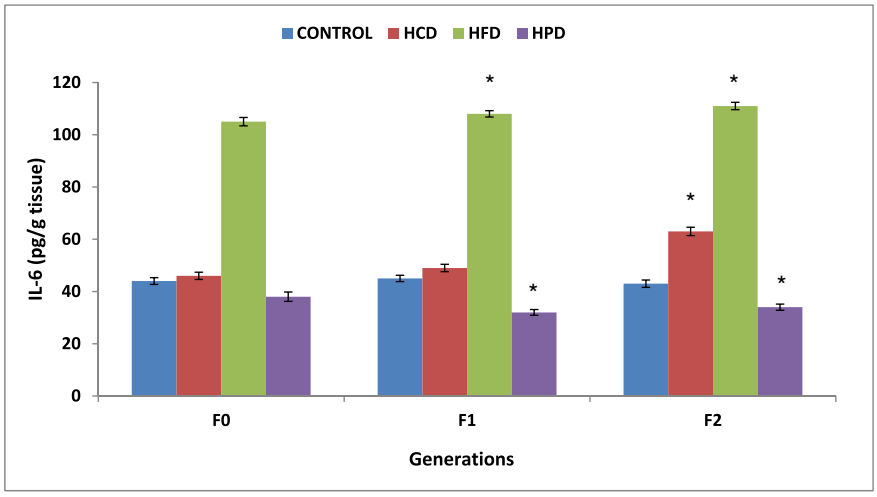

Fig. 6. Comparison of interleukin-6 concentration in F0, F1 and F2 generations.
Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

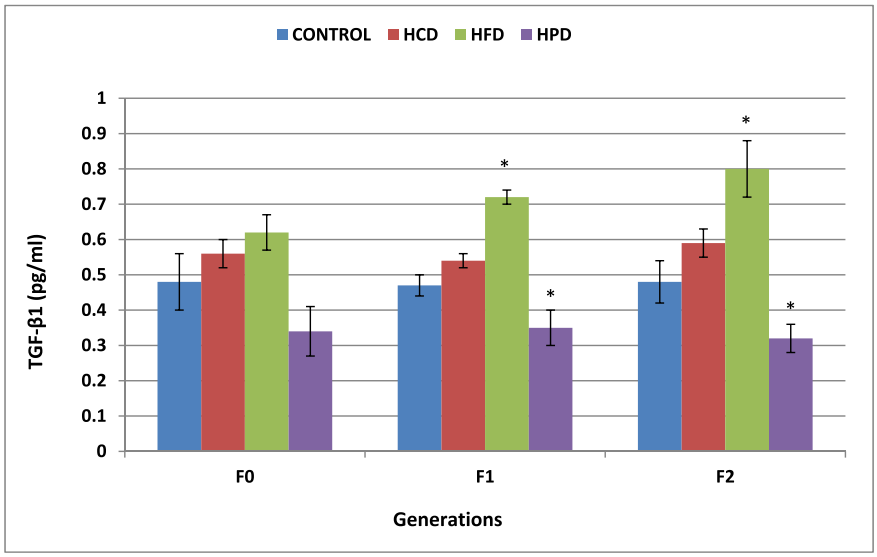

Fig. 7. Comparison of tissue growth factor – β1 concentration in F0, F1 and F2 generations.
Values are mean ± SEM for ten 12-week old male animals per dietary group; $P < 0.05$.
* = significant in comparison with control.

experimental dietary groups (HCD, HFD, HPD) in both F1 and F2 generations. In different studies under different dietary conditions, lipid peroxidation has been shown to progress at varying rates in the liver and our results confirm earlier reports that obesity is an independent risk factor that elevates lipid peroxidation and decreases activity of cytoprotective enzymes [29,30]. Oxidative stress and lipid peroxidation are essential pathophysiological mechanisms involved in NAFLD [31], a disorder associated with obesity, insulin resistance, Type II diabetes mellitus, and hyperlipidemia [32].

Inflammation has been implicated in the pathogenesis of many metabolic disorders and in liver damage [33]. Concentrations of inflammatory markers, especially interleukin-6 (IL-6) and tissue growth factor-β (TGF-β), are directly linked with insulin resistance, metabolic syndrome and chronic liver disease [34,35]. Dietary intake is a main modifiable lifestyle factor that affects inflammation [36]. In this study, F0 and F1 generation HFD-fed rats responded to the diet with an elevation in IL-6 concentration, while the HPD fed animals had a decline in levels of the marker (Fig. 5). Increased IL-6 in animals in response to high-fat feeding has been reported in previous studies [37], while decreased level with high-protein feeding has also been shown in other studies [38]. Interestingly, we report that in the F2 offspring, the offspring from each dietary group again showed an increase (HCD and HFD) and a decrease (HPD) in IL-6 concentration. Considering that the F2 generation animals were not fed on any of the diets, this again indicates that the offspring were predisposed to the changes observed in their parent generations through developmental programming. This was again observed when TGF-β was measured. The F1 generation had a similar decrease in this marker in HFD- fed rats but an increase in HPD-fed animals. As observed with IL-6, the F2 offspring showed a similar trend when compared individually with the generational control. TGF-β is an important inflammatory mechanism that leads to cellular growth and inflammation, however, it plays an important role in the pathogenesis of obesity, influencing the release of inflammatory mediators, increasing remodelling and collagen deposition in the adipose tissue [39].

5. Conclusion

The macronutrient composition of the diet consumed by parents has an effect on phenotypic outcome in offspring, programming changes in antioxidant activity and inflammatory status in offspring.

Acknowledgements

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