



Basic nutritional investigation

Multigenerational effects of dietary macronutrient intake on the metabolic phenotype of male Wistar rats

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ABSTRACT

Objectives: Gene–nutrient interactions are implicated in metabolic phenotypes like metabolic syndrome. The aim of this study was to examine the effects of diet-induced metabolic phenotypes in rats and investigate the effects of these phenotypes in three successive generations.

Methods: Three generations of rats were fed on different diets and mated. Blood glucose, adiposity, lipid profile, insulin, adipocytokines, ghrelin, and corticosterone concentrations were determined in F0, F1, and F2 generations using standard methods.

Results: In comparison with control across generations, glucose (32%), triacylglycerols (52%), and insulin (10%) were significantly elevated in the high-fat diet (HFD)-fed rats; total cholesterol was higher in HFD and high-carbohydrate diet (HCD)-fed groups; whereas high density lipoprotein was higher in the HFD rats but lower in the HPD rats. Adipocytokines were significantly higher in the HCD and HFD groups but lower in the high-protein diet group, whereas ghrelin only declined in HFD rats.

Conclusion: This study revealed that different dietary macronutrients induced distinctive metabolic phenotypes, which had variable effects in different generations.

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Introduction

The thrifty phenotype hypothesis proposes that poor nutrition in utero results in poor fetal growth and increased susceptibility to metabolic syndrome (MetS) [1]. This is because, when the in utero environment is malnourished or overnourished, metabolic adaptations are introduced into the phenotype of the fetus to allow for optimal utilization of resources to survive the harsh, unnatural environment. However, when the newborn is then exposed to a normal environment at birth with adequate optimal nutrition, these metabolic phenotypes continue manifesting and lead to imbalances. This is further explained by the Developmental Origins of Health and Disease, which states that a stimulus at a critical period of developmental plasticity in utero causes disruptions in normal growth and development [2,3]. Thus, a single genotype can give rise to many different phenotypes in response to different environmental conditions during the stages of development [4]. Of all the different possible phenotypes, MetS is the most commonly

manifested, and there is much information and research readily available about it. As a phenotype, its etiology is traceable to over-nourishment; however, undernourished phenotypes also are reported in literature [4].

As a metabolic phenotype, MetS is a constellation of disorders and has become a problem of epidemic proportions, not only in developed countries, but also increasingly so in developing societies. Overall, MetS is believed to be a major health problem and often presents a confounding therapeutic challenge [5]. MetS is characterized by an aggregation of interconnected factors that increase predisposition to coronary heart disease and other forms of cardiovascular disorders, as well as type 2 diabetes mellitus. Elements comprising this syndrome include elevated triacylglycerols (TGs) and apoprotein B–containing lipoproteins, reduced high density lipoprotein (HDL), arterial blood pressure elevation, and imbalances in glucose homeostasis [6]. The main manifestations of MetS, however, are insulin resistance (IR) and abdominal obesity [6]. Obesity, the main predisposing factor to MetS, continues to be one of the leading causes of overall morbidity and mortality in Western societies and its prevalence continues to increase worldwide [7]. It is estimated that by 2030, the number of overweight and obese adults will be ≥ 1.35 billion and ≥ 573 million, respectively [8]. Other abnormalities have been

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added to these factors, including non-alcoholic fatty liver disease and sleep apnea and prothrombotic and proinflammatory conditions [9].

Studies on MetS suggest that the development of a metabolic phenotype is strongly influenced by genetic factors, which explains the increased predisposition of offspring of parents with components of the MetS to it [10,11]. It is now well accepted that early-life insults increase the susceptibility of the offspring to develop several adverse conditions in later life, especially metabolic disorders [10–12], IR [10,11,13], and hypertension [14]. These insults to the offspring are usually consequences of adverse maternal conditions before or during pregnancy and each of these has a negative influence on the gestational milieu leading to important consequences for the growth and development of the fetus and the health of the offspring in adulthood.

Study into other possible forms of diet-induced metabolic phenotypes, especially those due to low-calorie diets, has not been well documented and research into this area is sparse. Because genetics seem to play a vital role in the incidence of these metabolic phenotypes, as seen in MetS, and environmental influences such as diet can influence gene expression of phenotypes, the relationship existing between dietary intake of an individual and the transfer of metabolic phenotypes to the progeny, its etiology, and its consequences, are of interest. This study focused on investigating the changes induced by different diets (composed of varying macronutrient compositions) on phenotype development in a generation of rats and their progeny while exploring the possible mechanisms via which these could occur.

Methods

Animals

Male and female Wistar rats from separate litters were obtained after weaning (postnatal day [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, under standard conditions of 12-h dark–light interval and between 20°C and 25°C maintained throughout the study. During this period, they were fed standard rat chow (Ladokun feeds, Nigeria Limited) and had free access to clean drinking water. They were acclimatized for 7 d before commencement of the dietary groupings. At the commencement of feeding in each dietary group, the body weights of the rats were measured, and this was continued weekly for the duration of the study. All female rats were nulliparous and the male rats that were used for 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.

Animal feed

The caloric composition of constituent macronutrients for the pelletized 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 fiber) amounting to 3.2 kcal/g
- group 2: high-carbohydrate diet (HCD; 20% protein, 58.5% carbohydrates, 17% fat, and 4.5% crude fiber) amounting to 4.4 kcal/g
- group 3: high-fat diet (HFD; 22% protein, 13.5% carbohydrates, 60% fat, and 4.5% crude fiber) amounting to 5.2 kcal/g
- group 4: high-protein diet (HPD; 55% protein, 25.5% carbohydrates, 15% fat, and 4.5% crude fiber) amounting to 3.3 kcal/g

The diets were designed so as to contain at least 20–25% total protein to provide the essential amino acids, which is in line with the recommendations of the American Institute of Nutrition [15].

Experimental protocol

For the F0 generation, 80 albino rats (40 males and 40 females) about 3 wk old were used. Only virgin female rats with normal estrous cycles were used. The estrous cycle of the rats were assessed daily as described by Marcondes et al. [16]. Proestrous female animals were kept isolated for the duration of the feeding period of 9 wk, but were mated with certified fertile males at a ratio of 1:1 overnight after week 9, and the presence of sperm in their vaginal or copulatory plug the next morning marked gestational day (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 on the same diets as their parents for a period of 9 wk, after a 3-wk weaning period. The sibling pairing and feeding process was repeated for a third generation (F2). Animals were sacrificed after each 12-wk period and samples collected for analysis (Fig. 1). All blood samples were collected after an overnight fast. In the final analysis, data from the male animals (F2) were used because a preliminary study in our laboratory demonstrated that female animals did not respond significantly to dietary changes within the experimental period [17].

Measurement of anthropometric variables

Body weight was measured weekly using an electronic weighing balance. The body weight of pregnant dams was also measured from days 1 to 20 starting from confirmation of mating in dams. Feed was weighed daily before it was given to animals in each generation, and the leftovers were weighed at the end of the day. Similar amounts of food was given to animals in each group, but animals were allowed to feed ad libitum. The difference between the two measurements was noted as the daily feed intake. Caloric intake was calculated as follows:

$$\text{Caloric intake (kcal} \cdot \text{g}^{-1}) = \text{daily food intake (g)} \times \text{total energy of food (kcal/kg)} / 1000$$

Measurement of adiposity

The abdomen was opened and the two main abdominal fat pads (epididymal and retroperitoneal), the subcutaneous fat pad, and the stripped carcass were

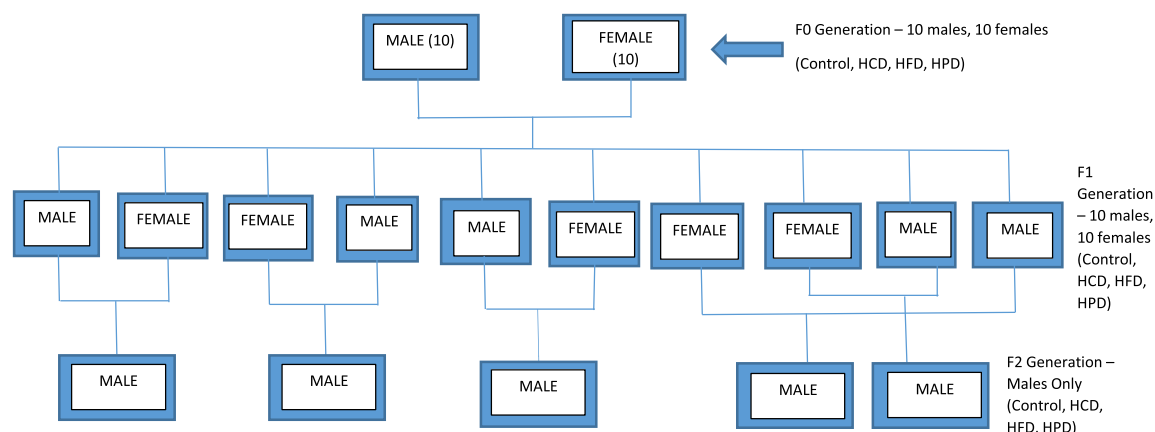


Fig. 1. Experimental design. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

quickly dissected and weighed. Total body fat was measured as the sum of epididymal fat + retroperitoneal fat + subcutaneous fat pad weights. The adiposity index was calculated as (total body fat/final body weight) \times 100.

Determination of fasting blood glucose

Fasting blood glucose was assessed based on the glucose oxidase method of determination [17,18]. Before assessing fasting blood glucose (FBG), the animals were fasted overnight (about 12 h), but were allowed free access to water. Overnight fasting nearly depletes liver glycogen stores in rats. This has the advantage of reducing variability in baseline blood glucose.

Serum collection and hormonal assay

Blood samples were collected from the retroorbital sinus (12 weeks after birth) into polythene tubes and allowed to clot for 1 h. The blood samples were then centrifuged at 3000g for 10 min in a cold centrifuge. Serum was then aspirated and stored at 4°C. The collection of the blood sample was done between 0800 h and 0900 h in the morning. Total cholesterol (TC), TGs, and HDL cholesterol (HDL-C) were determined using a lipid profile kit (Randox Laboratories, Crumlin, UK). Insulin, leptin, adiponectin, and corticosterone were measured using enzyme-linked immunosorbent assay (ELISA). Insulin, leptin, and adiponectin ELISA kits were procured from Sigma-Aldrich (St. Louis, MO, USA). Serum corticosterone was measured using rat corticosterone ELISA kit (Cloud-Clone, Abcam, Cambridge, UK).

Statistical analysis

Data obtained were expressed as mean \pm SEM. The significance of the results for dietary groups within each generation was evaluated using analysis of variance and the means were compared using the Tukey–Kramer Multiple comparison Test. $P < 0.05$ was regarded as statistically significant.

Results

Effects of different diets on body weight over three generations of rats

In the F0 generation, weight was significantly higher in the HCD and HFD groups but lower in rats fed the HPD compared with control. In the F1 generation, HPD was lower, whereas HFD was higher in independent comparisons with their control. By the F2 generation, HCD and HFD again became higher but the HPD was lower in comparison with the control (Table 1).

Effect of different diets on FBG in Wistar rats across generations

In individual comparison with the control, the results obtained show that FBG was significantly elevated ($P < 0.05$) in HCD- and HFD-fed rats in each generation when compared individually with the controls for each generation (Table 2).

Effect of different diets on TG levels in Wistar rats across generations

Serum TG concentration was significantly ($P < 0.05$) higher in HFD-fed animals in each generation of animals in comparison with corresponding generational control animals. The HPD resulted in a lower serum TG concentrations in F0 and F1 generations compared with their controls (Table 3).

Table 1
Body weight (g) in three generations after week 9*

	F0 generation	F1 generation	F2 generation
Control	228.2 \pm 7.1	204.2 \pm 1.9	205.5 \pm 1.2
HCD	257.6 \pm 2.2 [†]	201.9 \pm 3	255.3 \pm 1.4 [†]
HFD	270.5 \pm 3.9 [†]	282.2 \pm 3.8 [†]	320.7 \pm 2.3 [†]
HPD	181.2 \pm 5.6 [†]	162.7 \pm 2.3 [†]	172.3 \pm 2.4 [†]

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.

[†]Significant in comparison with control.

Table 2
Blood glucose level (mg/dL) in three generations after week 9*

	F0 generation	F1 generation	F2 generation
Control	96.9 \pm 8.2	95.3 \pm 5.3	93.2 \pm 6.7
HCD	150 \pm 8.7 [†]	143.1 \pm 7 [†]	147.3 \pm 2.4 [†]
HFD	143.2 \pm 4.8 [†]	170.4 \pm 1.5 [†]	189.1 \pm 4.9 [†]
HPD	112 \pm 5	110.2 \pm 9.4	95.2 \pm 9.1

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.

[†]Significant in comparison with control.

Table 3
Triacylglycerol concentration (mmol/L) in three generations after week 9*

	F0 generation	F1 generation	F2 generation
Control	1.26 \pm 0.23	1.24 \pm 0.10	1.27 \pm 0.40
HCD	1.97 \pm 0.36	2.00 \pm 0.50	2.20 \pm 0.20
HFD	2.90 \pm 0.20 [†]	3.60 \pm 0.50 [†]	4.40 \pm 0.20 [†]
HPD	0.71 \pm 0.06 [†]	0.80 \pm 0.10 [†]	1.00 \pm 0.20

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.

[†]Significant in comparison with control.

Effect of different diets on HDL-C level in Wistar rats across generations

HDL-C was significantly lower ($P < 0.05$) in HFD-fed rats in the F0, F1, and F2 generations, respectively, when individually compared with control animals. HCD- and HPD-fed animals showed a significant elevation in HDL levels by the F2 generation when compared individually with their controls (Table 4).

Effect of different diets on TC levels in Wistar rats across generations

In the F0 generation, TC content was significantly elevated ($P < 0.05$) with HCD and HFD feeding, but it was lower in HPD compared with control. In the F1 and F2 generations, it was higher only in the HFD group but lower in HPD rats (Table 5) compared with controls in each generation.

Effect of different diets on body weight of dams during gestation in Wistar rats across generations

In the F0 generation, the HPD-fed rats reflected significantly lower gestational body weights compared with control on each day of the gestational period (Table 6). Beginning at GD 9, HCD-fed animals had higher body weight than controls, however, higher gestational weight in HFD-fed animals was observed from GD 3. F1 generation animals fed on HCD showed no significant difference in body weight on each day of the gestational period, whereas HFD-fed rats had higher weights from GD 1,

Table 4
HDL cholesterol concentration (mg/dL) in three generations after week 9*

	F0 generation	F1 generation	F2 generation
Control	29 \pm 1.3	30 \pm 0.6	28 \pm 0.5
HCD	30 \pm 2.1	32 \pm 0.9	33 \pm 0.8 [†]
HFD	23 \pm 1.9 [†]	20 \pm 1.3 [†]	17 \pm 1.2 [†]
HPD	30 \pm 0.9	32 \pm 0.5	33 \pm 0.3 ^{*,†}

HCD, high-carbohydrate diet; HDL, high-density lipoprotein; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.

[†]Significant in comparison with control.

Table 5

Total serum cholesterol (mg/dL) in three generations after week 9*

	F0 generation	F1 generation	F2 generation
Control	82 ± 4.1	78 ± 1.4	76 ± 3.1
HCD	98.2 ± 4.6 [†]	71 ± 6.2	74 ± 4.5
HFD	99.7 ± 3.2 [†]	107 ± 7.7 [†]	127.2 ± 8.2 [†]
HPD	73.5 ± 5.3 [†]	64.6 ± 6 [†]	57.8 ± 5.8 [†]

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.[†]Significant in comparison with control.

which lasted until GD 21. The HPD-fed F1 rats reflected similar lowering of daily gestational body weight at each time point as observed in F0.

Effect of different diets on food intake of dams during gestation in Wistar rats across generations

Daily food intake during gestation was significantly lower beginning at GD 9 in the F0 generation dams fed an HCD (Table 7). The HFD-fed F0 dams only had lower intake on GD 15, whereas HPD-fed animals in this generation showed a constant lessening of food intake at each measured time point throughout gestation. In F1 generation animals, the HCD group had a gain in food intake on GD 12, 18, and 21. The HFD-fed rats only had higher food intake on GD 1; however, HPD-fed animals reflected a sustained daily lowering in food intake on all gestational days.

Table 6

Body weight of Dams during gestation (g)*

F0 generation	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	249 ± 7.1	251 ± 5.4	262 ± 5.3	272 ± 2.3	278 ± 2.7	288 ± 6.8	294 ± 4.6	315 ± 3.4
HCD	249 ± 2.2	257 ± 3.5	271 ± 5.4	290 ± 3.3 [†]	316 ± 4.4 [†]	325 ± 4 [†]	331 ± 6.2 [†]	345 ± 6.9 [†]
HFD	260 ± 3.9	272 ± 4.2 [†]	280 ± 1.8 [†]	310 ± 3.3 [†]	329 ± 3.5 [†]	335 ± 4 [†]	346 ± 2.8 [†]	361 ± 6.2 [†]
HPD	180 ± 5.6 [†]	190 ± 8.2 [†]	214 ± 4.5 [†]	222 ± 7.6 [†]	234 ± 4.8 [†]	246 ± 5.5 [†]	259 ± 3.6 [†]	270 ± 5.6v
F1 generation	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	243 ± 1.9	246 ± 2.8	262 ± 3.2	271 ± 2.8	277 ± 2.4	289 ± 4.8	300 ± 5.7	319 ± 4.1
HCD	245 ± 3	255 ± 4.3	263 ± 3.8	273 ± 4.1	289 ± 5.4	296 ± 3.8	310 ± 3.4	332 ± 2.7
HFD	273 ± 3.8 [†]	285 ± 5.6 [†]	295 ± 3.8 [†]	314 ± 5.3 [†]	346 ± 5.5 [†]	356 ± 4.4 [†]	370 ± 3.8 [†]	388 ± 5 [†]
HPD	152 ± 2.3 [†]	162 ± 4.6 [†]	177 ± 5.7 [†]	188 ± 6.3 [†]	201 ± 6 [†]	212 ± 3.2 [†]	221 ± 8.9 [†]	232 ± 6.4 [†]

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.[†]Significant in comparison with control.**Table 7**

Food intake of Dams during gestation (g)*

F0 generation	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	18.6 ± 0.4	18.8 ± 0.6	20 ± 0.3	21.8 ± 0.5	23.1 ± 0.4	24.4 ± 0.4	25.3 ± 0.5	28.4 ± 0.6
HCD	18 ± 0.8	18.2 ± 0.4	19 ± 0.5	19.4 ± 0.6 [†]	19.6 ± 0.4 [†]	21.1 ± 0.6*	23.5 ± 0.3 [†]	26.2 ± 0.7
HFD	16.8 ± 0.7	18.4 ± 0.6	19.5 ± 0.4	20.8 ± 0.6	21.7 ± 0.9	22.6 ± 0.5 [†]	24.6 ± 0.3	26.7 ± 0.6
HPD	14.9 ± 0.5 [†]	15.5 ± 0.7 [†]	15.4 ± 0.5 [†]	16.2 ± 0.4 [†]	16.8 ± 0.5 [†]	16.6 ± 0.4 [†]	18.2 ± 0.7* [†]	19.5 ± 0.8 [†]
F1 generation	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21
Control	18.2 ± 0.5	18.4 ± 0.2	19.4 ± 0.5	20.2 ± 0.6	22.4 ± 0.4	23.8 ± 0.5	24.1 ± 0.3	26.9 ± 0.2
HCD	18.4 ± 0.5	18.4 ± 0.6	19.2 ± 0.4	19.6 ± 0.8	19.9 ± 0.6 [†]	21.8 ± 0.5	22.2 ± 0.5 [†]	24.3 ± 0.3 [†]
HFD	16.6 ± 0.5 [†]	17.8 ± 0.5	19.1 ± 0.3	20.2 ± 0.4	21.2 ± 0.4	23.1 ± 0.6	25.4 ± 0.8	27.6 ± 0.5
HPD	15.4 ± 0.3 [†]	16.2 ± 0.5 [†]	17.2 ± 0.3 [†]	17.1 ± 0.7 [†]	16.9 ± 0.6* [†]	18.2 ± 0.8 [†]	19.2 ± 0.4 [†]	20.1 ± 0.4 [†]

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.[†]Significant in comparison with control.**Table 8**

Average food intake of offspring*

F1 generation Group	Food intake (g)	Calorie intake kcal•g ⁻¹ •d ⁻¹
Control	18.3 ± 0.6	58.6 ± 2.3
HCD	18.2 ± 0.4	80.1 ± 3.8
HFD	16.8 ± 0.6	87.4 ± 3.4
HPD	13.7 ± 0.8 [†]	45.2 ± 1.8
F2 generation Group	Food intake (g)	Calorie intake kcal•g ⁻¹ •d ⁻¹
Control	18.4 ± 0.7	58.9 ± 2.8
HCD	18.3 ± 0.6	80.5 ± 4
HFD	17 ± 0.4	88.4 ± 3.2
HPD	15.2 ± 0.6 [†]	50.2 ± 2.1

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.[†]Significant in comparison with control.

Effect of different diets on food intake of offspring

In both F1 and F2 generations, the offspring in the HPD-fed groups showed lower food intake when compared individually with their respective controls (Table 8).

Effect of different diets on insulin concentration in Wistar rats across generations

Insulin concentration was significantly higher ($P < 0.05$) in the HFD and HPD groups in the F0 generation compared with control.

Table 9

Insulin concentration (pmol/L) in three generations after week 9*

	F0 generation	F1 Ggeneration	F2 generation
Control	518 ± 34	521 ± 21	514 ± 24
HCD	592 ± 41	604 ± 38 [†]	610 ± 28 [†]
HFD	789 ± 20 [†]	801 ± 22 [†]	868 ± 19 [†]
HPD	960 ± 44 [†]	943 ± 46 [†]	878 ± 33 [†]

HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet.

*Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$.[†]Significant in comparison with control.

This was also significantly higher in all dietary groups in the F1 and F2 generations (Table 9).

Effect of different diets on adiposity in Wistar rats across generations

Epididymal fat, retroperitoneal fat, and white adipose tissue were significantly higher ($P < 0.05$) in the HFD groups in each generation, respectively, than the corresponding control (Figs. 2–4).

The HCD, however, showed an elevation in these variables only by the F2 generation. Subcutaneous fat content was higher only in the HFD in the F1 and F2 generations in individual comparison with the control (Fig. 5).

Adiposity index was significantly higher ($P < 0.05$) in the HFD groups in all generations in individual comparison with the control (Fig. 6). This was also higher in the F2 generation of animals fed on the HCD.

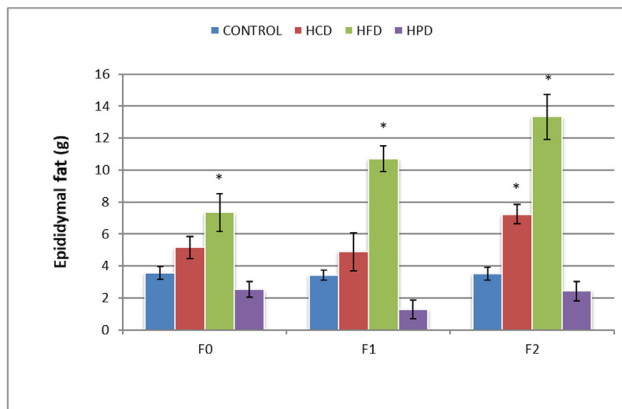


Fig. 2. Comparison of epididymal fat content across three generations. Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

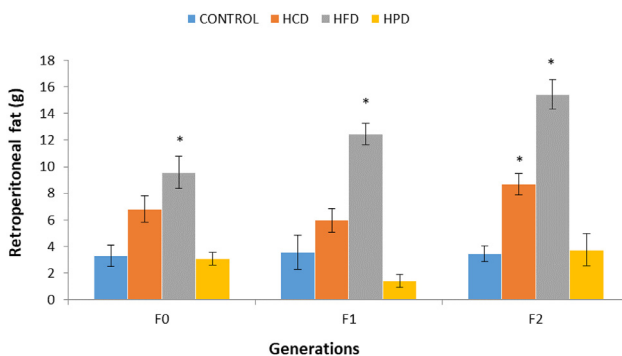


Fig. 3. Comparison of retroperitoneal fat content across three generations. Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

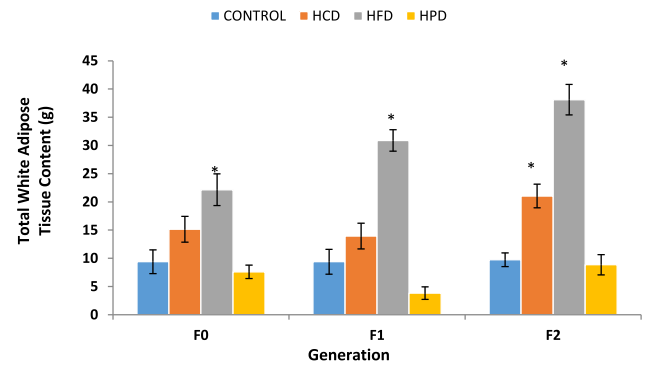


Fig. 4. Comparison of total white adipose tissue content across three generations. Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

Effect of different diets on leptin concentration in Wistar rats across generations

In comparison with F0 control, leptin levels were significantly higher ($P < 0.05$) in the HFD group but lower with HCD feeding (Fig 7). In the F1 and F2 generations, leptin concentration was also

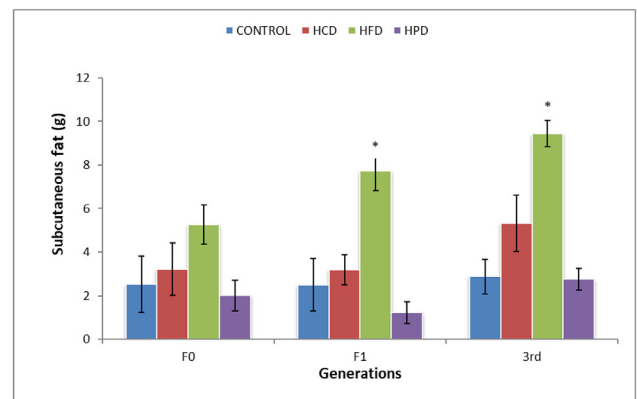


Fig. 5. Comparison of subcutaneous fat content across three generations. Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

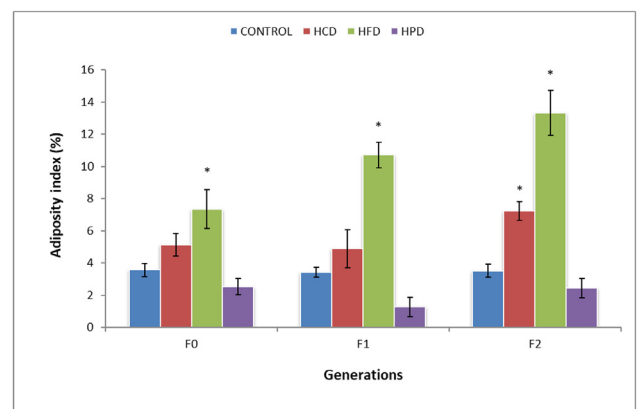


Fig. 6. Comparison of adiposity index across three generations. Values are mean ± SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

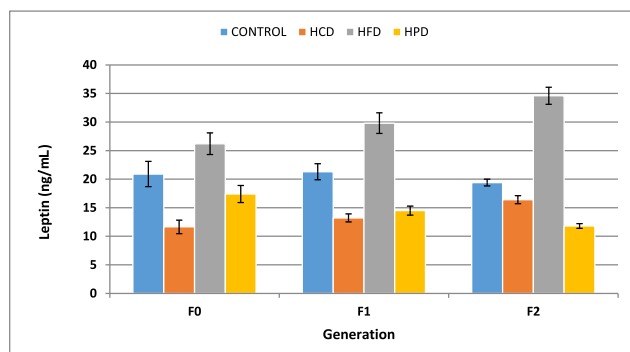


Fig. 7. Comparison of leptin concentration across three generations. Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

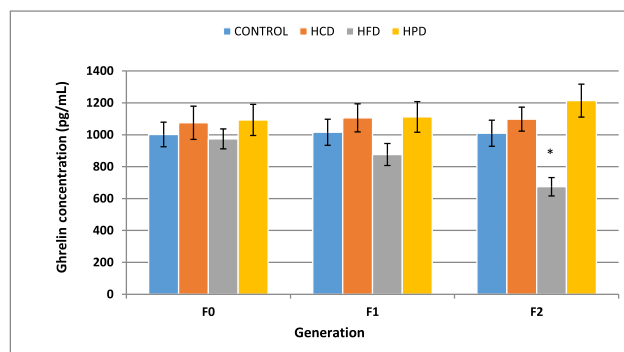


Fig. 9. Comparison of ghrelin concentration across three generations. Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

higher in the HFD group but lower in both HCD and HPD groups than in the control group.

Effect of different diets on adiponectin concentration in Wistar rats across generations

In each of the three generations, with individual comparison with their respective controls, the HCD and HFD groups had significantly higher ($P < 0.05$) concentrations of adiponectin (Fig. 8). However, within each generation, the HPD rats had lower adiponectin levels.

Effect of different diets on ghrelin concentration in Wistar rats across generations

Ghrelin concentration was only significantly lower ($P < 0.05$) in the F2 generation of rats fed with the HFD in individual comparison with the control animals for each generation (Fig. 9).

Within each generation, HPD feeding resulted in significantly higher levels of corticosterone ($P < 0.05$). In HFD-fed rats, there was lower corticosterone concentration in the F0 and F1 generations (Fig. 10).

Discussion

Structural changes can take place in DNA that do not change the sequence of the DNA and can bring about alterations in gene

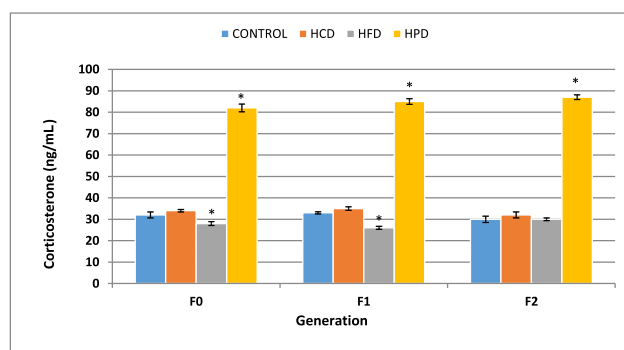


Fig. 10. Comparison of corticosterone concentration across three generations. Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

expression [19]. Basically, these changes, which are due to external environmental influences such as diet, do not alter the genotype but result in altered expression of phenotypes. These modifications have been implicated in the etiology of many non-communicable diseases, including type 2 diabetes [20]. This regulation was initially thought to occur only during the early developmental stages of the germ cell and in embryos before implantation, and to be sustained throughout the life span [21]; however, new research has shown that such events can occur in response to many different environmental influences that persist throughout life and do not necessarily have to be permanent modifications [21].

The increase in anthropometrics, hyperglycemia, hyperinsulinemia, hypertriglyceridemia and decreased HDL-C levels observed in the HFD-fed animals was consistent with the development of MetS [5]. This is increased across generations, suggesting an enhancement of the phenotype and increased predisposition to MetS owing to the influence of this diet, resulting in typical physical manifestations. However, interestingly, the HCD groups reflected the MetS phenotype in the F0 generation, but this was eliminated in the F1 generation only to resurface in the F2 generation. The HPD groups also showed a characteristic phenotype suggesting an adaptation to the macronutrient content of diet over generations; however, HDL-C concentrations in each generation increased with this diet. The implications of these results is that these dietary types exhibit singularly different phenotypes, which confirms the influence of diet on phenotype and that this influence is sustained (depending on the diet) and is higher with different

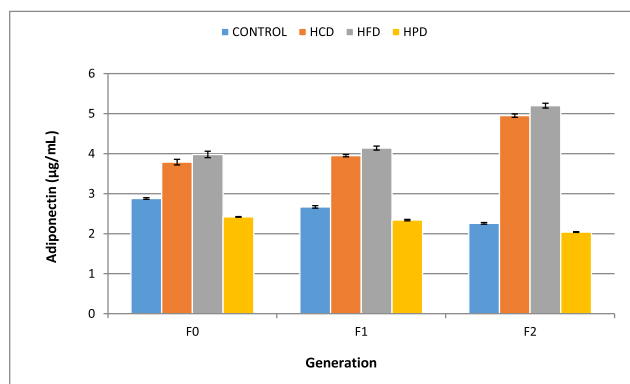


Fig. 8. Comparison of adiponectin concentration across three generations. Values are mean \pm SEM for 10 12-wk-old male animals per dietary group; $P < 0.05$. HCD, high-carbohydrate diet; HFD, high-fat diet; HPD, high-protein diet. *Significant in comparison with control.

effects over generations. This might, in part, be attributed to dietary intake in the mothers in each generation. Results shown in Table 4 indicate that pregnant F0 and F1 dams consumed the diets in large quantities in the control, HCD, and HFD groups; however, HPD animals consumed a low quantity of their feed during gestation. This resulted in the low body weight observed in pregnant dams fed the HPD diet (Table 3). This was also reflected in their offspring, as results showed low intake of the HPD with relatively high consumption in all other diets (Table 5). It is noteworthy that the HPD, in comparison with the HCD and HFD, did not have low-caloric content, and as such the effects observed would be due to the macronutrient itself and an ability to compound the effects of low energy intake because of low consumption on various metabolic factors in the mothers and metabolic programming in offspring in each generation [22,23].

The macronutrient composition of diet affects adipose tissue hormones such as leptin [24]. Leptin is believed to function as a negative feedback signal essential for the regulation of energy balance. Animals fed the HPD and HCD had lower leptin levels than control rats in all generations; however, it was observed that leptin was increased in the HFD over generations. The observed hyperleptinemia in HFD-fed rats is buttressed by the increased and sustained adiposity in these animals across generations. Furthermore, in the HPD group, leptin levels were decreased continuously over generations, with the F2 generation showing the lowest concentration compared with the other two generations (F0 and F1). Leptin prevents the build-up of lipid in adipocytes by increasing the turnover of TGs, inhibiting basal and insulin-stimulated *de novo* lipogenesis while stimulating glucose and free fatty acid oxidation [25]. Leptin also directly exerts an inhibitory effect on insulin secretion from the pancreas [26]. A high leptin concentration, as observed in the HFD rats (MetS), is a feature of leptin resistance, which occurs in obese animals. Leptin has been shown in several studies to have no effect on food intake or body composition of animals that expressed leptin receptors but were obese. These animals all manifested elevated circulating concentrations of endogenous leptin [27,28]. Hyperleptinemia, which was observed in the F0 generation, can be added to the increased adiposity in the animals. However, our studies showed that by the next generation (F1), the concentration of leptin was higher and this was same in the F2, suggesting that leptin resistance was intensified across generations. This could in part be due to the increase in adiposity seen from one generation to the next, but also might suggest an increased predisposition and reaction to leptin resistance in the animals over generations. Maternal metabolic states, which earlier were added to differences in feed intake and calorie content of each diet, is another factor that could affect leptin signaling in progeny [29]. The HCD also resulted in an increase in leptin concentration in each generation, which is directly related to a generational increase in adiposity. This suggests that increased predisposition is more likely responsible for leptin resistance seen in the both the HCD and the HFD groups, which would suggest a change in phenotype toward increased hyperleptinemia. The HPD resulted in a decline in leptin levels across generations. This is consistent with low adiposity in this dietary group, but also could point to a resultant phenotypic adaptation to the diet.

Adiponectin levels were elevated in the HCD and the HFD across generations. The finding in the HFD contrasts with reports that the HFD had an association with decreased adiponectin concentration [30]. However, it confirms studies in humans that showed a positive association between total fat intake and adiponectin level [31], and also studies in animals [32]. Adiponectin is produced and secreted

by adipocytes [33,34] predominantly in the intraabdominal compartment and this adipokine is positively associated with insulin sensitivity [35,36]. An increase in intraabdominal fat, as observed in the HFD in this study, decreases adiponectin, which results in an antagonism of insulin's effects in liver and peripheral tissues, primarily muscle. As adiponectin enhances insulin's effect to suppress hepatic glucose production, reduced adiponectin will decrease the effect of insulin to inhibit hepatic glucose production [37]. Recent studies have shown that epigenetic changes might result in modifications of adiponectin gene in MetS, which give rise to multigenerational changes in levels of adiponectin [38]. This eventually results in abnormalities in glucose and lipid metabolism because of genetic variations that lead to genotypic and phenotypic associations with MetS. Accordingly, the high levels of adiponectin observed in this study suggest that in each generation, under obese conditions, there is a change in the adipokine's phenotype, a possible adaptation by adiponectin-producing cells to maintain insulin action. Insulin sensitivity, as discussed, is maintained by adiponectin, as such, the results indicate that in a bid to maintain this action, levels of adiponectin increase, suggesting an alteration in phenotype that results in resistance to adiponectin actions in MetS. This suggests that just like leptin resistance occurs in obesity and MetS, hyperadiponectinemia and adiponectin resistance could be a consequence of HCD and HFD feeding. Protein diets have an insulinotropic effect and promote insulin secretion [39]. The result of this study shows that over generations, adiponectin level was decreased, which suggests that in stimulating insulin action, the HPD caused a decrease in adiponectin level, which as discussed earlier, will result in an increase in insulin stimulation and sensitivity.

Chronic injection of ghrelin stimulates food intake and decreases energy expenditure leading to overweight development [40]. This hormone is also sensitive to the fat and carbohydrate content of the diet [41]. Ghrelin is an endogenous orexigenic peptide produced in the stomach and involved in short-term regulation of food intake. Its concentration in plasma increases before meals and decreases very strongly postprandially [42]. In the F1 generation of rats in this study, a ghrelin decrease in the HFD group was observed. By the F2 generation, ghrelin levels showed greater decreases, which was highly significant, and is also observable to less effect in the F0 and F1 generation. This is in line with the known responses of ghrelin secretion to increased adiposity. This is an indication that exposure of ghrelin to fat and increased obesity resulted in phenotypic changes in ghrelin action from one generation to the next and points to an increase in sensitivity of genes responsible for ghrelin action. In the present study, high-carbohydrate intake did not have an effect on ghrelin release contrary to the results of Soriano-Guillen et al. [43].

Corticosterone concentration showed significant increase in each generation of both HFD- and HPD-fed animals. However, the HFD caused an initial decrease in corticosterone level in the F0 and F1 generations compared with the control, and then subsequently showed an increase by the F2, but this increase was not significant compared with the control. Pathophysiological levels of glucocorticoids as observed in HPD-fed animals, result in muscle protein breakdown as well as systemic IR, whereas normal physiological levels are crucial for mobilizing stored fuel for the "fight or flight response" as well as for replenishing fat stores when insulin levels rise with feeding. Glucocorticoids have diverse effects on adipose tissue biology and are required for induction of lipogenic genes, regulation of lipolysis [44], and adipose endocrine function [45]. They also play an important role in restraining adipose tissue inflammation in obesity [46]. The corticosterone increase might

contribute cooperatively with ghrelin to replenish some of the energy stores through their combined action in adipose tissue [47]. However, HPD rats might be more susceptible to weight regain after a dietary change by stimulating fat intake through their high corticosterone status [48]. In addition, corticosterone increase is a sign of activation of the hypothalamic-pituitary-adrenal (HPA) axis. This HPA activation is partly dependent on the macronutrient composition of the diet [49,50]. These results agree with previous experiments showing the absence of variations in basal corticosterone levels after exposure to a HFD [49,51,52]. However, it is impossible to exclude a change in sensitivity to stress in the HFD animals as HFD augments HPA responsivity to restraint stress with an effect that may vary with the duration of exposure to the high-fat content [51–53]. The high generational basal corticosterone measured in the HPD rats was unexpected, although corticosterone increase has been observed after a high-protein meal [54]. This can be adduced to an alteration of the HPA axis in these animals. It is therefore possible that these rats will react differently, with increasing generations, to environmental cues that are either related to nutrition or to other behaviors (e.g., fear, aggressiveness, etc), when compared with other dietary groups. Maternal corticosterone status also has been reported to have a significant influence on offspring corticosterone level [55].

Conclusion

The dietary macronutrient composition of a diet has a significant effect on the phenotype expressed; phenotypes that are observed from one generation to the next, predisposing individuals to certain metabolic variations; some in adaptation to correct the effects of abnormal nutrition in offspring, others further complicating metabolic states. Further studies are needed to determine the mechanisms involved in these different diet-induced metabolic phenotypes and their inheritance.

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