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Research article

# Soda intake influences phenotype, antioxidants and inflammatory status in high protein-fed wistar rats

Temitope Gabriel Adedeji *, Sikirullai Olatunde Jeje, Tosan Peter Omayone, Boluwatife Olamide Dareowolabi

*Department of Physiology, School of Basic Medical Sciences, Federal University of Technology, Akure, Nigeria*

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ABSTRACT

An increasing population of people, especially young adults who exercise, consume high protein diets along with carbonated drinks. While there are numerous studies on the effect of high protein diets, there is a need to understand how protein diets in combination with carbonated drinks impact physiology. In order to assess these effects on wistar rats' phenotype, antioxidants and inflammatory profiles, 64 wistar rats were divided into dietary groups of 8 male and 8 female animals each. The animals were fed standard diet as control (chow), chow and carbonated soda, a high protein diet (48.1% energy from protein) and a high protein diet with carbonated soda according to their groups.

Body measurements, blood glucose levels, serum insulin levels, lipid peroxidation, antioxidant activity, adipokines and inflammatory markers concentrations were all determined. At the end of the study, body measurements, inflammatory markers and adipokine concentration were increased in animals fed the high protein diet and high protein-soda diet. There was a decrease in antioxidant and lipid peroxidation levels in protein fed male and female animals but those fed protein in combination with soda had increased lipid peroxidation levels.

In conclusion, high protein diet in combination with carbonated soda impacts physiology differently from a high protein diet alone, and may stimulate weight gain, oxidative stress and HPD-related inflammation in Wistar rats.

## 1. Introduction

Most meals, in both developed and developing countries, are a combination of the different food groups-carbohydrates, proteins, fats and oils, mineral salts, vitamins and water [1] in the right proportions with carbohydrates making up about 45–65% of total energy intake [2]. However, meals have changed over time as a result of environmental influences, changing economic conditions, and most importantly, health factors [3]. Health or more often ill-health is an important factor that determines dietary choices in humans. In order to combat diseases like obesity, cancers, neurodegenerative disorders like Alzheimer's disease and epilepsy, and diabetes mellitus, customized or modified diets are taken into consideration [4]. One of such diets is the high protein diet, a diet in which intake of protein exceeds 2.0 g/kg/day [5].

In many parts of the world and especially in West Africa, the prevalent food group is carbohydrates, with the most common source of this being rice in countries like Nigeria [6]. Recently however, diets high in protein are becoming increasingly popular among

* Corresponding author.
*E-mail address:* tgadedeji@futa.edu.ng (T.G. Adedeji).

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certain groups of people: obese individuals [7] and young adults looking to build muscles or following a workout regimen [8]. High protein diets are recommended for obese people because of the ability of protein to increase satiety [9] and its high thermic effect [10], which lead to a negative energy balance, calorie deficit and consequently, weight loss [11] For people looking to gain muscle mass, protein, in the form of shakes, smoothies, powder and bars, is often recommended [12], because its constituent amino acids are involved in repair and maintenance of muscle mass [13].

A growing population of young adults combine sugar sweetened beverages with their meals [14]. The average consumption of Coca-Cola, one of the leading manufacturers of soda, in 2012 was about 94 portions per person per year [15]. These drinks, commonly called soda, are a primary source of added sugar in the diet as they make up more than 40% of the added sugar in many diets [16], even though the recommended daily allowance for added sugar is about 25% of total caloric intake [17]. Most sugar sweetened beverages contain high fructose corn syrup [18], a type of sweetener that contains about 45–55% fructose, which has been implicated in the rise of obesity and metabolic syndrome [19]. Sugar sweetened beverages have been implicated in the development of obesity, metabolic syndrome and even increased systolic blood pressure [20].

Different combinations of diets are thought to affect the phenotypes of organisms, and studies have been carried out using experimental models to discover the effects of different dietary combinations on physiology and pathology. For instance, fatty diets may increase the risk of oxidative stress [21], while high carbohydrate diets and sugary foods (of which soda is a major example) are main contributors to the development of obesity and diabetes [22]. Both high protein diets [23] and sugar sweetened beverages [24] have been linked to insulin resistance, a major feature of diabetes [25], which can develop as a result of inflammation, obesity [26] etc.

In this study, we assessed the effect(s) of a diet high in protein (48.1% of total energy derived from protein) combined with a sugar sweetened beverage (soda) on body measurements, mediators of inflammation and the reactive oxygen species as shown in Table 2. Our main aim was to investigate if the impact of a high protein and soda diet on these variables is different from that of a high protein diet only, while determining if the resultant effects exhibit sexual dimorphism.

## 2. Materials and methods

### 2.1. Animals

A total of 64 Wistar rats (32 males and 32 females) with average initial weights of between 60 and 80 g were used in this study. Rats of the same sex were randomly sorted into groups of eight animals each at the beginning of the experimental period. The animals were housed in standard, well-lit and well-ventilated cages. Acclimatization period for the animals was the first twenty-one days of the study in which animals had access to standard rat chow and pure water *ad libitum*. Total duration of the experimental feeding period was fourteen (14) weeks.

### 2.2. Ethics statement

Animal care was conducted according to the National Institute of Health guidelines for the care and use of laboratory animals. All experiments were approved by the Ethics Committee of the Federal University of Technology Akure, and the protocols were designed and conducted to minimize suffering and discomfort to all animals involved.

### 2.3. Animal feed composition

The feed composition is shown in Table 1. Diet ingredients were obtained and pelletized by Ladokun Feeds® to facilitate even and easy consumption by all animals within each group. Pellet preparation was done with minimal heat treatment at every step of the process to prevent denaturing of essential vitamins and amino acids. The diets were designed by an in-house nutritionist in line with recommendations from the American Institute of Nutrition as previously described [27].

**Table 1**
Feed composition (SD-Standard Diet; HPD-High Protein Diet).

| COMPONENT (kg) | SD | HPD |
|---|---|---|
| Groundnut cake | 10.0 | 10.0 |
| Soya | 10.0 | 10.0 |
| Palm Kernel cake | 4.0 | 4.0 |
| Maize | 10.0 | 5.0 |
| Wheat offal | 10.0 | 5.0 |
| Fish Meal | 4.0 | 14.0 |
| Bone Meal | 2.0 | 2.0 |
| Methionine | 0.1 | 0.1 |
| Lysine | 0.1 | 0.1 |
| Premix | 0.1 | 0.1 |
| Salt | 0.1 | 0.1 |
| Butter | 0.0 | 0.0 |
| Total | 50.4 | 50.4 |

### 2.4. Proximate analysis

Proximate analysis for the macronutrient composition of the diets was done as previously described [28]. Moisture content of the diet was assessed by drying 5 g of feed in a pre-weighed crucible placed in an oven, heating to 100 °C followed by cooling repeatedly until a constant weight was observed. Moisture content (%) was calculated using Eq. (1) below:

$$\frac{Initial\ weight(g) - Final\ weight(g)}{Weight\ of\ sample(g)} \times 100 \tag{1}$$

To determine the fat content of the diet, we mixed 1 g of the diet sample, methanol and chloroform (2:1) for 20 min using a vortex mixer, followed by addition of 1 ml of chloroform and 1.8 ml of distilled water. The mixture was centrifuged to evaporate the organic layer and the fat content determined by the weight differences before and after the procedure.

For protein content determination, we mixed 100 mg of the diet sample with 1 g of a digestion mixture of Copper sulphate, Selenium and Potassium sulphate (1:1:20). This mix was digested along with 20 ml of concentrated sulfuric acid in a Kjeldahl flask. 10 10 ml of the digested sample and 10 ml of 40% Sodium hydroxide were distilled and the ammonia released was collected into a container with 25 ml of 4% Boric acid and methylene blue. Using 0.02 N Hydrochloric acid, we back-titrated the resultant mixture as well as a reagent blank, and then calculated protein content using Eq. (2) as follows:

$$\frac{Acid\ required\ to\ neutralize\ diet\ sample\ (ml)\ X\ Acid\ required\ to\ neutralize\ blank\ (ml)\ X\ Final\ volume}{Weight\ of\ sample\ (g)\ X\ aliquot\ volume\ (ml)} \tag{2}$$

To determine crude fibre content of the diet, we boiled a mixture of 2 g of moisture- and fat-free samples and 200 ml of 0.255 N Sulfuric acid for 30 min. After washing the residue with hot water, it was boiled with 200 ml 0.313 N Sodium hydroxide for 30 min. The precipitate was washed with hot water again, followed by washing with alcohol and ether wash (We) before drying overnight at 80–100 °C and weighing. The crucible was heated again in at 600 °C for 2–3 h, cooled and weighed again (Wa). The difference in the weights (We-Wa) represented the weight of crude fibre. This was calculated using Eq. (3) below:

$$\text{Crude fibre} = \frac{\{[100 - \text{Moisture (g)} + \text{Fat (g)}]\ \text{X (We} - \text{Wa)}\}}{\text{Weight of moisture and fat} - \text{free sample}} \tag{3}$$

For ash content of the diet, 5 g of feed was heated for 3–5 h at 600 °C repeatedly until a constant weight was obtained and the ash was white or grey white in colour.

Carbohydrate content was calculated using Eq. (4):

$$100 - [\text{Moisture content (g/100g)} + \text{Protein content (g/100g)} + \text{Fat content (g/100g)} + \text{Ash content (g/100g)} + \text{Crude fibre content (g/100g)}] \tag{4}$$

The caloric composition of constituent macronutrients for the pelletized feeds in each dietary group was determined and is shown in Table 2. The quantity of each purified macronutrient (carbohydrates, fats, and proteins) per 100 g, from the mix, was used to calculate the calorie contribution of each diet.

Calorie content = quantity (g) x energy content of 1 g of macronutrient (kcal)

where:

$$1\text{g fat} = 9\text{kcal}, 1\text{g carbohydrate} = 4\text{kcal}, \text{and } 1\text{g protein} = 4\text{kcal}. \tag{5}$$

$$\text{Total diet calorie content} = \text{kcal (CHO} + \text{protein} + \text{fat)} \tag{6}$$

$$\text{Calories (\%) from a macronutrient} = \text{kcal macronutrient/kcal (CHO} + \text{protein} + \text{fat)} \tag{7}$$

$$\text{Energy intake / rat} = \text{Daily ingestion (g) x macronutrient calorie content} \tag{8}$$

A popular high fructose corn syrup-sweetened carbonated soda drink was given *ad libitum* to animals in the treatment groups and continued throughout the study. Animals taking water also had *ad libitum* access to clean water throughout the entire duration of the study. A serving 100 ml size bottle of carbonated soda according to the manufacturer contains 1.79 kJ/ml; 0.11 g/ml carbohydrate, 0.05 mg/ml of sodium.

**Table 2**
The caloric composition of constituent macronutrients for the pelletized feeds.

| | Standard Chow (% of diet/E%) | High Protein Diet (% of diet/E%) |
|---|---|---|
| Protein | 26.5%/20.1% | 55%/48.1% |
| Carbohydrates | 40%/30.4% | 25.5%/22% |
| Fat | 29%/49.5% | 15%/30% |
| Crude Fiber | 4.5%/0% | 4.5%/0% |
| Total Calories | 5.27 kcal/g | 4.57 kcal/g |

### 2.5. Animal groupings

Animals of both sexes were divided into four dietary groups (control, control + soda, high protein diet (HPD), and high protein diet + soda (HPD + soda) as shown in Table 3.

### 2.6. Experimental procedure

The sixty-four weanlings were grouped according to their dietary interventions after three weeks of acclimatization (Table 3). Each group had eight animals of the same sex and a total of eight groups (four for each sex) were formed. 150 g of each feed was weighed and served to each group each day, and at the end of each 24-h period, the feed remaining was weighed. At the end of the study, the average feed, soda and calorie intake of the animals in each dietary group was calculated (Table 4).

A common carbonated soda was provided *ad libitum* to the animals in the soda groups while the animals being treated with water had free access to clean water throughout the day. Body measurements comprising of body weight, thoracic circumference and abdominal circumference were conducted weekly. At the end of the period of study, the animals were euthanized by $CO_2$ inhalation, and tissues were collected for experimental analysis.

### 2.7. Body measurements

At the end of each week, the body weight of each animal was taken with the aid of a Camry® electronic weighing scale, which has a capacity of 1500 g and an accuracy of 1 g. To measure the abdominal and thoracic circumference, a non-elastic tape measure was used. The largest zone of each animal's abdomen was measured with the non-extensible tape to assess the abdominal circumference while thoracic circumference was measured at the site immediately behind the fore-leg.

### 2.8. Measurement of blood glucose

Blood glucose was assessed once every third week with the aid of an Accuchek® glucometer through the capillary blood glucose method [29].

### 2.9. Collection of blood and serum

After thirteen weeks of study, blood samples were collected from each animal via the retro-orbital sinus. The blood samples were centrifuged at 8000 g for 10 min, serum was aspirated and then stored at $-20^{o}$c for further assays.

### 2.10. Sacrifice of animals

At the end of the 14-week period, animals were euthanized by $CO_2$ inhalation. The liver of each animal was harvested for antioxidant and inflammatory assays.

### 2.11. Determination of hormone levels

Serum concentrations of insulin, TNF-α, leptin and adiponectin were determined via Enzyme Linked Immunosorbent Assays (ELISA) [30]. The kits for this procedure were purchased from Bioassay Laboratory technology in Wuhan, China.

### 2.12. Assessment of lipid peroxidation

Malondialdehyde levels were determined via the Thiobarbituric acid (TBA) method [31].

### 2.13. Assessment of antioxidant levels

Catalase levels were determined using Claiborne's method which assesses the decomposition of $H_2O_2$ at 240 nm [32], while Superoxide dismutase was evaluated by the indirect spectrophotometry method of Pyrogallol [33]. Glutathione levels were determined by the method of Beutler et al. [34].

**Table 3**
Animal groupings according to Dietary interventions (HPD- High Protein Diet).

| S/N | Animal Groupings | Dietary Intervention |
|---|---|---|
| 1 | Chow Group | Standard Chow and water |
| 2. | Soda Group | Standard chow and carbonated soda |
| 3. | HPD group | High Protein Diet and water |
| 4. | HPD + Soda group | High Protein Diet and Carbonated Soda |

**Table 4**
Average feed and calorie intake.

| Diet | Average Feed Intake (g) | Feed Calorie intake kcal.g.d$^{-1}$ | Total (feed + soda) Calorie intake kcal.g.d$^{-1}$ |
|---|---|---|---|
| Chow (M) | 18.3 ± 0.6 | 96.3 ± 6.1 | 96.3 ± 6.1 |
| Chow + Soda (M) | 14.2 ± 1.2* | 74.8 ± 7.5* | 111.8 ± 5.6 |
| HPD (M) | 13.7 ± 0.8* | 62.5 ± 5.4*** | 62.5 ± 5.4* |
| HPD + Soda (M) | 11.8 ± 1.4*** | 53.7 ± 6.2**** | 83.7 ± 7.3 |
| Chow (F) | 15.6 ± 1.7 | 82.2 ± 5.8 | 82.2 ± 5.8 |
| Chow + Soda (F) | 14.8 ± 2.0 | 77.9 ± 5.5 | 109.1 ± 7.7* |
| HPD (F) | 12.9 ± 1.2 | 59.0 ± 6.8* | 59.0 ± 6.8 |
| HPD + Soda (F) | 11.5 ± 0.9 | 52.6 ± 5.0** | 80.1 ± 8.2 |

(M-Male; F-Female; HPD-High Protein Diet).
Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. n = 8.

### 2.14. Statistical analysis

For data analysis, GraphPad prism version 9.0 was used. The values were presented as Mean ± SEM and analyzed using one or two-way ANOVA. Tukey's posthoc Multiple comparisons was used to detect significant differences between the means with $P < 0.05$.

## 3. Results

### 3.1. Feed consumption, soda and calorie intake

In male rats, average feed intake was lower in all treatment groups when compared individually with control, with the lowest feed consumption observed in HPD-soda rats (Table 4). Calorie intake from feed was also significantly decreased in all groups, even without soda consumption. Total calorie intake (a combination of feed and soda calorie contributions) was significantly decreased only in HPD diet rats, with increased intake observed in the soda group. Soda intake (Table 5) was high in all soda groups; however HPD-soda consumed less soda than the soda group fed with chow.

There was no significant change in feed consumption of female rats and soda intake was relatively lower compared to male animals. However, just like male animals, female rats fed the HPD-soda combination consumed less soda than the soda group and therefore had a lower calorie contribution from soda. Total calorie intake was significantly elevated in the soda group when compared with the control.

### 3.2. Effect(s) of diet on body weight of wistar rats

A steady increase in body weight was recorded throughout the duration of the experiment. An increase in body weight among male rats was recorded starting from the 8th week in the soda group when compared with the control group. Significant body weight elevation was also seen in the HPD-soda group starting from the 9th week, while the HPD group had lower body weight when compared with the control. The weight of the female animals also increased over the course of the 14 weeks, however a significant increase was recorded only in the HPD-soda group in relation to the control group (Fig. 1).

### 3.3. Diet-induced changes in abdominal circumference of wistar rats

There was a significant increase in the abdominal circumference of male animals in the Soda group in comparison with control animals. In the HPD group, abdominal circumference spiked in weeks 8,9,10, but the overall effect by the end of the study was lower abdominal circumference in rats fed the diet. HPD-soda animals, on the other hand, recorded a significant increase in abdominal circumference in the final weeks of the study. In female rats fed the HPD soda diet, abdominal circumference decreased significantly

**Table 5**
Average soda intake.

| Diet | Average Intake of Soda (ml/day) | Average Energy Intake (kcal.g.d$^{-1}$) |
|---|---|---|
| Chow (M) | – | – |
| Chow + Soda (M) | 86.4 ± 2.2 | 37.0 ± 0.8 |
| HPD (M) | – | – |
| HPD + Soda (M) | 69.8 ± 1.8 | 30.0 ± 0.6 |
| Chow (F) | – | – |
| Chow + Soda (F) | 72.9 ± 1.4 | 31.2 ± 0.6 |
| HPD (F) | – | – |
| HPD + Soda (F) | 64.3 ± 1.2 | 27.5 ± 1.2 |

(M-Male; F-Female; HPD-High Protein Diet).
Values are Mean ± SEM for male and female animals per dietary group. n = 8.

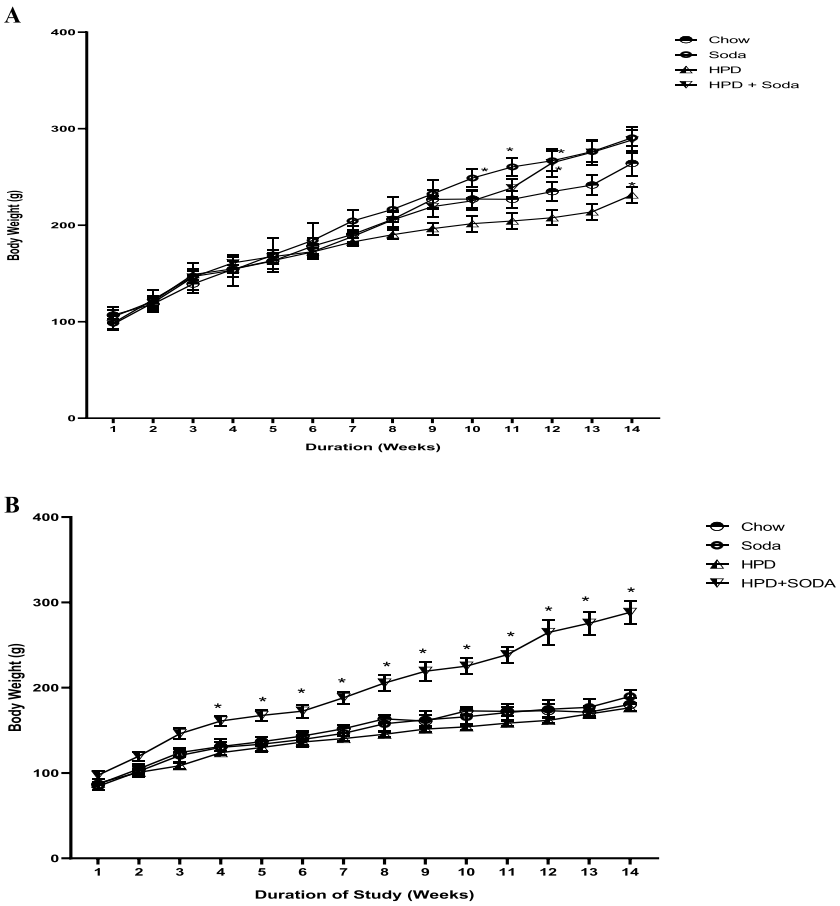


**Fig. 1.** Diet induced changes in weight of Wistar rats (**A**-male, **B**-female). Values are Mean ± SEM for male and female animals per dietary group; P < 0.05. n = 8 *Significant in comparison with control (Chow).

from about the 7th week of feeding. (Fig. 2).

### *3.4. Diet-induced changes in thoracic circumference of wistar rats*

In both Soda and HPD-Soda fed male animals, the thoracic circumference was significantly increased when compared independently with the control rats. The HPD-fed rats however began to show a significant decline in thoracic circumference from the 10th week of feeding. In female rats, the HPD-Soda group had decreased thoracic circumference from the 6th week when compared with the control group (Fig. 3).

### *3.5. Diet-induced changes in blood glucose levels of wistar rats*

Blood glucose in male rats spiked in the early weeks of the test period but subsequently declined to sub-control levels by the end of the study. We however observed a sustained decline in blood glucose in both HPD groups (HPD and HPD-Soda), which, although increased by the midpoint of the experimental period, ended lower than the control group. In female rats, the soda-fed rats had higher blood glucose concentration by the midpoint of the experimental period, eventually ending with higher blood glucose level when compared with the control group. Both HPD groups initially had increased blood glucose by the first test week when samples were taken. Subsequently, both the HPD and HPD-soda groups had a decline in their glucose concentrations (when individually compared with the control) until the end of the study. The HPD-soda group however had a higher decline in blood glucose concentration towards the latter parts of the study period, even though both groups had similar blood glucose concentrations at the end of the test period. (Fig. 4).

### *3.6. Diet-induced changes in serum insulin levels of wistar rats*

Only male soda group animals reflected a significant decrease in serum insulin concentration. In female rats, the HPD-soda group

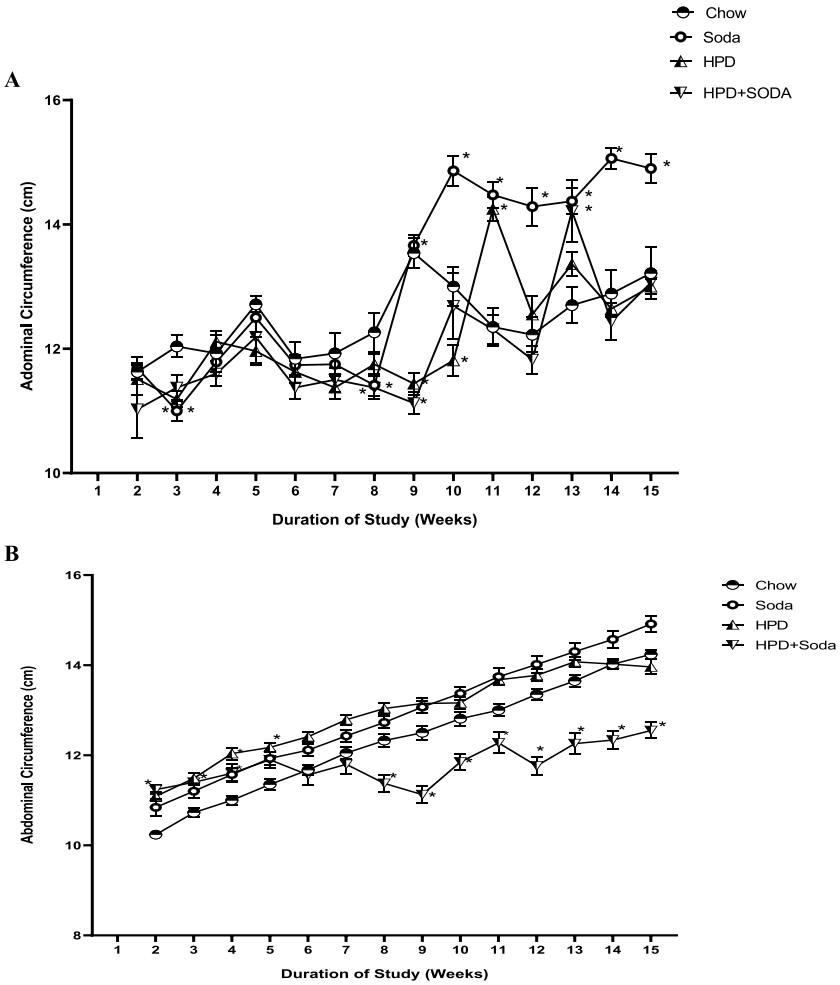


**Fig. 2.** Diet induced changes in Abdominal circumference of Wistar rats (**A**-male, **B**-female). Values are Mean ± SEM male and female animals per dietary group; $P < 0.05$. $n = 8$ *Significant in comparison with control (Chow).

showed a significant increase in serum insulin concentration. (Fig. 5).

### *3.7. Diet-induced changes in malondialdehyde (MDA) levels of wistar rats*

All male animals in the dietary groups (soda, HPD, HPD-soda) had a significant decrease in malondialdehyde levels, however no significant change was recorded in female animals. (Fig. 6).

### *3.8. Diet-induced changes in antioxidant levels of wistar rats*

A significant decrease in catalase concentration was recorded in male animals of the soda group. In female animals, a significant decrease in catalase was seen only in the HPD-soda group.

No significant changes were recorded in reduced Glutathione levels.

There were no significant changes in Superoxide dismutase activity in male animals, however female animals in the HPD-soda group experienced a significant decrease in SOD levels (Table 6).

### *3.9. Diet-induced changes in leptin levels of wistar rats*

In both male and female dietary groups, no significant changes in leptin concentration were recorded. (Fig. 7).

### *3.10. Diet-induced changes in adiponectin levels of wistar rats*

Although a significant increase in adiponectin level was recorded in the male animals fed the high protein diet, no significant

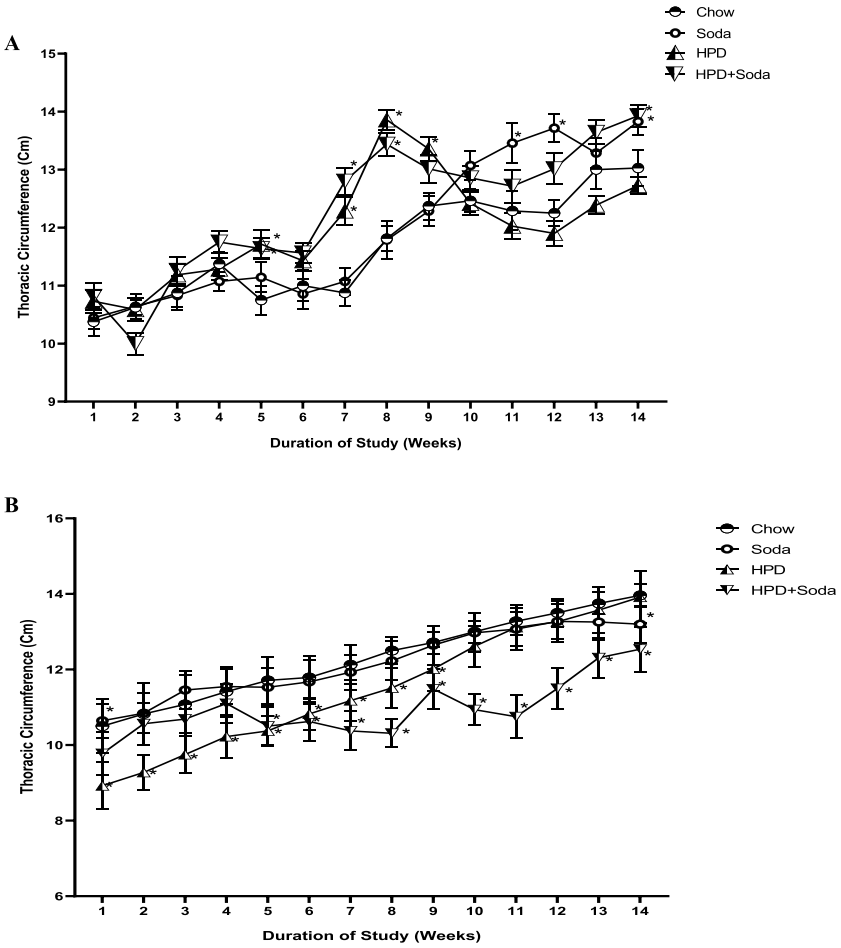


**Fig. 3.** Diet induced changes in Thoracic Circumference of Wistar Rats (**A**-male, **B**-female). Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. $n = 8$.

changes were seen in female animals. (Fig. 8).

### *3.11. Diet-induced changes in TNF-α levels of wistar rats*

Male animals fed the high protein and high protein-soda diets had significant increases in TNF-α concentrations. (Fig. 9).

### *3.12. Diet-induced changes in Interleukin-6 levels of wistar rats*

Only male animals fed the high protein diet showed a significant increase in Interleukin-6 levels. (Fig. 10).

## 4. Discussion

To achieve healthy-eating goals and outcomes, an increasing population (especially young people) in both developed and developing societies are opting for diets high in protein [35], with diets like the Atkins diet, the south beach diet and others, becoming increasingly popular [36]. These diets hold the promise of reduced body weight, higher muscle mass and disease (e.g diabetes) management [37]. While most protein diets are often accompanied by a restriction in carbohydrates, a large proportion of the age group that consume this diet for its health and exercise benefits are also high consumers of fructose-sweetened drinks such as carbonated soda [38]. In this study, we examined the effects of a high protein diet accompanied by increased added dietary sugar in the form of carbonated soda.

High protein diets increase satiety which leads to lesser consumption of food, and consequently reduced caloric intake (Table 4) and lesser weight [39], as we confirm in this study. Average feed intake declined in all the groups, but the effect was most pronounced in the HPD groups, especially the HPD-soda group. Our results suggest that high protein content reduced how much of the diet was consumed, although soda also seems to have an additional effect, as observed in the soda group which consumed copious amounts of

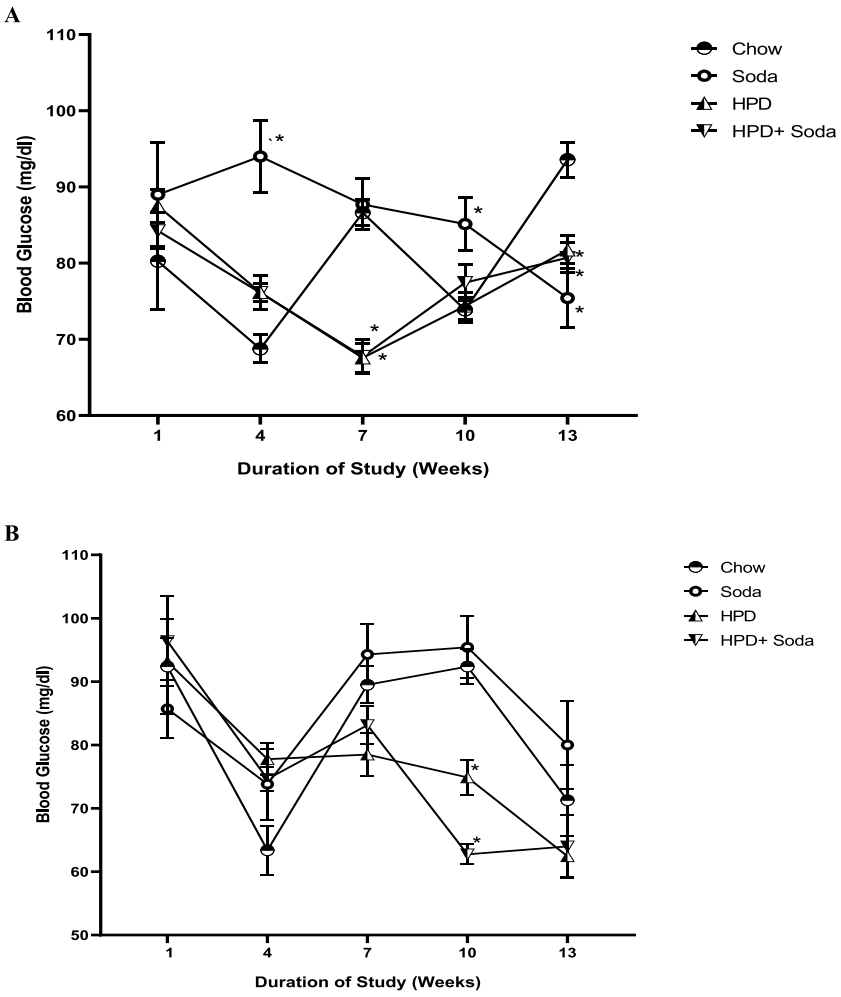


**Fig. 4.** Diet induced changes in blood glucose levels of Wistar rats (**A**-male, **B**-female). Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. $n = 8$ *Significant in comparison with control (Chow).

soda but had significantly lower chow intake. This effect of proteins has been attributed to their ability to increase stimulation and production of satiety hormones including glucagon, glucagon-like peptide-1, and peptide YY 3–36. The composition of the high-protein diet in our study also incorporates a reduction in fat content compared to the control diet. Studies have shown that diets low in fat promote a decrease in weight by altering several factors including: reduction in satiety from foods rich in fat, reduced thermic effect of fat that leads to lower energy expenditure and a positive energy balance [40], increased absorption of fat from the intestine and higher number of calories in 1 g of fat than in carbohydrates or protein [41]. Our study reports increase in both weight and abdominal circumference in male HPD + soda animals. The soda component of the diet, although lowering feed intake, was consumed in large quantities and contributed in large part to the increase in total calorie intake observed in male rats. Carbonated soda, a source of added sugar in the diet is associated with increased weight gain [42]. Energy-rich foods in liquid form provide low satiety [43], which would cause animals to consume more food, increasing calorie intake and consequently, favouring a positive energy balance that ultimately leads to weight gain [43]. A constant increase in weight gain overtime results in obesity [44], a condition associated with inflammation and insulin resistance [45]. These are implicated in several disorders including diabetes mellitus [46] and cardiovascular diseases [47]. Of interest is the observed sexual dimorphism in the observed effects of the HPD-soda diet in male and female animals. Female rats consumed less amounts of soda compared to the male animals, and since the average feed intake was lower in HPD groups (similar to male rats), this accounted for the lower total calorie intake we report. Although, both males and females had increased body weight when fed the HPD-soda diet, thoracic circumference was increased in males but decreased in female rats. This is also true for the soda-fed rats, the male animals had an increase in thoracic circumference, but a decrease was observed in female animals. Sexual dimorphism has been well reported in literature in response to diet treatment in rats [48–50] This study suggests that male animals seem to respond more to diet treatment, in this case a HPD-soda diet.

Consumption of soda added between 25 and 40 kcal (depending on group's average daily intake) extra to the average daily calorie intake of experimental animals. Feed consumption data suggest that consumption patterns were lower in the soda groups fed on chow,

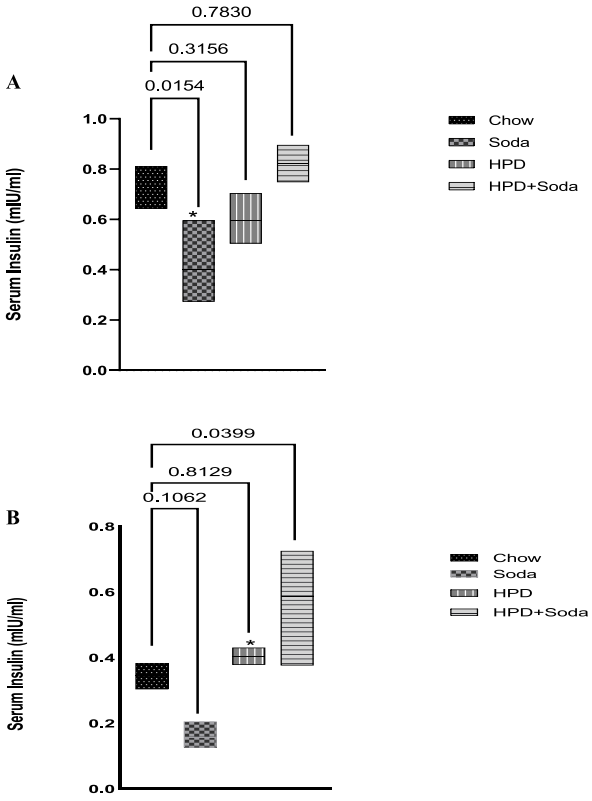


**Fig. 5.** Diet induced changes in Serum Insulin levels of Wistar rats (**A**-male, **B**-female). Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. $n = 8$ *Significant in comparison with control (Chow).

however this group had higher rates of soda intake, which would account for the increased body weight in males, and maintenance of body weight in spite of lower feed consumption in females. The HPD especially reflects the increased calorie intake from soda and its resultant effects. This group of animals had increased body weight despite reduced intake of feed (common to all the HPD fed animals). This effect can be adduced to their increased intake of soda, with a resultant increase in calories [51], obvious on individual comparisons with the HPD group, whose body weight decreased significantly.

Protein diets have been shown to have an insulinotropic effect, promoting the secretion of insulin [52]. In this study, there was no significant change in insulin concentration in the HPD, although in female rats fed the HPD-soda diet, insulin level was significantly elevated. This suggests that this combined diet might have an enhancing effect on insulin secretion within the experimental timeline we employed in this study. This can be attributed to the fact that high protein diets [53] and sugars [54] increase the release of GLP-1, an incretin hormone that increases insulin secretion [55]. Increased insulin levels promote better clearance of blood glucose [56] which was observed in male animals fed the HPD-soda diet. This could be an indication that a diet high in protein could have a balancing effect on the spike in glucose gotten from added dietary sugars, especially since there were significant increases in glucose concentration in male and female rats in the soda group. It is important to note however that some studies have suggested that a prolonged diet of high protein is linked to the development of type 2 diabetes as a result of insulin resistance [57].

We assessed the influence of the experimental diets on a factor that can increase the risk of insulin resistance: inflammation [58]. According to Wu and Ballantyne, inflammation and insulin resistance have a direct relationship with each other [59]. In order to evaluate the effects of our formulated diets on this relationship, we assayed the tissue samples for TNF-α and IL-6 in male wistar rats. These inflammatory markers were both increased in animals fed the high protein and high protein + soda diet; diets that can stimulate an increased secretion of insulin. While diets with reduced fat content are thought to reduce inflammation, our experimental protein diet, which is low in fat caused an increase in both TNF-α and IL-6. This effect can be attributed to the long-term consumption of diets rich in protein in relation to other dietary constituents [60] Although, high sugar intake is thought to increase the secretion of inflammatory markers [61] and we report that animals in both high protein and high protein + soda diet groups had significantly elevated TNF-α levels, an increase in IL-6 was only observed in the HPD-fed rats. This suggests that the HPD has a greater impact than the soda in the diet in influencing inflammation. This corroborates reports by Reitman et al. [52].

The composition of a diet is important for several reasons one of which is the secretion and function of hormones [62]. In this study, we assessed the effects of different diet combinations on a pair of adipokines, leptin and adiponectin. Leptin is a hormone secreted from fat cells [63] that regulates energy homeostasis and metabolism [64]. Increased insulin secretion is one of the factors that stimulate the secretion of leptin through a posttranscriptional mechanism or through glucose metabolism [65]. Leptin is also a factor in combating

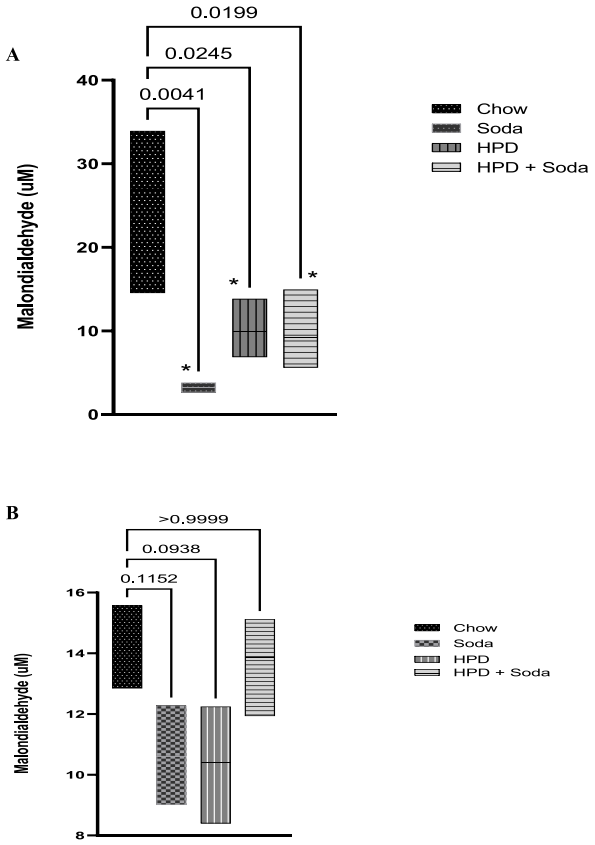


**Fig. 6.** Diet induced changes in Malondialdehyde levels of Wistar rats (**A**-male, **B**-female). Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. n = 8 *Significant in comparison with control (Chow).

**Table 6**
Diet-induced changes in Antioxidant Levels of Wistar rats.

| Dietary groups (Male and Female) | Superoxide Dismutase (u/ml) | Catalase (umol/ml/mins) | Reduced Glutathione (mM) |
|---|---|---|---|
| Chow (M) | 1.646 ± 0.0846 | 90.45 ± 0.10 | 2.127 ± 0.147 |
| Chow + soda (M) | 1.710 ± 0.124 | 73.44 ± 5.57* | 2.017 ± 0.294 |
| HPD (M) | 1.514 ± 0.069 | 86.82 ± 1.074 | 1.978 ± 0.263 |
| HPD + Soda (M) | 1.416 ± 0.076 | 83.50 ± 11.06 | 2.122 ± 0.246 |
| Chow (F) | 1.794 ± 0.082 | 97.34 ± 0.164 | 0.994 ± 0.036 |
| Chow + Soda (F) | 1.721 ± 0.063 | 97.15 ± 0.105 | 1.138 ± 0.111 |
| HPD (F) | 1.689 ± 0.038 | 92.35 ± 3.538 | 1.530 ± 0.086 |
| HPD + Soda (F) | 1.493 ± 0.016* | 75.17 ± 11.53**** | 1.475 ± 0.156 |

(M-Male; F-Female; HPD-High Protein Diet).
Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. n = 8 *Significant in comparison with control.

insulin resistance. In our study, leptin levels in both males and females remained unchanged. Adiponectin is another adipokine that has roles in insulin sensitivity and metabolism [66] Adiponectin has an indirect relationship with obesity [67] and low levels of adiponectin are implicated in the development of type 2 diabetes [68] however, circulating adiponectin was significantly higher in male animals fed the High protein diet-animals that had the lowest average weight.

Malondialdehyde is a biomarker of oxidative stress, a condition implicated in diseases like cardiovascular disorders, cancers and diabetes [69]. Oxidative stress occurs as a result of an imbalance between free radicals, of which malondialdehyde is an example, and antioxidants [70]. In this study we investigated the effect our experimental diets on malondialdehyde levels in both male and female animals. The low MDA levels in high protein diet groups confirms previous findings that protein has an inverse relationship with lipid peroxidation as previous research has shown that diets low in protein increase markers of lipid peroxidation [71,72].

To battle the effects of oxidative stress, antioxidants such as catalase, GSH and superoxide dismutase protect against reactive oxygen species [73]. Both catalase and superoxide dismutase were reduced in the HPD-soda groups of both male and female animals, which shows a consistent effect in both sexes. Catalase was also decreased in the Soda group. This would suggest an effect that might be more dependent on the effect of the soda in the diets, an effect which would enhance the damaging effect of reactive oxygen species as

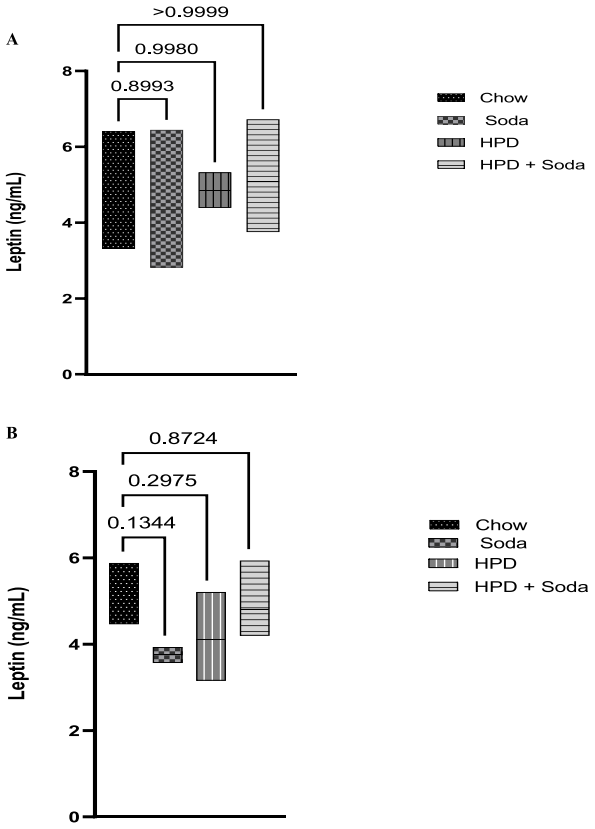


**Fig. 7.** Diet-induced changes in Leptin levels of Wistar rats (**A**-male, **B**-female). Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. $n = 8$ *Significant in comparison with control (Chow).

a result of its fructose content [74]. GSH, the reduced form of glutathione [75] reduces lipid peroxidation in cells [76]. While significant changes were not recorded in GSH concentration, the male and female animals in the HPD and HPD-soda groups had a higher GSH concentration.

Studies in rodents immediately after weaning report that quantity of aerobic and anaerobic bacteria in feces increased when the protein level in diet is increased two-fold (100–200 g/kg). They describe increased *Lactobacilli* and decreased Coliforms and Staphylococci abundance in these animals. Reportedly, there are elevated populations of pathogens such as Coliforms, Streptococcus and Bacillus [77], while probiotics such as Lactobacilli, Bifidobacteria [78] and Saccharolytic bacteria such as Megasphaera, Mitsuokella decrease in abundance. Populations of butyrate-producing bacteria [79,80] are also decreased. This is important because prebiotics function as anti-inflammatory agents [81], which proffers a possible mechanism for the observed HPD-related increases in inflammatory mediators we report in this study. In humans, high protein diets in healthy participants have been associated with increase in Bacteroides spp-predominant microbiota [82], while in mice, studies have revealed changes in the gut microbiome, favoring bacteria with an inverse correlation with body fat mass [83], adiposity and hyperglycemia [84,85]. Conversely, consumption of a refined sugar diet (e.g. high fructose corn syrup found in soda) is negatively correlated with abundance of Christensenellaceae, a member of the bacteria phylum Firmicutes [86]. This bacterial group is negatively correlated with body weight and visceral fat content. This provides a possible explanation for the increased body weight HPD + soda groups, which can be explored in future studies.

## 5. Conclusion

The results of this study suggest that high protein/soda dietary combination increases body weight and worsens body measurements in Wistar rats. This combination influences the antioxidant defence systems negatively and might also increase inflammation. Thus, high protein diet in combination with carbonated soda impacts physiology differently from a high protein diet alone, and may stimulate weight gain, oxidative stress and HPD-related inflammation in Wistar rats.

## Author contribution statement

Temitope Gabriel Adedeji: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

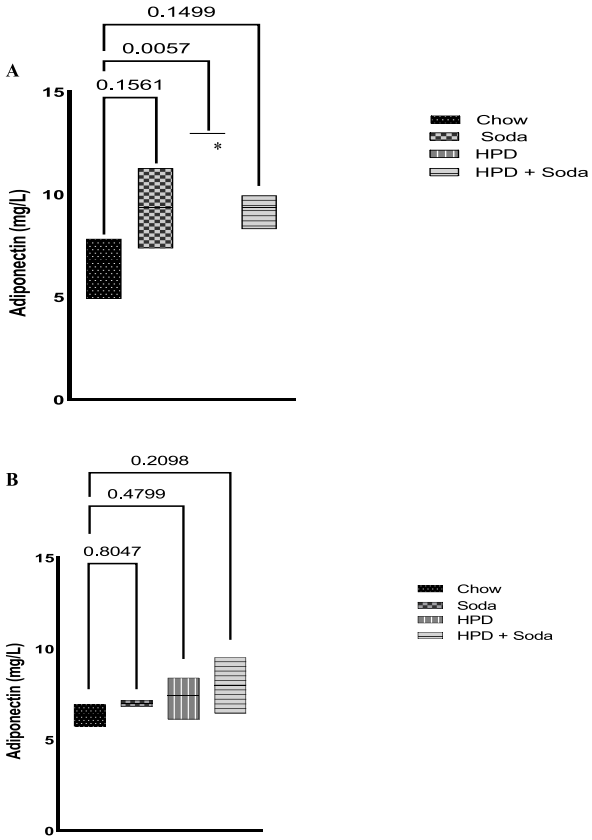


**Fig. 8.** Diet induced changes in Adiponectin levels of Wistar rats (**A**-male, **B**-female). Values are Mean ± SEM for male and female animals per dietary group; $P < 0.05$. $n = 8$ *Significant in comparison with control (Chow).

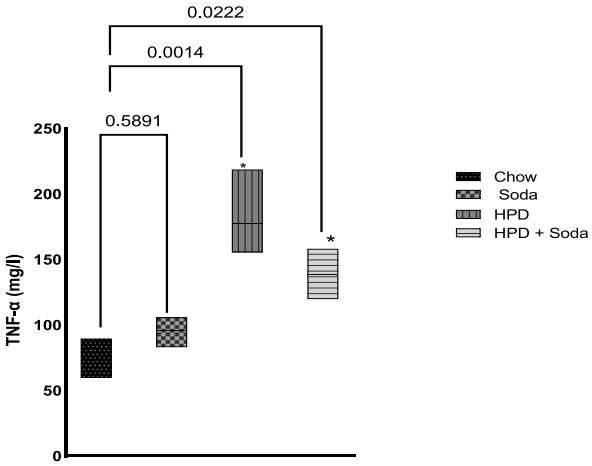


**Fig. 9.** Diet induced changes in TNF-α levels of male Wistar rats. Values are Mean ± SEM for male animals per dietary group; $P < 0.05$. $n = 8$ *Significant in comparison with control (Chow).

Sikirullai Olatunde Jeje; Tosan Peter Omayone: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Boluwatife Olamide Dareowolabi: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

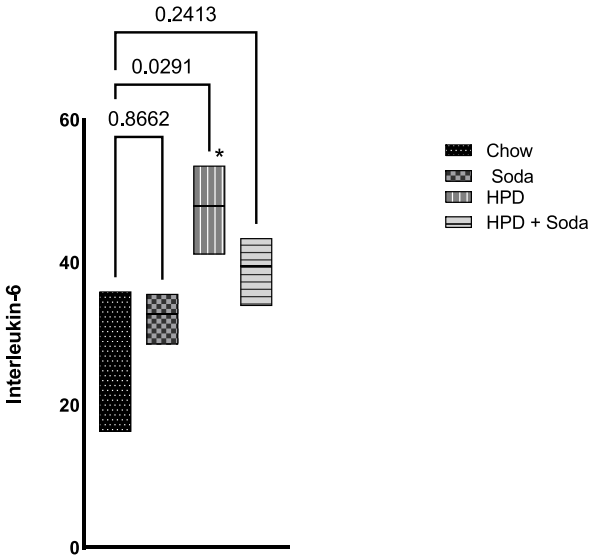


**Fig. 10.** Diet induced changes in Interleukin-6 levels of male Wistar rats. Values are Mean ± SEM for male animals per dietary group; $P < 0.05$. n = 8 *Significant in comparison with control (Chow).

## Data availability statement

Data included in article/supplementary material/referenced in article.

## Declaration of competing interest

The authors declare that there are no known competing or conflicting financial interests or personal relationships that would have an influence on the work we have reported.

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