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Coconut water alters maternal high fat diet induced changes in hormones and pup morphometry of Wistar rats

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Abstract

Background: Maternal high fat diet (HFD) during gestation adversely programmes foetal metabolism and cardiovascular function for the development of obesity and its related cardiovascular diseases in adult life. The hypolipidemic actions of coconut water (CW) in the presence of HFD have been reported. This study examined the effects of oral administration of CW on lipid panel, hormone profile, pup and placental morphometry of dams fed HFD during gestation.
Methods: Twenty-four pregnant Wistar rats were assigned to four groups (n=6) and treated daily from gestation day (GD) 1 to 21 as follows; Group 1: 1ml/100g b.wt. distilled water; Group 2: 1ml/100g b.wt. CW; Group 3: HFD (70% standard rat feed plus 30% butter); Group 4: HFD + 1ml/100g b.wt. CW. Animals were sacrificed on GD 21. Random blood glucose was measured using tail blood. Caesarean section was performed to remove the pups and their placentas which were immediately measured. Oxidative stress status of the placentas; serum lipid and hormone profiles of dams were assessed.
Results: HFD+CW resulted in significant ($P<0.05$) reductions in pup weight and morphometric indices when compared with pups from HFD. These changes were accompanied by significant improvements in maternal serum lipid profile, alterations in hormone levels and higher placental lipid peroxidation.
Conclusion: These results suggest that coconut water is protective against maternal high fat diet-induced changes. Further studies are on-going to determine the actions of coconut water of maternal high fat diet induced foetal programming of adult health.

Keywords; *Maternal high fat diet; coconut water; morphometry*

Résumé

Contexte: Le régime maternel riche en haute graisse (RRHG) pendant la gestation défavorablement programme le métabolisme fœtal et la fonction cardiovasculaire pour le développement de l'obésité et ses maladies cardiovasculaires liées dans l'âge adulte. Les actions hypo lipidiques de l'eau de noix de coco (EC) en présence de RRHG ont été rapportées. Cette étude a examiné les effets de l'administration orale d'EC sur panneau lipidique, profil hormonal, souriceaux et morphométrie placentaire de barrages nourris avec RRHG pendant la gestation.
Méthodes: Vingt-quatre rats Wistar gestantes ont été réparties en quatre groupes (n = 6) et traitées tous les jours depuis le jour de gestation (JG) 1 à 21 comme suit; Groupe 1: 1 ml / 100 g de poids corporel eau distillée; Groupe 2: 1 ml / 100 g de poids corporel EC; Groupe 3: RRHG (70% d'alimentation standard de rat plus 30% de beurre); Groupe 4: RRHG + 1 ml / 100g de poids corporel EC. Les animaux ont été sacrifiés le JG 21. Glucose de sang aléatoire a été mesuré en utilisant le sang provenant de la queue. La césarienne a été effectuée pour enlever les souriceaux et leur placenta qui ont été immédiatement mesurées. L'état de stress oxydatif des placentas; lipide sérique et profils hormonaux de barrages ont été évalués.
Résultats: RRHG + EC ont entraîné des réductions significatives (P <0,05) du poids des souriceaux et indices morpho-métrique par rapport aux souriceaux de RRHG. Ces changements ont été accompagnés par des améliorations significatives en profil maternel des lipides sériques, des altérations dans les niveaux d'hormones et plus haute peroxydation de lipide placentaire.
Conclusion: Ces résultats suggèrent que l'eau de coco est protectrice contre les changements induits par le régime riche en graisses maternels. D'autres études sont en cours pour déterminer les actions de l'eau de noix de coco de régime maternel riche en graisse de programmation fœtal induit de la santé d'adulte.

Mots-clés; *Régime maternel riche en graisses; eau de noix de coco; morphométrie*

Introduction

Obesity is a global health problem; its negative impact cuts across socioeconomic class and national development. Although the aetiology of obesity is complex and probably not fully understood, it has been known to involve the integration of social, behavioural, cultural, physiological, metabolic and genetic factors [1]. Maternal exposure to any of these

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factors can program future generations of offspring to develop obesity irrespective of the offspring's exposure [2,3]. The foetal programming hypothesis proposes that modifications in maternal nutrition and endocrine status could result in developmental adaptations of the foetus which permanently alter its structure, physiology and metabolism [4]. This has since been proven by several epidemiological studies and also through human and animal experimental models [5–8]. Morphometric indices such as body weight, height, head and abdominal circumferences, are very useful in assessing foetal/neonatal growth and development [9]. For instance, a u-shaped curve association between the risk of adult disease and birth weight has been well established [10].These measures are often standardized as ratios such as the Ponderal Index (PI), waist-to-height ratio (WHtR), waist-to-hip ratio (WHR) and head circumference-to-abdominal circumference ratio (HC/AC); thereby enabling comparison between populations and discoveries of associations between body size and disease risk [11–14].

Maternal obesity or consumption of high caloric diets during pregnancy, leads to foetal programming of offspring, thereby predisposing them to the development of obesity along with its related complications in later life [3,6,15–17]. The deleterious effects of maternal high fat diet on the neuroendocrine, metabolic, cardiovascular and reproductive health of offspring have been widely reported [18–31]. Therefore, it stands to reason that campaigns for healthy maternal nutrition should be intensified. However, the prevailing socioeconomic circumstances in developing countries inadvertently sabotage the efficacy of such campaigns. The population of females in the work force has remarkably increased since the 1960 to 1980 era when women first joined the labour market, with some countries recently recording over 80% female participation [32,33]. This implies that the only diet options for several mothers during the perinatal period are the readily available, energy dense, high fat containing, fast foods [34].Feasible solutions to maternal high fat diet induced foetal programming should therefore incorporate more convenient alternatives in addition to dietary modifications. Studies suggest that such adverse programming of offspring can be potentially reversed by nutritional or targeted therapeutic interventions especially during the period of developmental plasticity [35].

Some of the reactions proposed for the adverse perinatal effects of high maternal fat diet include oxidative stress, lipotoxicity and inflammation [31,36–38]. The hypolipidemic, hypoglycaemic and antioxidant effects of coconut water, the liquid endosperm of the coconut (*Cocos nucifera*L.) fruit, have been reported [39–42]. Coconut water has no reported toxicity and can hence be considered as a safe and convenient option for career mothers. This study was therefore designed to investigate the effects of coconut water administration and maternal high fat diet during gestation on maternal lipid profile and pup morphometry in Wistar rats.

Materials and methods

Plant material

Coconut (*Cocos nucifera* L.)fruits were obtained from a coconut plantation in Oyo state, Nigeria and verified by a botanist from the Department of Botany, University of Ibadan, Ibadan, Nigeria. Coconut water was obtained by piercing the soft "eye" of the coconut (the germination pore) with a sterile screw driver and decanting the water into a large sterile container. Fresh coconut water was used each day. Coconut water was administered via oral gavage at a daily dosage of 1 ml/100g body weight using blunt-tipped oral cannulas attached to 2ml syringes. Administration of coconut water was done between 8.00-9.00am daily.

Animals

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the guidelines for laboratory animal care of the National Institute of Health (NIH publication no. 85-23, revised 1996).Virgin female rats obtained from the Central Animal House, University of Ibadan were mated with proven breeder male rats from the Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, University of Ibadan. Pregnancy was confirmed by the presence of spermatozoa in vaginal smears and the day of observation of spermatozoa was taken as gestation day (GD) 1 for each female. Pregnant rats were then randomly divided into four groups, namely; control, coconut water (CW), high fat diet (HFD) and high fat diet plus coconut water (HFD+CW).

Treatments

High fat diet consisted of 70% standard rodent diet (Ladokun Feed Mills, Ibadan, Nigeria) and 30% butter (Real brand, Chellarams, Lagos, Nigeria). With the exception of the HFD and HFD+CW groups which received high fat diet during gestation, all rats were fed standard rodent diet before mating and during gestation. The animals had access to food and drinking water *ad libitum*.

Caesarean section

On the twenty-first day of gestation (GD21), ether anaesthesia was induced by placing the rat in an airtight desiccator containing a ball of cotton wool moistened with a few drops of diethyl ether for about one minute until the eyelid closure reflex was lost. A drop of tail blood was collected for random blood glucose measurements which were done using an automated glucometer (On Call Plus®, ACON Laboratories Inc., USA). Respiratory movements were monitored visually by observing for regular chest and abdominal undulations. The anaesthetized dams were cut open from the linea alba of the anterior abdominal wall to the thoracic cavity to expose the heart. Blood was collected via cardiac puncture using sterile needles and syringes and emptied into plain tubes. The abdominal cavity was then dissected to remove the gravid uterus.

Pup and placental morphometry

The pups and their placentas were immediately removed from within the uteri and weighed individually on an electronic scale (Lisay, China). Crown-to-rump length, abdominal diameter and head diameter of each pup; diameter and thickness of each placenta were measured using a digital Vernier calliper (Mitutoyo, Japan). The diameter was measured along the length of each placenta, while the thickness was measured at the centre when the placenta was placed on a horizontal plane.

Placenta redox status

The largest placenta from each dam was homogenized in 4ml of phosphate buffer (pH 7.4) per gram of placental tissue. The supernatant was obtained after centrifuging at 3000rpm for 15 minutes and was used for the determination of malondialdehyde (MDA), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) levels using standard assay techniques [43–45].

Hormone assays

Serum collected was used to assay for follicle stimulating hormone (FSH), luteinizing hormone (LH), oestrogen, testosterone (Fortress Diagnostics Limited, UK), corticosterone (Oxford Biomedical Research, USA), insulin and leptin (Ray Biotech Inc. USA) using the ELISA technique. They were determined using kits according to the manufacturer's instructions.

Statistics

Data are expressed as Mean ± Standard Error of Mean (SEM). Significance of difference of means was analysed using one-way ANOVA followed by post hoc analysis where necessary. $P<0.05$ was considered significant.

Results

Body weight, serum lipid and blood glucose levels of dams at GD21

High fat diet led to a significant gain in body weight during gestation when compared to the control and coconut water (CW) groups (Fig.1). High fat diet (HFD)dams showed a statistically significant ($p<0.05$) increase in serum triglyceride, total cholesterol and low density lipoprotein (LDL) cholesterol concentrations during gestation (Fig. 2) which was not evident in HFD+CW dams. Maternal high density lipoprotein (HDL) cholesterol was reduced in all the groups when compared with control (Fig.2). Leptin secretion was significantly increased in HFD dams and reduced in HFD+CWdams (Fig.3). CW dams showed a significant reduction in random blood glucose levels (Fig. 4), while serum insulin levels were significantly increased in the HFD dams when compared with CW dams (Fig.5)

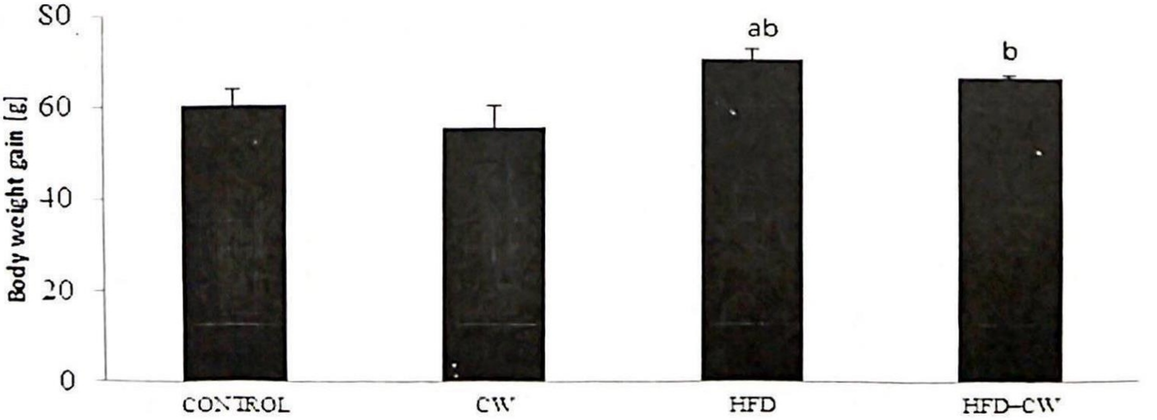

Fig.1: Body weight gain of dams during gestation. Initial body weight was measured on GD1 while final body weight was measured on GD21. P<0.05 was considered significant when compared with [a]control and [b]CW groups respectively.

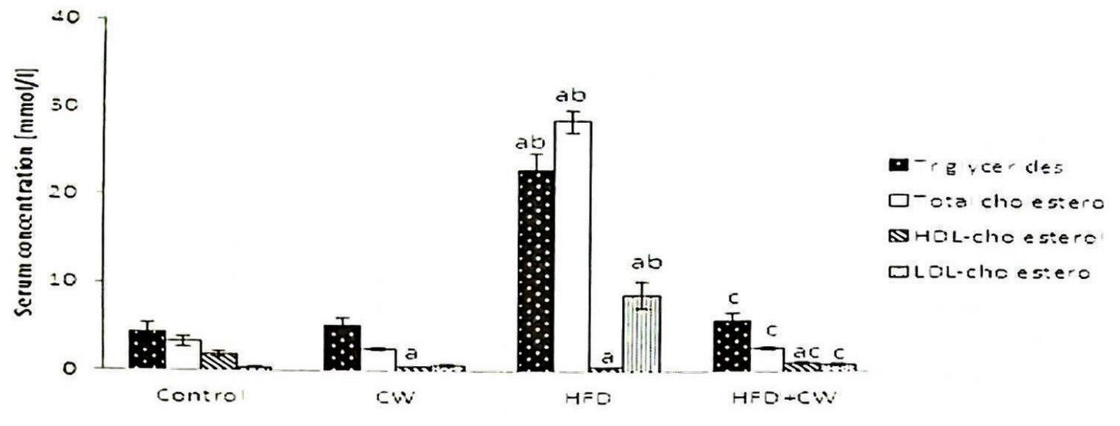

Fig.2: Serum lipid profile of dams on GD21. P<0.05 was considered significant when compared with [a]control,[b]CW and [c]HFD+CW groups respectively

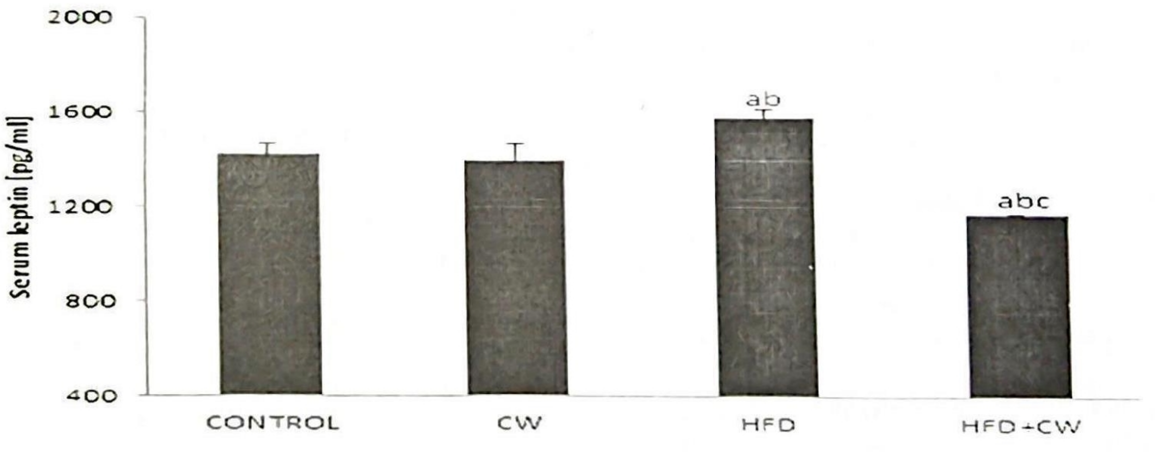

Fig.3: Serum leptin levels of dams on GD 21. P<0.05 was considered significant when compared with [a]control,[b]CW and [c]HFD+CW groups respectively.

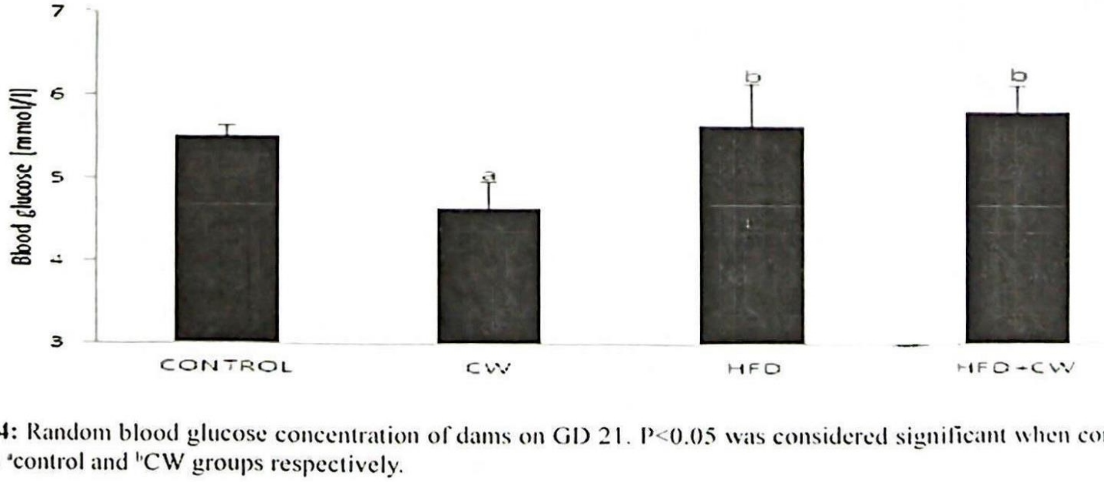

Fig.4: Random blood glucose concentration of dams on GD 21. P<0.05 was considered significant when compared with [a]control and [b]CW groups respectively.

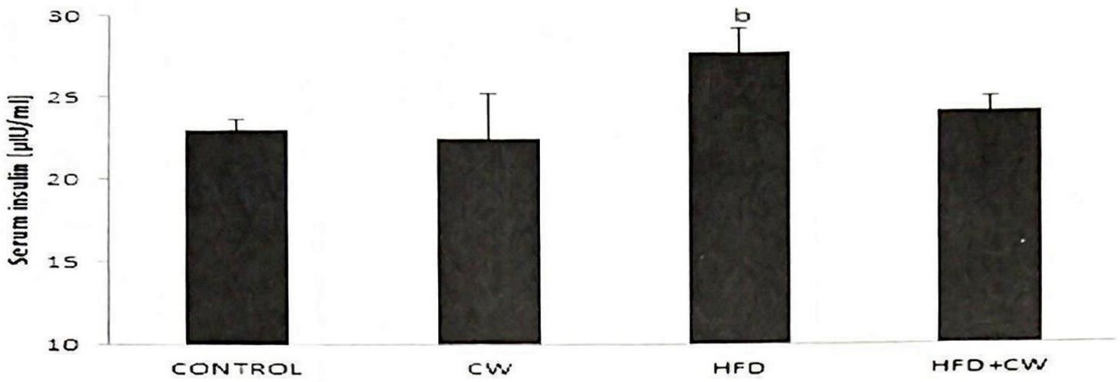

Fig.5: Serum insulin concentration of dams on GD 21. P<0.05 was considered significant when compared with [b]CW group.

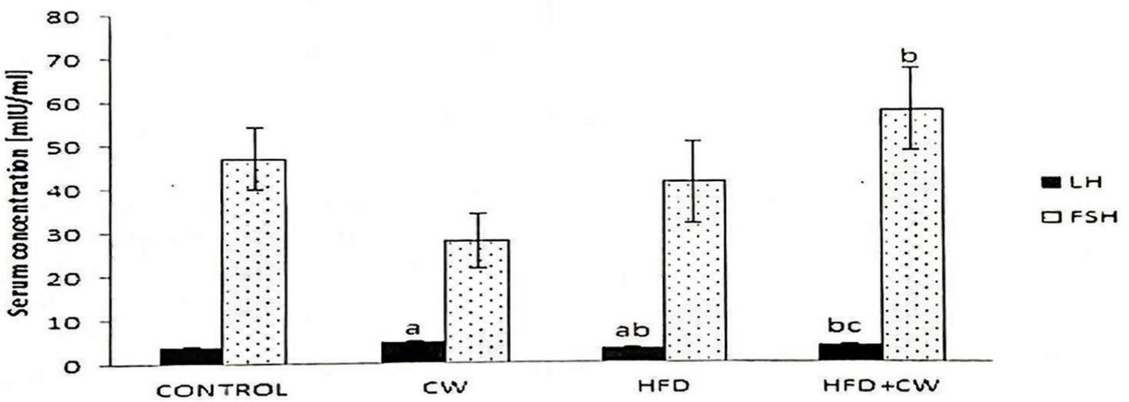

Fig.6. Serum levels of the gonadotropins; Luteinizing hormone (LH) and Follicle Stimulating hormone (FSH) on GD 21. P<0.05 was considered significant when compared with [a]control, [b]CW and [c]HFD+CW groups respectively.

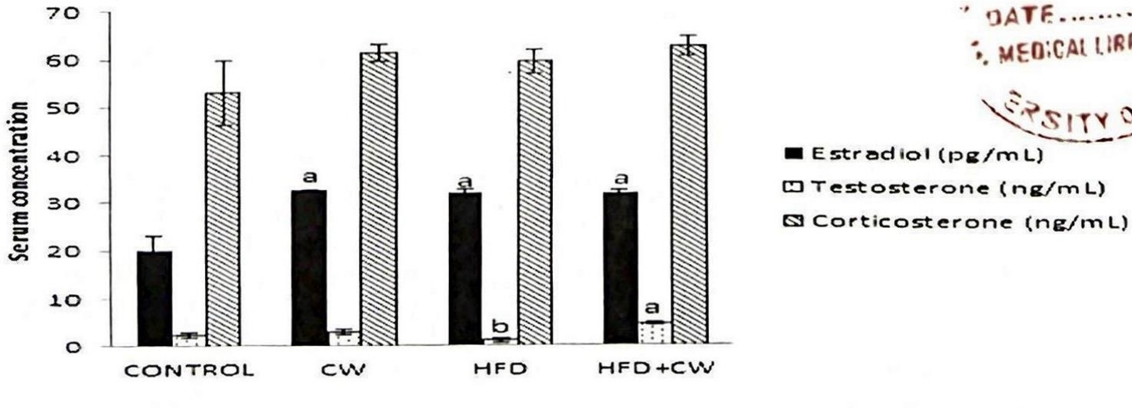

Fig.7: Serum levels of the steroid hormones; Estradiol, Testosterone and Corticosterone, on GD 21. P<0.05 was considered significant when compared with [a]control and [b]CW groups respectively.

Hormone profile of dams on GD21

Serum Luteinizing hormone (LH) levels were significantly increased in CW and reduced in HFD dams (Fig.6). Serum Follicle Stimulating hormone (FSH) levels were significantly increased in HFD+CW dams when compared with CW dams (Figure 6). Serum levels of estradiol on GD21 were significantly increased in all the test groups (Fig.7).

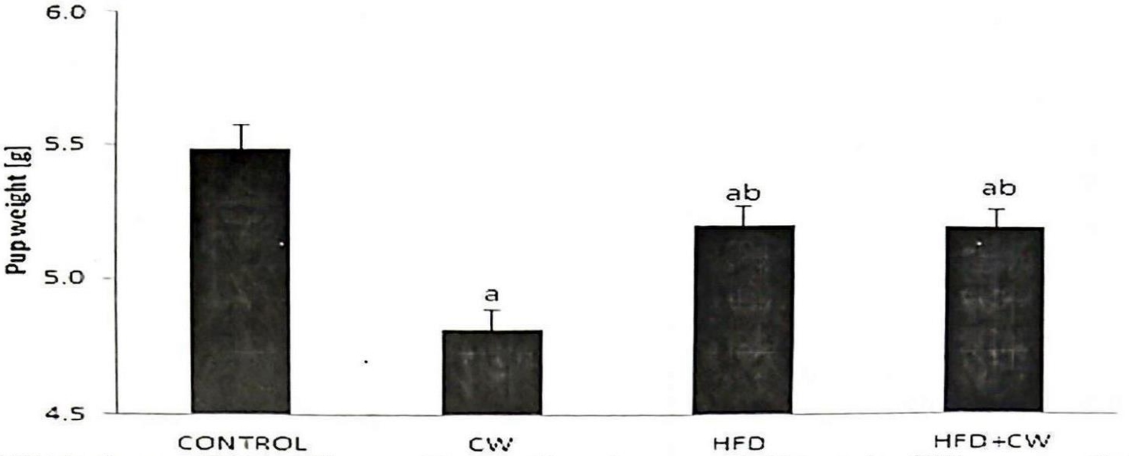

Fig.8: Weight of pups on GD 21. P<0.05 was considered significant when compared with [a]control and [b]CW groups respectively.

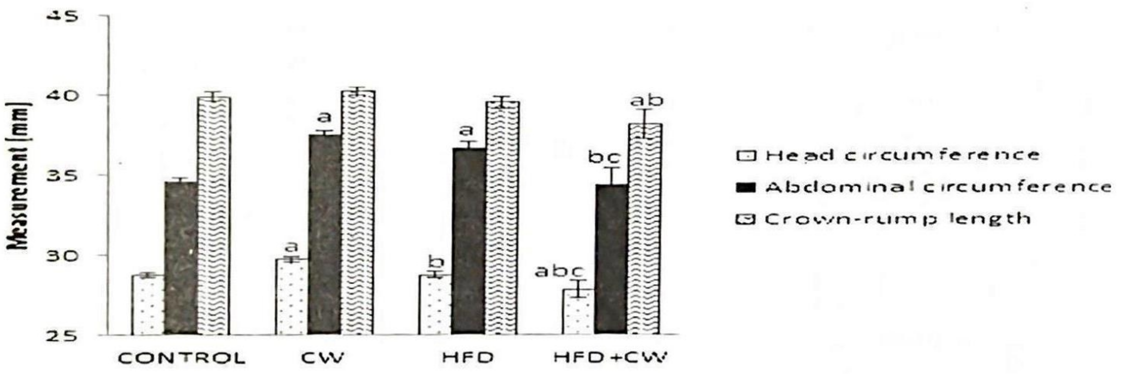

Fig.9: Head circumference, abdominal circumference and crown-to-rump length (height)of pups on GD 21. P<0.05 was considered significant when compared with [a]control, [b]CW and [c]HFD groups respectively.

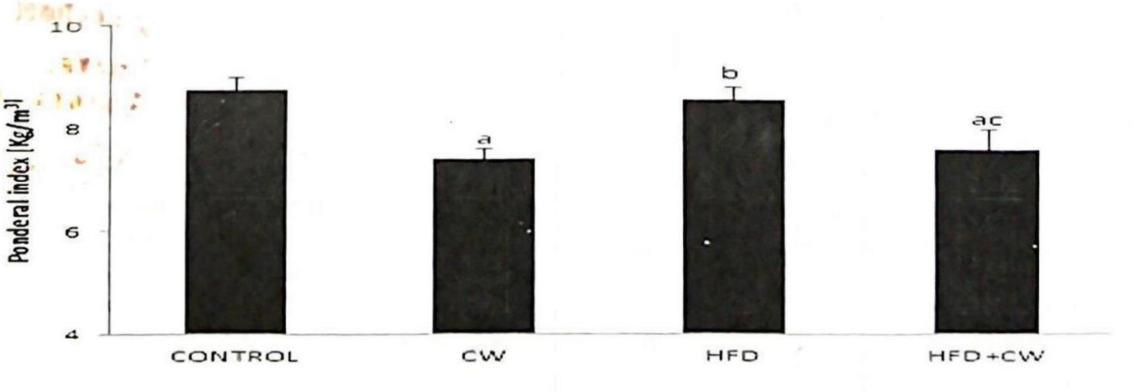

Fig.10: Ponderal index (PI) of offspring on PND 1. P<0.05 was considered significant when compared with [a]control, [b]CW and [c]HFD groups respectively.

Serum testosterone levels were increased in HFD+CW dams when compared with the controls (Fig.7). Compared with the CW dams, HFD dams showed a decrease in serum testosterone levels (Fig. 7).Serum corticosterone levels were not significantly affected in this study (Fig.7).

Pup morphometry

There was a statistically significant decrease in the weight of pups from all the groups on GD21, with coconut water offspring showing the most pronounced reduction (Fig.8). The head circumference of offspring on PND 1 was

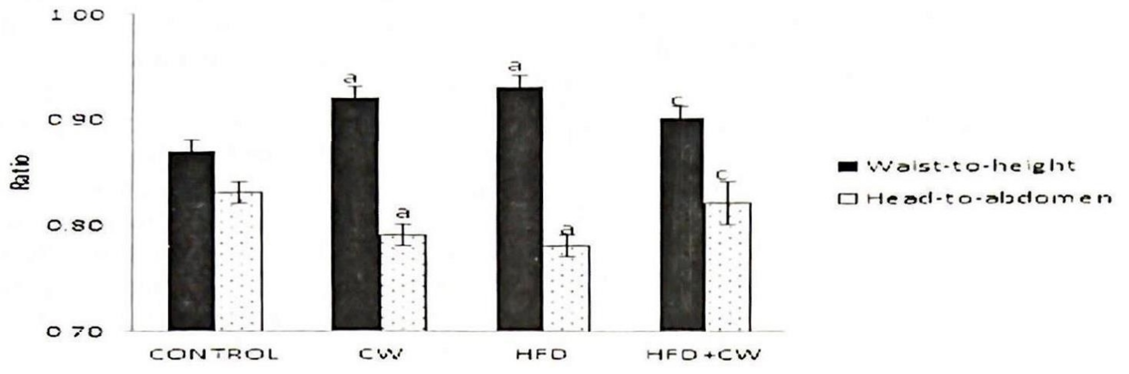

Fig.11: Waist-to-height and Head-to-abdomen ratios of pups on PND 1. $P<0.05$ was considered significant when compared with [a]control, [b]CW and [c]HFD groups respectively.

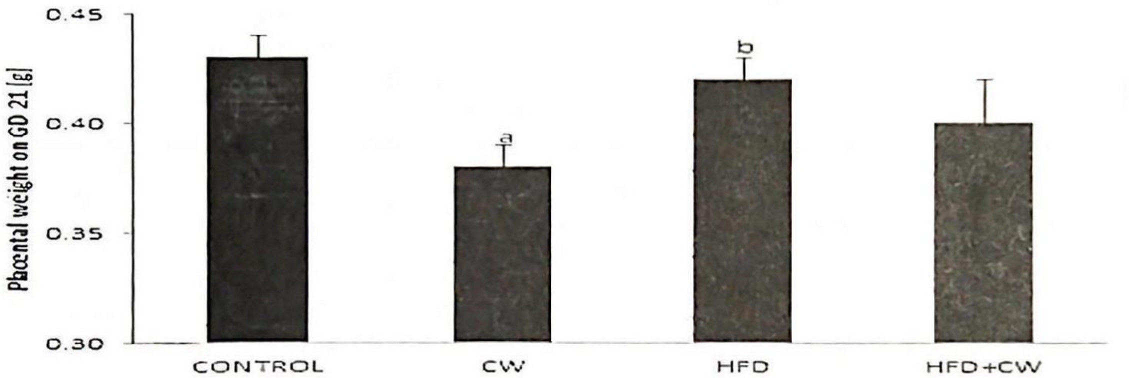

Fig.12: Placental weight on GD21.$P<0.05$ was considered significant when compared with [a]control and [b]CWgroups respectively.

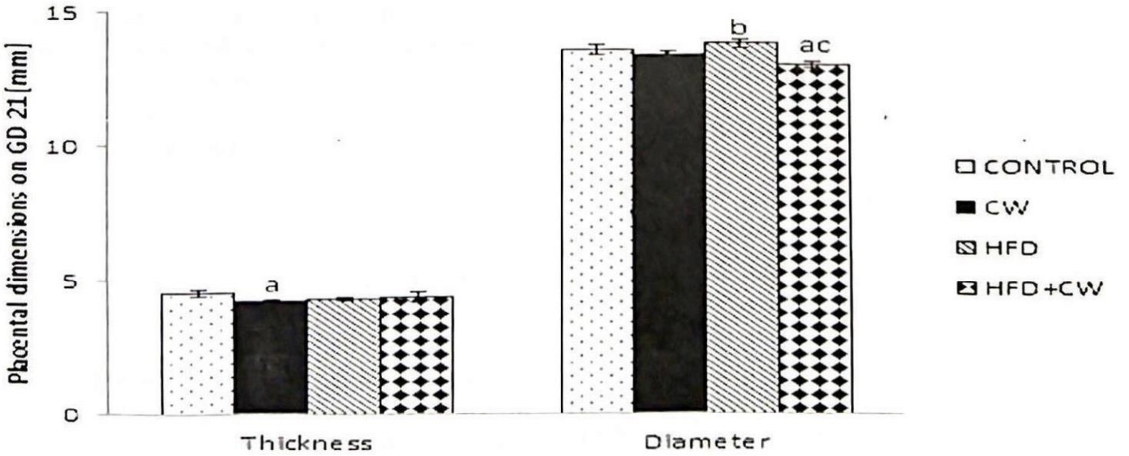

Fig. 13: Placental thickness and diameter on GD21. $P<0.05$ was considered significant when compared with [a]control, [b]CW and [c]HFD groups respectively

significantly increased by maternal CW administration and reduced by HFD+CW, while the abdominal circumference was increased in both CW and HFD (Fig.9). The crown-to-rump length (height) of pups was significantly reduced in HFD+CW (Fig.9). Ponderal index (PI) at birth was significantly increased in HFD offspring, while the CW and HFD+CW offspring showed a reduction in

PI (Fig.10). An increase in waist-to-height ratio and a reduction in head-to-abdomen ratio were observed in CW and HFD offspring; no significant difference was however observed for both variables in HFD+CW offspring (Fig.11).

Placental morphometry and redox status

The weight and size of the placenta was significantly reduced in CW group (Figs.12 and 13). There was significantly more lipid peroxidation in both the CW and HFD+CW groups as indicated by the increased malondialdehyde concentration (Table 1). However, this process did not adversely affect the concentration of antioxidants in the placenta of these groups on GD 21 (Table 1). Placentas from the HFD group had significantly higher glutathione peroxidase and significantly lower superoxide dismutase concentrations than the control placentas (Table 1).

resultant health complications [51]. This occurs as a result of an increase in neurogenesis in the hypothalamic third ventricle and the increased expression of orexigenic peptides in the developing foetus [51]. Maternal HFD is thus a major cause for concern as dietary-induced obesity is becoming more prevalent among women than men in all regions of the world [52]. Hence, multi-interventional approaches are required to protect future generations from an impending obesity epidemic.

Coconut water is a natural, pleasant-tasting and sterile drink which contains several biologically active compounds which include; L-arginine, ascorbic acid, calcium, magnesium and potassium [41,53,54]. Numerous health benefits have been ascribed to coconut water [42,53,55,56]. The antioxidant, antidiabetic and hypolipidemic effects

Table 1: Placental malondialdehyde and antioxidant levels on GD 21.

Groups	MDA (μmol/g tissue)	CATALASE (U/g tissue)	SOD (U/g tissue)	GPx (U/g tissue)
Control	1.35±0.07	23.30±1.72	3.47±0.20	1.78±0.12
Coconut Water	2.34±0.41[a]	27.73±4.14	2.65±0.24	2.24±0.42
High Fat Diet	1.52±0.12	19.98±0.38[b]	2.09±0.36[a]	3.95±0.10[ab]
High Fat Diet + Coconut Water	2.62±0.42[ac]	22.96±2.43	3.08±0.61	2.46±0.47[c]

MDA=malondialdehyde, SOD=superoxide dismutase, GPx=glutathione peroxidase. P<0.05 was considered significant when compared with [a]control, [b]CW and [c]HFD groups respectively.

may hold promise for the development of sustainable therapies in metabolic dysfunction.

Discussion

Foetal programming is a concept which refers to how maternal nutrition and environmental exposures result into *in utero* alterations in foetal structure and function which permanently modify adult physiology of offspring [4,46]. Maternal obesity and high fat diet during gestation programme offspring for the development of obesity, metabolic syndrome and cardiovascular dysfunction among other related diseases [16,22,47,48]. Pregnancy is normally associated with a gain in body weight which could be associated with the growing foetus(es) and/or an increase in maternal food intake to cater for the increased metabolic requirements. A high fat diet (HFD) during gestation upsurges the pregnancy-induced weight gain and portends a negative impact on the future health of the unborn offspring [48–50]. Maternal HFD during gestation programmes the offspring with alterations which predispose them to overweight, hyperphagia (along with a preference for fat) and hyperlipidaemia together with the

The hypolipidemic effect of coconut water reflected in this study was observed only when the concentration of dietary fats was increased. This supports previous findings [40]. The results of this study also suggest that coconut water potentiates the actions of leptin when there is an abundance of circulating fat, without affecting leptin synthesis. Leptin is an adipokine which regulates maternal to foetal metabolic interactions during pregnancy [57]. The mechanism via which coconut water alters the interplay of leptin, insulin and energy balance requires further investigation as these results suggest the absence of central leptin resistance and significant insulin levels (which are normal features in late gestation) [58]. The sex hormone activity on serum leptin levels is also contrary to what was reported by Ahima and Flier [59] that testosterone decreases and oestrogen increases leptin levels, thereby suggesting an independent action of coconut water on lipid metabolism. These results also imply that coconut water may have long-acting effects as the rats were given coconut water only once a day and at a dose much lower than what had been previously reported [39,40]. Sandhya and Rajamohan [39,40] have proposed several mechanisms of action for the hypolipidemic effects of coconut water in the presence of dietary fats. Notable among these are the enhanced hepatic lipid metabolism reflected by an increase in the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (which catalyses the rate-limiting step in cholesterol synthesis) and a reduction in lipogenic enzyme activity along with an increased conversion of available cholesterol to bile acids and an increased excretion of these bile acids [39,40,60].

No toxicity has been reported with the use of coconut water and the zero maternal mortality observed in this study further supports that fact [61]. The increase in serum estradiol observed in CW may be due to the presence of phytoestrogens in coconut water [62]. Phytoestrogens have been promoted as natural oestrogen replacement therapy [63].The increase in estradiol level observed with HFD may be due to an increase in steroidogenesis as a result of increased availability of precursors and the concomitant reduction in serum LH levels supports a negative feedback theory. The increase in LH concentration observed in CW supports previous reports of a hypothalamo-pituitary axis activity of coconut water [64]. Nair and Rajamohan [54] proposed that the L-arginine and ascorbic acid in coconut water stimulate the production of nitric oxide which in turn stimulates LH and FSH synthesis.

The reduction in size and increased lipid peroxidation in the placenta caused by maternal coconut water intake suggests that coconut water reduces placental efficiency and thereby compromises maternal health [65,66]. However, the significant differences in pup morphometry observed in the HFD+CW offspring suggests that coconut water has a protective effect on the pups in the presence of maternal high fat diet and may be detrimental when excess fat is not available in maternal diet. Morphometric indices such as low/high birth weight and a relative disproportion of body parts have been suggested to act as pointers of inclination to disease development in later life [12,67]. It was therefore concluded that coconut water may protect against maternal high fat diet induced foetal changes during gestation, and that coconut water should not be consumed by pregnant females on a healthy balanced diet as its hypolipidemic effects may adversely alter the foetal environment. Further studies are on-going to establish the foetal programming outcomes of the adult offspring since the foetal programming hypothesis relates to health of offspring in later life [35,46,68].

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