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Review

Dietary supplementation with natural honey promotes growth and health of male and female rats compared to cane syrup

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The modern human diet contains refined sugars mainly fructose, with culpability in the pathogenesis of metabolic diseases. We investigated the effects of natural honey (NH) as a source of fructose on metabolism in growing animal models. This was to determine whether NH can substitute refined sugars, without adverse effects. Fifty-nine suckling rats were fed diets containing NH or cane syrup (golden syrup, GS) from age 7 to 91 days. Thereafter the rats' general health indices and growth parameters were obtained. Compared to GS, NH increased ($p < 0.01$) body weight gain, and linear growth in the males while none of the diets influenced females' growth. Dietary GS increased ($p < 0.0001$) visceral fat weight, and caused hepatomegaly in the males. There was neither visceral fat weight increase in the NH-fed and female rats. The liver functionality was preserved in the honey-fed rats. Unlike cane syrup, NH prolonged intake by infants could not compromise the rats' health status in later life. NH is a healthy source of dietary sugars, and female rats were less susceptible to metabolic health problems. Extrapolating data from male rats to females should be done with caution due to potential gender differences in response to dietary manipulations.

Key words: Cane syrup, dietary supplements, disease, growth, health, metabolism, natural honey, obesity, sugar, rats.

INTRODUCTION

The dietary pattern can alter gastro-intestinal functions (Pacha, 2000), with consequent morphological and physiological changes in the viscera (Fukuchi et al., 2004; Rutledge and Adeli, 2007), and affecting human health. The major components of a modern human diet include added refined sugars such as fructose (Melanson et al., 2007). The increasing global consumption of refined sugars is associated with an increased incidence and prevalence of metabolic disorders and other deleterious effects in the human populace (Deen, 2004; Ford and Giles, 2003; Mahgoub et al., 2002). These consequently increase susceptibility to diabetes mellitus and renal diseases (Ford and Giles, 2003) leading to poor health, and children are also at risk (Birch et al., 1989). A high sugar diet decreases protein intake and retards growth (Ruottinen et al., 2008), and leads to

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obesity (Cao et al., 2007; Ludwig et al., 2001) in infants and children. Unfortunately, refined sugars mainly fructose is currently a major source of energy for metabolic activities. There is need to find an alternative source of this important dietary element for normal metabolism without compromising human health.

The substitution of refined carbohydrates by honey has been shown to be beneficial (Ajibola et al., 2007, 2012; Busserolles et al., 2002). Natural honey (NH) is a sweet liquid food of high nutritional value (Ajibola et al., 2012; White and Doner, 1980), and provides immense health benefits (Ajibola et al., 2007, 2012; Truswell, 1992; Gheldof and Engeseth, 2002; Chepulis and Starkey, 2008). The constituents of NH are sugars, water, amino acids, antibiotic-rich inhibine, proteins, phenol antioxidants, and micronutrients (Gheldof and Engeseth, 2002; White and Doner, 1980). The sugars in NH give more energy than artificial sweeteners (Ajibola et al., 2007; Truswell, 1992), and fructose is the most abundant sugar in honey (White and Doner, 1980). Despite its high fructose content, previous studies show that eating honey does not cause health hazards (Ajibola et al., 2012; Busserolles et al., 2002; Truswell, 1992), due to its phytochemical and other constituents (White and Doner, 1980). Honey consumption has been shown to be gastroprotective. NH ameliorates the lesions in adult rats exposed to gastrointestinal assaults by ulcerative and toxic agents such as acetic acid, ethanol, indomethacin and acidified aspirin (Gharzouli et al., 2002; Mahgoub et al., 2002). The antioxidants in honey have also been shown to control oxidative damage by activating the antioxidant potential of the liver (Gheldof and Engeseth, 2002; White and Doner, 1980). Despite the plethora of investigations conducted on honey's influence on health (Ajibola et al., 2012; Chepulis, 2007; Chepulis and Starkey, 2008; Gharzouli et al., 2002; Mahgoub et al., 2002; Schramm et al., 2003), most studies have concentrated on adult mammals. The few studies that took cognisance of the response of honey-fed young animals commenced with weaned animal models (Busserolles et al., 2002; Chepulis, 2007). However, it is known that dietary interventions in the pre-weaning phases may have long lasting effects on an individual, causing either precocious maturation of the gastrointestinal tract and/or some deleterious effects (Fukuchi et al., 2004; Pacha, 2000; Ruottinen et al., 2008; Rutledge and Adeli, 2007). Similarly, we are also not aware of any published data in which the long-term dietary effects of honey and cane syrup was compared on a gender basis. A survey of the literature reveals that most dietary studies use mainly male weaned rats as animal models (Ajibola et al., 2007; Al-Waili et al., 2006). The few data on neonates are from short term infant feeding (less than 4 weeks), and devoid of consideration of gender differences (Ramenghi et al., 2001). However, there are anecdotal reports of feeding honey to newborn babies, supported by cultural and traditional practices (Al-Bukhari, 1976; MacMillan, 1999). The inclusion of refined sugars in infant formula has also been documented (Rivero-Urgell and Santamaria-Orleans, 2001).

Therefore, our study was undertaken to determine the long term effects of consumption of honey vis-à-vis cane syrup on the growth and health profiles of male and female growing Sprague-Dawley rats fed from infancy to adulthood (7 to 91 days).

METHODOLOGY

The study was approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, South Africa (AESC approval number– 2010/29/2B), and performed according to the "Guidelines for the use and care of animals in Experimental, Education and other Scientific Procedures" of the University. The experiments were done in the animal unit of Central Animal Services (CAS) and the School of Physiology at the University of the Witwatersrand, South Africa.

Animals

Six dams and their pups (litter size 8 to 12) provided by the CAS were used for the study. The 59 neonatal (7-day old) male (n = 30) and female (n = 29) Sprague-Dawley rats had a mean body weight (BW) of 17.9 (SEM 0.28), (range 14 to 22) g. The mean BW of male pups was 18.1; and not significantly different from that of females (17.7).

Housing

During the pre-weaning period, the pups with their respective dams were housed as a family in solid bottom plastic cages (425 x 270 x 140 mm) with beddings of wood shavings. The dams were given commercial rat feed (Epol, Johannesburg, South Africa) and tap water *ad libitum*. After weaning, the dams were returned to stock and the weaned rats were housed in pairs in the plastic cages. The animals were placed on a 12 hour light-dark cycle (lights on 07.00 to 19.00 h) and environmental temperature of 22 ± 2°C.

Dietary treatments

Pre-weaning (7 to 21 days)

Five pre-weaning diets were prepared, in addition to suckling. One control diet, which comprised gavaging with distilled water and four treatment diets comprising gavaging with a low dose (10 ml/kg BW) or with a high dose (20 ml/kg BW) of a 50 % solution weight / volume (w / v) of either GS or NH. The four treatment diets were named GS low (GSL), NH low (NHL), GS high (GSH) and NH high (NHH). The pups in each litter were randomly assigned to the five groups, and gender based replication was achieved for each of the treatment diets and the control (n = 6 for each diet, except NHH female (n = 5)). The pups in each litter were treated for the first 3 days (age 7 to 9 days) with 0.1 ml of either cane syrup (golden syrup® (GS), Illovo Sugar Ltd, Natal, South Africa), or NH, or distilled water according to their assigned dietary treatments or control, in order to familiarise them with the experimental procedures of weighing and gavaging. The rats were gavaged with the above diets or distilled water (control) from 10 to 21 days using a plastic orogastric gavaging needle and 1ml hypodermic syringe. The NH used was raw honey from commercial source, (Willy's B's

Table 1. Nutritional composition (dry matter basis) of the undiluted forms of natural honey (NH) and golden syrup (GS) used for orogastric gavaging of the rats during pre-weaning phase (d 10 to 21) and to formulate the diets for the weaned animals.

Proximate analyses	Natural honey (NH)	Golden syrup (GS)
Dry matter (%)	84.08 ± 0.07	83.38 ± 0.01
Energy (MJ/Kg)	15.56 ± 0.21	15.55 ± 0.06
Protein*(%)	0.42 ± 0.06	0.25 ± 0.05
Fat (%)	0.53 ± 0.01	0.62 ± 0.00
Ash (%)	0.53 ± 0.00	0.17 ± 0.00

Nutritional composition of the undiluted NH and GS as provided by the South African National Accreditation Systems (SANAS) accredited Analytical laboratory (ARC-Irene Analytical Services, Irene 0062, South Africa). Data are expressed as means ± SEM. *Obtained by multiplying nitrogen content by the factor of 6.25.

Table 2. Nutritional composition (dry matter basis) of treatment and control diets used during the post-weaning period of the 12-week study (from d 22 to 91).

Nutrients	CRF	GSL	NHL	GSH	NHH
Dry matter (%)	74.79 ± 0.09^{a}	83.18 ± 0.22^{b}	83.49 ± 0.21^{b}	76.75 ± 0.48^{a}	76.61 ± 0.02^{a}
Energy (MJ/Kg)	16.47 ± 0.04^{a}	17.03 ± 0.24^{a}	16.56 ± 0.06^{a}	17.11 ± 0.19^{a}	17.87 ± 0.01^{b}
Protein*(%)	19.33 ± 1.49^{a}	17.00 ± 0.08^{a}	17.50 ± 0.46^{a}	11.43 ± 0.57^{b}	11.85 ± 0.30^{b}
Fat (%)	3.71 ± 0.01^{a}	4.43 ± 0.03^{b}	3.51 ± 0.02^{c}	2.08 ± 0.02^{d}	2.45 ± 0.01^{e}
Ash (%)	7.74 ± 0.04^{a}	5.95 ± 0.06^{b}	6.30 ± 0.07^{c}	3.72 ± 0.08^{d}	4.42 ± 0.02^{e}

Nutritional composition of the diets as provided by the South African National Accreditation Systems (SANAS) accredited Analytical laboratory (ARC-Irene Analytical Services, Irene 0062, South Africa). Results are expressed as means ± SEM. Data in the same row with different superscripts are statistically significant ($p < 0.05$). *Obtained by multiplying nitrogen content by the factor of 6.25.

Farm, Eikenhoff 1872, South Africa); whilst the GS was cane syrup in form of golden syrup, Illovo Sugar Ltd, Illovo, Natal 4150, South Africa. The GS and NH were diluted to avoid gavaging the young rats with thick fluids that could obstruct their respiratory tracts. Table 1 shows the nutritional composition of the undiluted honey and golden syrup as determined by an accredited Analytical laboratory. The NH and GS were iso caloric and each had less than 0.5% protein content (Table 1). All the rats were kept with their respective dams and allowed to suckle *ad libitum* between gavage and weighed daily before weaning to monitor growth.

Post-weaning diets (22 to 91 days)

Five post-weaning diets were also prepared. The GS or NH was added to the RF at the low level of 20% of the diet (volume/weight, v/w) for GSL and NHL, and at the high level of 50% (v/w) for the GSH and NHH. The control diet was the commercial RF (CRF) to which 20% (v/w) tap water was added. The pups were weaned at 22 days of age on to their post-weaning diets. The diets were prepared daily, weighed and freshly served in clean bowls. The nutritional composition of the adult diets as determined by the accredited Analytical laboratory is given in Table 2. The rats were supplied with tap water *ad libitum*. During this period (post-weaning), the rats were weighed twice weekly to monitor BW gain.

Blood sample collection

The feeding regimens were terminated at the age of 13 weeks, and the animals were fasted for 12 hours overnight prior to terminal intervention. The rats were euthanized with pentobarbitone (150 mg/kg intra peritoneal, Euthanaze, Centaur labs, Johannesburg, South Africa), and 10 mL of blood was collected by cardiac puncture using 10 mL syringes and 21G needles and divided equally into plain and heparin coated tubes (Greiner Bio-one GnebH, Austria) for clinical chemistry. The heparin coated tubes were gently inverted so that the blood mixed with heparin to prevent the blood from clotting, the blood samples were subsequently centrifuged to obtain plasma, while serum was obtained by centrifuging the blood collected in the plain tubes. The blood samples were centrifuged with a Sorvall® RT 6000B (Du Pont, USA) at 4°C and 5000xg for 15 min. The serum and plasma collected were frozen at –20°C for later analyses.

Visceral measurements

The rats were then meticulously dissected to collect the liver, kidneys and visceral fat for weighing using a Precisa 310M digital balance (Precisa®, Vadodara, Switzerland). Portions of the caudate lobe were cut from each liver sample and stained with haematoxylin and eosin (H & E) for histological examinations. The liver histology was observed using a light microscope (LM) (Reichert®, Austria) at x100 and x400 magnifications. The liver micrographs were then obtained with a Kodak C143 digital camera (Kodak®, China) mounted on the LM eye piece.

Clinical biochemistry

The activity of the liver enzyme aspartate transaminase (AST) was determined using the stored serum samples on a calibrated Reflotron machine (Roche Diagnostics Ltd, Burgess Hill West Sussex, UK) according to manufacturer's instructions. The other liver function tests: alanine transaminase (ALT); alkaline

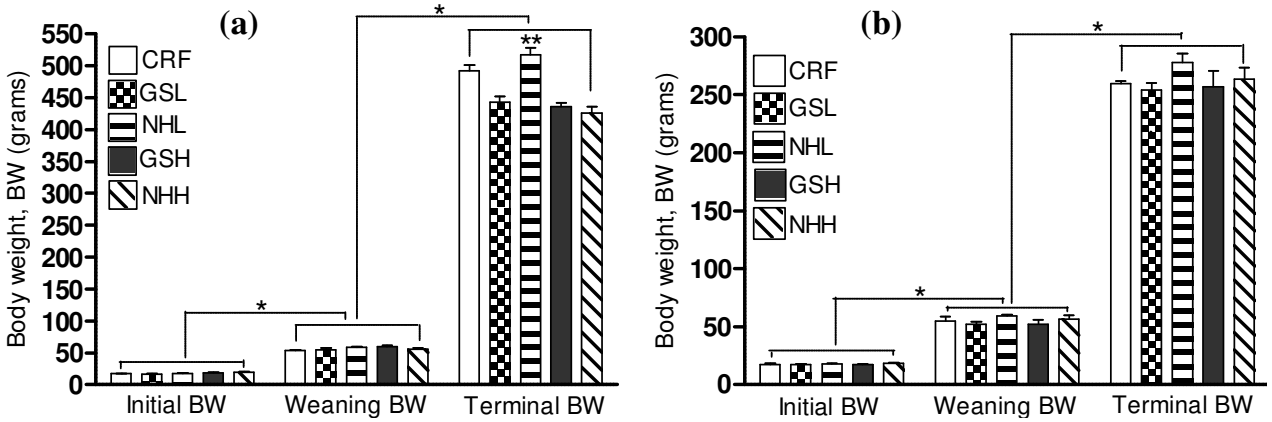

Figure 1. Growth phases of male experimental rats fed NH and GS supplements for 13 weeks: Initial body weight (BW) at day 7; Weaning BW (day 21); and Terminal BW at 13 weeks old: *BW significantly higher than BW of previous phase. (a) **TBW significantly higher ($p < 0.01$) than TBW of other groups. (b) No difference in TBW among the groups.

phosphatase (ALP); total protein (TP); albumin; globulin and total bilirubin (T bil) were done colourimetrically from 300 µl plasma using health profile discs on a Idexx Vet Test Chemistry Analyzer (Diamond Diagnostics® Holliston MA, USA). The other clinical parameters obtained from assaying the plasma sample on the Idexx Chemistry Analyzer were plasma concentrations of creatinine; urea; calcium; phosphorus and amylase using their respective discs.

Linear growth

The right femoral head was cut away from the acetabulum at the hip joint and soft tissues were removed from the long bones (femur and tibia). The length of the femur and tibia was then measured. Afterwards, the long bones were dried in a Salvis vacucenter oven (Oakton®, USA) at 40°C for 7 days (until constant weight) and weighed to obtain their dry weight. The bone density was estimated grossly by the formula of Monteagudo et al. (1997): Bone density (mg/mm) = dry bone weight (mg)/bone length (mm); and also obtained through radiographs using Fuji film X-ray machine.

Statistical analyses

Data were expressed as mean ± SEM. GraphPad Prism for Windows Version 5.02 (GraphPad Software, San Diego, California, USA) was used for data analyses. Student's t-test was used for the analysis of NH and GS shown in Table 1. The data from BW changes were analysed by repeated measures two-way analysis of variance (ANOVA) with Bonferonni's post hoc test. The means of the other parameters were analysed by one-way ANOVA with Neuman-Keul's post hoc used as a multiple comparison test. Level of significance was set at $p < 0.05$.

RESULTS

Dietary supplements

The analyses of natural honey and cane syrup data with Student's *t*-test showed that both dietary supplements were isocaloric and that the other components of both substrates (NH and GS) were not significantly different ($p < 0.05$). All the adult diets were iso-caloric; dose-matched diets were iso-nitrogenous; control diet, CRF was also iso-nitrogenous with low-dose diets.

Growth

Body weight gain (BWG)

The growth patterns of the male and female experimental animals from day 7 to 91 are shown in Figures 1a and 1b. All the treatment diets induced BWG in both sexes. At weaning (3 weeks old), there was no difference in the body weight among all the groups (Figures 1a and 1b).

All rats gained weight significantly over the 12-week study period, with the highest BWG in the NHL male group. The growth pattern of the male animals showed that honey-fed rats had significant terminal BWG of 16.6% relative to GS-fed rats (NHL *vs* GSL); and marginal (5.0%) relative to control rats (NHL *vs* CRF) at low dose (Figure 1a). At high dose, GSH gained more body weight (2.3%), albeit insignificant than NHH in males.

Results of terminal BWG in the females (Figure 1b) showed that the honey-fed gained 9.0% more than those fed low dose cane syrup diet (NHL *vs* GSL); and 6.9% more than control (NHL *vs* CRF) at the end of the study respectively. There was no difference ($p \geq 0.05$) in terminal BWG among the female groups at high dose-diets.

Linear growth

The results given in Table 3 showed that NH induced significant femur elongation and greater weight, and heavier tibia ($p < 0.05$) in males at low dose (NHL rats); whilst at high dose (NHH), only the femur elongation ($p < 0.05$) was observed. The densities of both long bones

Table 3. Weight, length and density of the femur and tibia in male and female experimental rats fed natural honey and cane syrup supplements for 12 weeks.

Diet	Sex	Femur			Tibia		
		Weight (mg)	Length (mm)	Density (mg/mm)	Weight (mg)	Length (mm)	Density (mg/mm)
CRF	M	848.0 ± 11.0	36.0 ± 0.45	23.56 ± 0.29	622.3 ± 13.4	43.7 ± 0.99	14.27 ± 0.29
	F	631.0 ± 31.7$^{\alpha}$	34.8 ± 0.48	18.10 ± 0.83$^{\alpha}$	458.5 ± 14.4$^{\alpha}$	39.3 ± 0.56$^{\alpha}$	11.66 ± 0.29$^{\alpha}$
GSL	M	813.2 ± 34.2	34.7 ± 0.61	23.44 ± 0.82	611.5 ± 28.3	41.8 ± 1.01	14.61 ± 0.56
	F	632.8 ± 13.3$^{\alpha}$	33.3 ± 0.56	18.99 ± 0.33$^{\alpha}$	469.0 ± 16.2$^{\alpha}$	38.7 ± 0.21$^{\alpha}$	12.13 ± 0.42
NHL	M	947.0 ± 20.5*	38.5 ± 0.72*	24.62 ± 0.57	689.8 ± 10.6*	44.2 ± 0.65	15.63 ± 0.21
	F	669.2 ± 29.1$^{\alpha}$	33.8 ± 0.60$^{\alpha}$	19.80 ± 0.87$^{\alpha}$	487.0 ± 35.6$^{\alpha}$	40.2 ± 0.79$^{\alpha}$	12.13 ± 0.85$^{\alpha}$
GSH	M	820.3 ± 24.3	35.8 ± 0.98	23.03 ± 1.14	580.3 ± 20.9	42.2 ± 0.31	13.76 ± 0.46
	F	594.7 ± 25.8$^{\alpha}$	34.7 ± 0.76	17.14 ± 0.56$^{\alpha}$	435.3 ± 23.0$^{\alpha}$	38.8 ± 0.47$^{\alpha}$	11.20 ± 0.49
NHH	M	827.7 ± 25.4	37.3 ± 0.95*	22.18 ± 0.46	624.8 ± 41.0	43.8 ± 0.70	14.23 ± 0.85
	F	617.8 ± 20.8$^{\alpha}$	35.4 ± 0.75	17.49 ± 0.73$^{\alpha}$	454.2 ± 14.7$^{\alpha}$	39.6 ± 0.51$^{\alpha}$	11.48 ± 0.39$^{\alpha}$

Data are expressed as mean ± SEM. Data in each column showing different superscripts is significantly different. *Significantly higher ($p < 0.05$) than value along the columns. $^{\alpha}$Value significantly different from the corresponding value of male group.

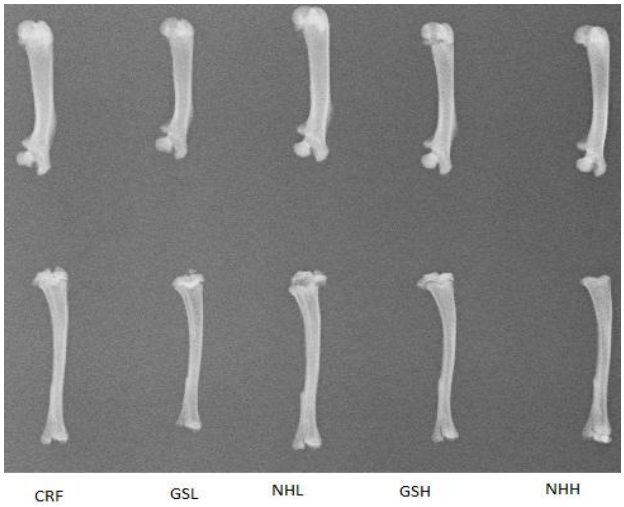

Figure 2. Radiograph obtained with Fuji film X-ray machine showing the long bones of male experimental rats at 12 weeks. Above: femur; below: tibia.

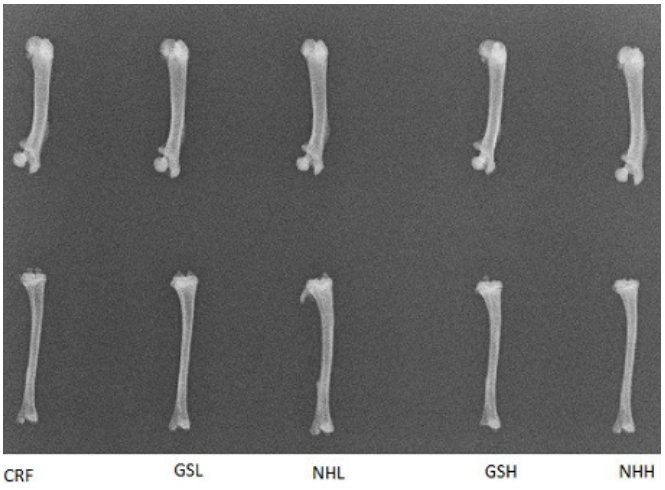

Figure 3. Radiograph obtained with Fuji film X-ray machine showing the long bones of female experimental rats at 12 weeks. Above: femur; below: tibia.

NHL were also denser than those of GSL (7.0%) and control (9.5%), while those of GSL were calculated to be denser than those of CRF marginally (2.4%). In the females, the density of the NHL femur was higher than those of GSL and CRF by 4.3 and 9.4% respectively, while GSL femur was denser than the control rat femur by 4.9% (Table 3). There were no gross differences in all the three parameters of linear growth (weight, length and density) measured in the tibias of all the female rats (Table 3). There were also no gross significant differences in the bones' densities of all the rats at high dose-diets. However, the graphical representation of the bones radiology showed that NH-fed rats had denser calculated as mg/mm showed that in males, NHL femurs were denser than those of GSL and the control (CRF) by 5.0% and 4.5% respectively while GSL femurs were 0.5% less dense than those of the control. The male tibias of bones than the other groups (Figures 2 and 3) in both sexes. Table 3 also showed the gender differences in linear growth. The male bones (femur and tibia) are heavier than the female bones; the tibia is longer in male rodents than the females amongst all groups; whilst the

Table 4. Weights of the liver and visceral fat in male and female experimental rats fed NH and GS supplements for 12 weeks.

Diet	Sex	Liver		Visceral fat	
		g	%	g	%
CRF	M	11.16 ± 0.34	2.57 ± 0.04	8.56 ± 0.50	1.97 ± 0.13
	F	7.02 ± 0.33$^{\alpha}$	2.67 ± 0.10	6.68 ± 0.66	2.56 ± 0.28
GSL	M	14.23 ± 0.56*	3.03 ± 0.15*	17.70 ± 0.73**	3.75 ± 0.14**
	F	7.44 ± 0.34$^{\alpha}$	2.55 ± 0.18$^{\alpha}$	10.25 ± 1.00 $^{\alpha}$	3.48 ± 0.34
NHL	M	12.57 ± 0.30	2.72 ± 0.13	11.11 ± 0.37	2.39 ± 0.07
	F	7.49 ± 0.30 $^{\alpha}$	2.70 ± 0.06	8.17 ± 0.66	2.96 ± 0.26
GSH	M	10.92 ± 0.32	2.57 ± 0.04	15.48 ± 0.85**	3.64 ± 0.14**
	F	7.74 ± 0.41 $^{\alpha}$	2.83 ± 0.05	10.09 ± 1.32 $^{\alpha}$	3.65 ± 0.36
NHH	M	10.24 ± 0.40	2.51 ± 0.06	10.21 ± 1.04	2.48 ± 0.21
	F	7.20 ± 0.28 $^{\alpha}$	2.63 ± 0.04	9.20 ± 0.92	3.39 ± 0.38

Data are expressed as mean ± SEM. *value significantly different ($p < 0.05$) across the column. **Significantly higher ($p < 0.0001$) than control (CRF) and other treatment groups in males. $^{\alpha}$Value significantly different ($p < 0.05$) from corresponding value in male groups.

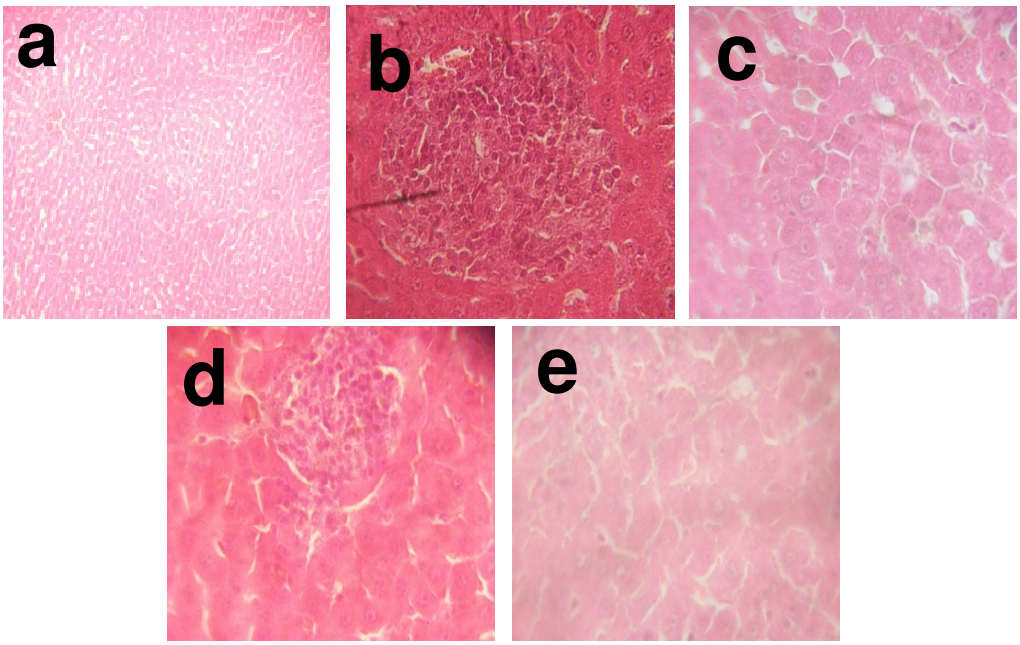

Figure 4. Photomicrographs of male rat liver (Haematoxylin & Eosin (H & E) stain) showing liver architecture: (a) CRF, control group (magnification (mag) = 100 x); (b) GSL (mag = 400 x); (c) NHL (mag = 400 x); (d) GSH (mag = 400 x); (e) NHH (mag = 400 x). There is accumulation of Kupffer cells around the central vein in the GSL and GSH livers due to inflammation.

density of the male femurs were also observed to be higher than that of the female rats.

Liver and Visceral fat

The weight of the liver and visceral fat was recorded in Table 4. The male GS-fed rats had higher absolute liver weight (g) at both (low and high) -diet doses, and relative to BW, only GSL rats had heavier livers (%) than the other male groups. There was no difference amongst the female rats in the measured absolute (g) and relative (%) weight of the liver. Cane syrup induced a highly significant ($p < 0.0001$) increased visceral fat weight in

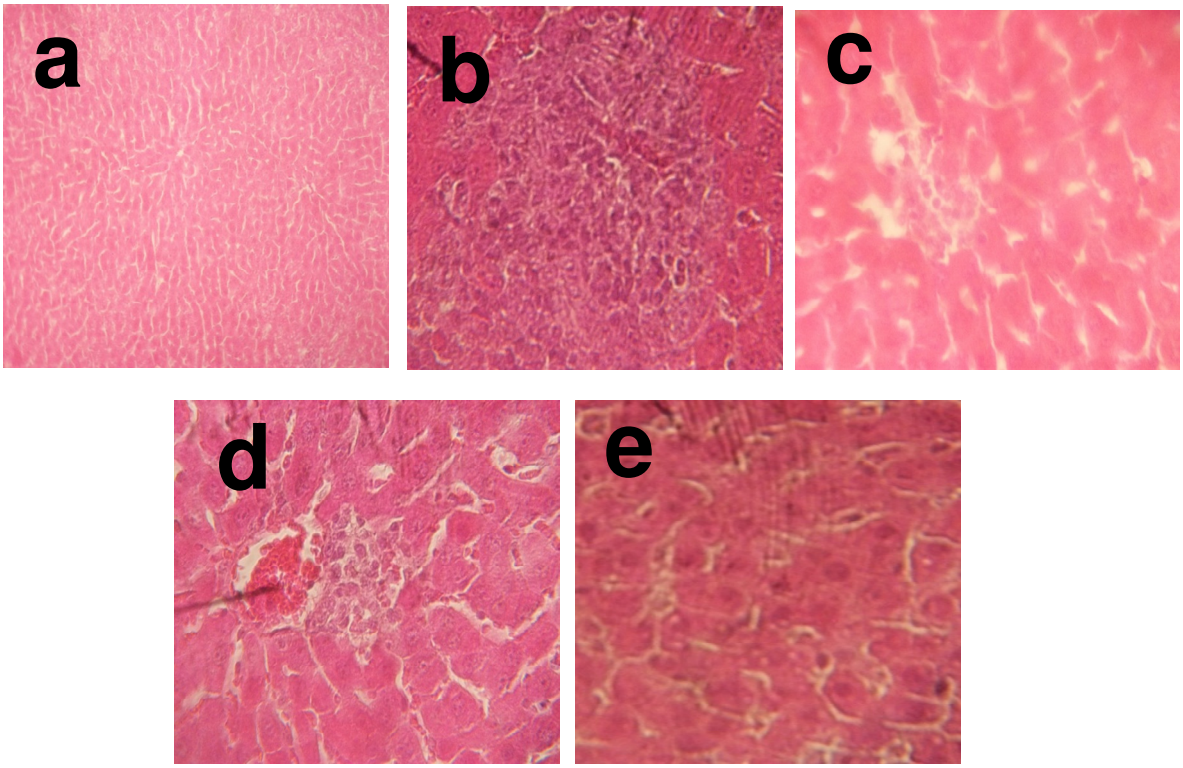

Figure 5. Photomicrographs of female rat liver (H & E stain) showing the liver architecture: (a) CRF, control group (mag = 100x); (b) GSL (mag 400x); (c) NHL (mag = 400x); (d) GSH (mag = 400x); (e) NHH (mag = 400x). There is accumulation of Kupffer cells around the central vein in GSL and GSH livers due to inflammation.

males relative to the other groups; whilst there was no difference ($p \geq 0.05$) in the visceral adiposity of all the female rats (Table 4). There was accumulation of Kupffer cells in the livers of cane syrup fed rats (Figures 4 and 5), an indication of inflammation.

Health indices

Table 5 showed the liver function test data; whilst the kidneys weight and renal function tests parameters are documented in Table 6. The other clinical biochemistry parameters showing the health profiles of the animals are documented in Table 7. The values of the liver marker enzymes, (except ALP) of the animals fed with cane syrup, especially in male rats were higher than normal (Table 5). All the values obtained from NH-fed rats were within the normal ranges. However, the differences in values of most of the hepatic, renal and other health indices obtained from the male and female experimental animals did not attain any statistical significance (Table 6). These are creatinine, calcium, phosphorus, alanine transaminase, total bilirubin, total protein and globulin. The albumin levels obtained from all the rats were slightly lower than normal values, reflecting the low protein levels of the experimental diets as shown in Table 2.

DISCUSSION

The results of our study confirmed that there are immense health benefits in eating natural honey compared to cane syrup. The GS and NH solutions fed to the neonatal animals were iso-caloric and each had less than 0.5% protein content, and as such the pups were exposed to substrates with similar macronutritional values. However, NH contains minerals, and other micronutrients than GS (White and Doner, 1980), as indirectly deduced from its (NH) ash content (Table 1). All the diets fed to the adult rats were iso-caloric (except NHH with higher energy value), and dose-matched diets were iso-nitrogenous. However, GSH and NHH had significantly ($p < 0.05$) lower protein content than others (Table 2). The energy value and protein content of CRF diet were similar to those of low dose diets. The control and low dose diets met the nutrient requirements of growing rats (NRC, 1972) whilst the protein contents of the high dose-diets were lower than recommended. The estimated feed intake of the low dose-diet groups was higher than the feed consumption by their high dose-diet counterparts (data not shown). The difference was probably due to preference for solid particles in low-dose diets, against the pasty nature of high-dose diets. This preferential dietary intake by rats has been reported

Table 5. Liver function tests in male and female experimental rats fed natural honey and cane syrup for 12 weeks.

Diet	Sex	AST (U/L)	ALT (U/L)	ALP (U/L)	Tbil (µmol/L)
		Normal range of values			
		45.7 – 80.8	15.7 – 30.2	57 – 128	3.42 – 12
CRF	M	67.2 ± 2.19	28.5 ± 2.46	99.2 ± 6.56	11.5 ± 3.39
	F	61.2 ± 4.47	27.3 ± 6.74	82.8 ± 4.32	9.7 ± 2.08
GSL	M	75.5 ± 4.56*	43.5 ± 9.57	97.7 ± 5.85	8.7 ± 0.99
	F	81.6 ± 4.04$^{\beta}$	30.5 ± 12.10	75.0 ± 2.65	7.7 ± 1.12
NHL	M	58.2 ± 4.19	27.5 ± 3.13	104.8 ± 8.83	7.2 ± 1.01
	F	66.6 ± 3.08	25.2 ± 3.09	74.2 ± 1.01$^{\alpha}$	9.0 ± 1.91
GSH	M	86.7 ± 8.15*	32.8 ± 8.68	128.3 ± 11.05*	8.2 ± 0.60
	F	84.6 ± 9.83$^{\beta}$	26.3 ± 3.89	69.8 ± 4.06$^{\alpha}$	7.3 ± 0.49
NHH	M	69.9 ± 2.88	25.8 ± 2.87	117.7 ± 8.02	9.7 ± 1.38
	F	76.9 ± 1.86	24.0 ± 4.99	68.6 ± 5.71$^{\alpha}$	9.4 ± 1.57

AST assayed from serum samples on Reflotron machine (Roche Diagnostics®, Burgess Hill, UK); Liver enzymes (ALT, ALP) activities and TBil obtained as part of the health profiles assayed from plasma on the Idexx Vet Test Chemistry Analyzer (Diamond Diagnostics®, Holliston MA, USA). Data are expressed as mean ± SEM. *values different ($p < 0.05$) in males across the column. $^{\beta}$Different from corresponding female values ($p < 0.05$). $^{\alpha}$different ($p < 0.05$) from corresponding value in male groups. AST = aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase; Tbil = total bilirubin.

Table 6. Kidneys weight, urea, creatinine and urea/creatinine ratio in the male and female experimental rats fed NH and GS supplements for 12 weeks.

Diet	Sex	Kidneys weight (g)	Urea (mmol/L)	Creatinine (µmol/L)	Urea/creatinine ratio
			Normal range of values		
			3.6 – 7.5	44.2 – 88.4	20.2 – 42.1
CRF	M	2.99 ± 0.07	5.7 ± 0.30	49.0 ± 4.77	30.1 ± 3.02
	F	1.96 ± 0.07$^{\beta}$	6.9 ± 0.37$^{\beta\alpha}$	57.8 ± 2.94$^{\alpha}$	29.6 ± 1.51
GSL	M	3.21 ± 0.11	6.0 ± 0.34	51.2 ± 1.82	28.9 ± 1.55
	F	1.91 ± 0.05$^{\beta}$	5.3 ± 0.36	52.3 ± 2.46	25.0 ± 1.33
NHL	M	3.11 ± 0.13	5.5 ± 0.25	51.0 ± 1.26	26.8 ± 1.57
	F	1.95 ± 0.08$^{\beta}$	5.1 ± 0.24	48.5 ± 3.32	26.7 ± 2.64
GSH	M	2.56 ± 0.08*	3.9 ± 0.46*	41.8 ± 1.80	22.9 ± 1.91
	F	1.95 ± 0.10$^{\beta}$	5.3 ± 0.23$^{\alpha}$	55.5 ± 4.35	24.5 ± 2.11
NHH	M	2.53 ± 0.07*	3.8 ± 0.20*	43.8 ± 2.85	22.0 ± 2.10
	F	1.82 ± 0.05$^{\beta}$	5.3 ± 0.34$^{\alpha}$	49.6 ± 3.28	26.9 ± 2.89

Data are expressed as means ± SEM. *values different ($p < 0.05$) in males across the column. $^{\beta}$ Different from corresponding female values ($p < 0.05$). $^{\alpha}$ different ($p < 0.05$) from corresponding value in male groups.

elsewhere (Sieck et al., 1978). The growth patterns of our male and female rats from the age of 7 days old to termination at 13 weeks showed the treatment diets induced significant BWG in both sexes. However, there was a notable differential response to dietary treatments and gender influence at termination (13 weeks) as shown

Table 7. Other health profile markers assayed in the plasma of the male and female experimental rats fed NH and GS supplements for 12 weeks.

Diet	Sex	Ca (mmol/L)	P (mmol/L)	TP (g/L)	Alb (g/L)	Glob (g/L)	Amyl (U/L)
		Normal range of values					
		2.00 – 3.25	1.71 – 2.68	56 – 76	38 – 48	18 – 28	326 – 2246
CRF	M	2.2 ± 1.00	2.4 ± 0.04	58.8 ± 1.11	31.2 ± 0.87	27.7 ± 0.67	1024.5 ± 81.2
	F	2.5 ± 0.17	2.2 ± 0.22	64.0 ± 3.65	34.8 ± 2.82$^{\alpha}$	29.2 ± 4.39	672.3 ± 69.0$^{\beta\alpha}$
GSL	M	2.1 ± 0.09	2.6 ± 0.16	62.5 ± 1.57	31.0 ± 1.46	31.0 ± 2.73	1021.5 ± 139.4
	F	2.3 ± 0.12	2.2 ± 0.21	62.8 ± 2.64	33.8 ± 2.2$^{\alpha}$	29.2 ± 1.78	774.0 ± 50.6$^{\beta}$
NHL	M	2.3 ± 0.04	2.4 ± 1.00	59.7 ± 0.67	30.8 ± 0.70	29.0 ± 0.37	1083.0 ± 57.6
	F	2.2 ± 0.05	2.0 ± 0.08	60.3 ± 1.26	32.3 ± 0.99$^{\alpha}$	27.8 ± 1.76	867.3 ± 60.1
GSH	M	2.2 ± 0.06	2.5 ± 0.07	58.5 ± 0.56	31.2 ± 0.60	27.3 ± 0.49	1354.8 ± 85.4
	F	2.4 ± 0.03	2.2 ± 0.09	61.3 ± 2.20	32.2 ± 1.40$^{\alpha}$	29.0 ± 3.30	1016.8 ± 31.6$^{\alpha}$
NHH	M	2.2 ± 0.05	2.4 ± 0.05	57.7 ± 1.09	31.2 ± 0.60	26.5 ± 0.89	1267.7 ± 68.2
	F	2.2 ± 0.09	2.0 ± 0.13	62.6 ± 2.42	35.2 ± 3.07$^{\alpha}$	27.4 ± 3.49	894.4 ± 18.6$^{\alpha}$

Health profile markers assayed colorimetrically from 300µl plasma on the Idexx Vet Test Chemistry Analyzer (Diamond Diagnostics®, Holliston MA, USA). Data are expressed as mean ± SEM. *values significantly different ($p < 0.05$) across the column. $^{\beta}$values different from corresponding female values ($p < 0.05$). $^{\alpha}$Value significantly different ($p < 0.05$) from corresponding value in male groups. CRF, Control group, Rat feed; GSL, Golden syrup Low; NHL, Natural honey Low; GSH, Golden syrup High; NHH, Natural honey High; n = 6rats per group except NHH female where n = 5. Ca = calcium; P = phosphorus; TP = total protein; Alb = albumin; Glob = globulin; Amyl = amylase; nr = normal range of values.

in Figures 1a and 1b. As is normal for Sprague-Dawley rats, we recorded highly significant gender differences in growth response with the males having 43% more terminal BW than females, despite their similar weight at birth, as well as being subjected to the same dietary treatments and experimental conditions. Sprague-Dawley male rats are noted for faster growth and higher body weight than females (Klinger et al., 1996). The eating of honey caused normal growth as that of the control rats (Figures 1a and 1b), and increased the terminal body weights of the male rats more than that of GS-fed male (Figure 1a). This observation was in conformity with previous findings that honey improves the growth of rats (Ajibola et al., 2007, 2012). In a previous study, Cheplius and Starkey (2008) had a different opinion on growth response by honey-fed rodents. These authors reported low body weight (BW) gain of honey-fed and those fed sugar-free (control) diet adult rats relative to sugar-fed animals. The conflicting observations might be due to the difference in growth phases of the rats used for the studies. In the studies reporting the growth influence of honey, young rats in their active growing phase were used, while Cheplius and Starkey used two months old rats for their study, and also administered the honey at a probably sub-pharmacological and lower (10%) dose than the amount used in the present study (20 and 50%). In addition, it was not known whether the high BW of the sucrose-fed rats by these authors was due to muscle growth or high fat weight (Cheplius and Starkey, 2008). It is noteworthy that, the other aspect of the findings from the same study suggests the latter, as these authors report a significantly higher level of body fat in the sugar-fed rats than the honey eaters (Cheplius and Starkey, 2008). In a previous study, excess consumption of refined sugars is said to be associated with retarded growth (Ruottinen et al., 2008).

The measurement of long bones is a better and more accurate method of growth assessment in animals and human subjects. The tibial length has been used in several studies (Fritton et al., 2005; Hunziker and Schenk, 1989; Tjaderhane and Larmas, 1998) as a more accurate indicator of linear growth instead of the BWG.

Our findings on linear growth in the present study further corroborated previous findings on the growth influence of honey in rodents. The long bones of the NH-fed rats were longer ($p < 0.05$) and of higher weights than those animals fed with golden syrup. The increase in the bone density of honey-fed rats also showed that honey consumption influenced linear growth, probably due to the high calcium component of natural honey (Ariefdjohan et al., 2008; White and Doner, 1980). According to one study (Tylavsky, 2004), the calcium constituent of a diet has an influence on bone growth. This was also buttressed by other authors (Cho et al., 2001; Fritton et al., 2005) that showed that bone mineralization could manifest as increased bone density. However, we did not analyze the content of calcium in the honey used in our study. The increased bone weight

observed in our rats could be due to an increase in organic components of the bone. The gender difference to the dietary treatments of our animals came to fore again as the males were observed to have higher linear development than the female rodents. Grossly, the mild differences in all the parameters of both long bones (weight, length and density) used to assess linear growth did not attain any statistical significance amongst our female rats. However, the radiographic representations (Figures 2 and 3) of the bones showed that NH-fed rats had denser bones than GS rats in both sexes.

The consumption of cane syrup induced higher liver weight in the rats than honey (Table 4). We established from this study that, unlike GS dietary supplements, the feeding of rats with natural honey from an early age did not cause any pathological changes. The higher values of the liver marker enzymes, AST and ALT obtained from the rats nurtured with cane syrup supplements showed that the liver functions of these GS rats could have been compromised. On the contrary, honey consumption did not threaten liver functionality as seen from the values of all the liver key enzymes (AST, ALT and ALP), as well as other health profile markers shown in Tables 5, 6 and 7. The values obtained were within the normal ranges showing that there were no health problems in our honey-fed rats in this study. In addition, NH did not impact negatively on the other hepatic and renal health indices. These strengthened the health potentials of honey consumption.

Our rats nurtured with cane syrup had higher ($p < 0.0001$) visceral fat weight than NH-fed rats. These contributed substantially to their (GS fed rats) body weight, and manifested as abdominal obesity, a central component of metabolic syndrome (MetS) (Das, 2002). However, the increased visceral fat weight was highly significant ($p < 0.05$) in the male than female groups (Table 4), and the low dose-diet fed rats had enhanced visceral adiposity than the other groups. Cheplius and Starkey (2008) also document higher visceral adiposity in their sucrose-fed than the honey-fed rats. The elevated visceral adiposity coupled with hepatomegaly could cause hepatic steatosis, with eventual progression to nonalcoholic fatty liver disease (NALFD) in the GS-fed male rats. The exposure of the rats to long-term GS dietary supplements also caused hepatic inflammation, which manifested as Kupffer cells accumulation in the liver (Figures 4 and 5). There are potential benefits of substituting honey for refined sugars such as cane syrup in animal feed and by extension human diet. Previous animal study in our laboratory shows these nutritional and therapeutic values of honey. In that study, we performed oral glucose tolerance test (OGTT) on rats given similar dietary treatments, and recorded glucose intolerance in the GS rats unlike NH-fed rats (Ajibola et al., 2012). The total area under the curve (AUC) of the OGTT calculated in that study also showed the GS-fed rats were hyperglycaemic while honey eaters were normoglycaemic (Ajibola et al., 2012). In addition, the metabolic parameters obtained from our earlier study show that GS cause hypertriglyceridaemia, hypercholesterolemia, hyperinsulinaemia, increased circulating free fatty acids and increased hepatic fat stores unlike honey (Ajibola et al., 2012). The retarded growth, abdominal obesity and hepatic inflammation of the GS rats documented in our present study, and the other components of MetS earlier observed were not associated with NH consumption (Ajibola et al., 2012). This showed that honey induced healthy growth in animals, compared to the refined sugars such as golden syrup. Nonetheless, there is need for further studies with prolonged feeding of honey to experimental animals and clinical trials involving human participants. This can be instrumental to establish the mechanisms behind the health benefits of honey.

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ABBREVIATIONS

ALP; Alkaline phosphatase, **ALT;** alanine transaminase, **AST;** aspartate transaminase, **BW;** body weight, **BWG;** body weight gain, **CRF;** commercial rat feed, **GS;** golden syrup, **GSH;** golden syrup High, **GSL;** golden syrup Low, **nr;** normal range of values, **NH;** natural honey, **NHH;** natural honey high, **NHL;** natural honey low, **T bil;** total bilirubin, **RF;** rat feed.

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