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EFFECTS OF DIETARY SUPPLEMENTATION WITH PURE NATURAL HONEY ON METABOLISM IN GROWING SPRAGUE-DAWLEY RATS.

Abdulwahid Ajibola

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the degree

of

Doctor of Philosophy

Johannesburg, South Africa

2013

DECLARATION

I, Abdulwahid Ajibola declare that the work contained in this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other University.



……

Abdulwahid Ajibola

2nd day of July 2013

DEDICATION

To

Almighty Allah

Who taught by the pen

He taught man what he knew not

and

Prophet Muhammad

who encouraged the search

for knowledge as distant as China

RESEARCH OUTPUTS

PUBLISHED JOURNAL PAPERS

Ajibola A, Chamunorwa JP, Erlwanger KH. 2012. Nutraceutical values and contributions of natural honey to human health and wealth.

Nutrition and Metabolism 9: 61.

Available online at http://www.nutritionandmetabolism.com/content/9/1/61

Ajibola A, Chamunorwa JP, Erlwanger KH. 2013. Dietary supplementation with natural honey promotes growth and health of male and female rats compared to cane syrup. *Scientific Research and Essays* 8 (14): 543 – 553.

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Ajibola A, Chamunorwa JP, Erlwanger KH. 2013. Comparative effect of cane syrup and natural honey on abdominal viscera of growing male and female rats. *Indian Journal of Experimental Biology* 51: 303 – 312. Available online at http://www.nopr.niscair.res.in/bitstream/123456789/16553/1/IJEB51(4)303-312.pdf

SUBMITTED MANUSCRIPTS

Ajibola A, Chamunorwa JP, Erlwanger KH. Metabolic health of growing male and female rats on long-term dietary supplementation with natural honey compared to cane syrup. *FASEB Journal* (January 2013).

Ajibola A, Chamunorwa JP, Erlwanger KH. Metabolic syndrome susceptibility of suckling male and female rats following oral administration of natural honey compared with cane syrup. *Journal of Apicultural Research* (January 2013).

INTERNATIONAL CONFERENCES AND SEMINAR PRESENTATIONS

Ajibola A, Chamunorwa JP, Makaula S, Erlwanger KH. Comparative effects of honey and sugar on the morphometry of viscera in growing Sprague-Dawley rats (*Rattus norvegicus*). Presented at the 31st Annual Scientific Conference, Physiological Society of Nigeria (PSN), Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria 7th – 9th September 2011. (Oral Presentation). *Book of Abstract* pp 9 – 10.

Ajibola A, Chamunorwa JP, Erlwanger KH. Long-term response of male and female Sprague-Dawley rats (*Rattus norvegicus*) to dietary golden syrup and pure natural honey. Presented at the 31st Annual Scientific Conference, Physiological Society of Nigeria (PSN), Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto, Nigeria 7th – 9th September 2011. (Oral Presentation). *Nigerian Journal of Physiological Sciences* 26(2): 232.

Ajibola Abdulwahid. “Metabolic syndrome: Aetiology, Causes, Symptoms, Sequalae, Management and Control”. Guest Speaker at a One day Faculty Seminar Orgarnised by Faculty of Biomedical Sciences, Kampala International University, Uganda at Kampala International University, Western Campus (KIU-WC), Bushenyi, Uganda on 7th October, 2011.

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Ajibola A, Chamunorwa JP, Makaula S, Erlwanger KH. Metabolic response of suckling male and female rats to oral administration with honey compared to cane syrup. Presented at the 32nd Annual Scientific Conference, Physiological Society of Nigeria (PSN), Department of Physiology, University of Calabar, Nigeria 22nd – 24th August 2012. (Poster Presentation). *Book of Abstract* pp 33.

Ajibola A, Chamunorwa JP, Makaula S, Erlwanger KH. Excessive consumption of cane syrup induces a hepatic inflammatory response in healthy male and female rats. 6th Congress of the African Association of Physiological Sciences (AAPS), Suez Canal University Hospital, Ismailia, Egypt 3rd – 5th September 2012. *Book of Abstract* pp 45.

ABSTRACT

The excessive consumption of refined sugars mainly fructose is linked to metabolic dysfunction. The early introduction of refined sugars in the diets of children has resulted in an increased incidence of childhood metabolic dysfunction. There are conflicting reports on the gender susceptibility to developing metabolic dysfunction. Natural honey (NH) has been shown to have health benefits when included in the diet. The neonate is sensitive to dietary manipulations which can have long lasting effects. The short and long term effects of inclusion of NH in the diets of neonates were investigated in rat models. This was with a view to determine whether NH can substitute refined sugars such as cane syrup (GS), without adverse effects, and whether there were any gender differences in response to the dietary modification.

In the long term study, 59 suckling Sprague-Dawley (SD) rats were fed with either NH- or GS-supplemented diets from age 7 (neonate) to 91 (adulthood) days. For the short term study to investigate the effects of NH in neonates, 69 SD pups were gavaged with NH or GS twice daily at 12-hour intervals and allowed to nurse freely in between from age 7 to 20 days. The rats in all groups were weighed daily during the period when they were gavaged and then twice weekly thereafter to assess body weight gain (BWG) over the study periods. The adult rats were subjected to an oral glucose tolerance test (OGTT) at 13 weeks of age before termination. The rats' growth was determined through their BWG and linear growth was assessed by measurements on the bones (femur and tibia) weight, length and density. Blood was collected for the assessment of clinical biochemistry and plasma markers of

general health including the circulating metabolic substrates (glucose, triglycerides (TGs), free fatty acids (FFAs)); hormones (insulin, leptin); liver and renal functions were also obtained. Morphometric measurements (weight, lengths and histology) were also done on the abdominal viscera. Hepatic storage of metabolic substrates (glycogen and lipids) was determined. The analysis of the proximate composition of the NH and GS as well as the diets was performed with the Student's *t*-test. A repeated measures two-way analysis of variance (ANOVA) with Bonferonni's post hoc test was used to analyse the BWG and OGTT, while the other parameters were analysed by one-way ANOVA with Neuman-Keul's post hoc test, and level of significance was set at $p < 0.05$.

The matched diets were found to be isonitrogenous and isocalorific. Following the long term study, the NH fed rats showed tolerance to an oral glucose load. GS increased fasting blood glucose (FBG), TGs ($p < 0.05$), FFAs ($p < 0.0001$), visceral fat weight ($p < 0.0001$), and caused hypercholesterolemia, hyperinsulinemia, hepatomegaly and fatty liver in the males. NH increased intestinal villi growth and preserved the liver integrity in both males and females. Although, the GS-fed female rats did not suffer multiple risks of metabolic syndrome (MetS), there were high FBG concentration and hypercholesterolemia induced at low dose and metabolic dyslipidemia shown as high TG levels at high dose. These findings were contrary to the notion about the females having a lower susceptibility to developing metabolic syndrome than males.

Some traditional rites include the feeding of honey to infants at birth, and there is evidence of the dietary inclusion of sugars in infant formulations. The need to evaluate the safety of honey consumption vis-à-vis refined sugars in children becomes imperative. Thus, after inducing metabolic syndrome in the rats through 12-week GS feeding, the effects of both diets on neonates were studied. In the neonates, there was no difference in all the parameters measured except the higher circulating non fasting FFAs ($p < 0.0001$) and hepatic storage of lipids ($p < 0.001$) in GS-fed than the NH-fed pups. Metabolic syndrome did not develop within the short term.

NH was thus found to be a healthy source of dietary sugars, improved glycaemic control and metabolic profiles. The study underscored the differential effects of dietary treatments in male and female rodents. This pointed to the advantages of gender based comparative studies in biomedical research. The study confirmed the nutraceutical value of NH, and advocated for its consumption as a healthy substitute to refined sugars. The consumption of refined sugars by infants should be discouraged, and females should also be cautious in excessive sugar intake, as they can also be susceptible to the metabolic adverse health effects of artificial sweeteners.

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To my mother, Alhaja Sidikat Ajibola, who laid the educational foundation I built on, I pray that Allah grants you long life to reap bountifully from your farm. This foundation was concretised by my mum, Alhaja Adiat Fahm whose son, Dr Jubril Fahm (formerly of Livingstone Hospital, Port Elizabeth, South Africa) facilitated my MSc degree in Pretoria, South Africa. My family starting from my elder wife, Mrs Khairat T. Ajibola; my ward, Rahmot Adeola Lamidi; and my children namely Abdullah, Aminat, Aishat, Awwal, Adiat, Hasibat, Attiyyah and Akbar were all very supportive, and sacrificed their needs for my attention during the study. I thank my amiable, affable, affectionate, young wife, Mrs Azeezat M. Ajibola, not only for her applaudable attitudes, serene support, perpetual prayers, persistent perseverance and enduring encouragement, but also for single-handedly taking care of my entire family at her own expense during my absence from home in pursuit of this course. In addition to sacrificing her needs and comfort, she lent me her hands fiscally.

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GLORIFICATION

So high was the aspiration

Journey too long for decimation

On the road there was obstruction

Fear and anxiety leading to sublimation

Alas the hurdles jumped with determination

Lofty heights subdued for prestigious elevation

That’s enough a cause for jubilation

But there is a need to exercise caution

Because this another form of examination

And there are tunnels ahead for penetration

Also numerous tasks still waiting for actualisation

Amongst these are kins’ high hope and expectation

With Almighty Allah all these will be easy manifestation

In His name oceans crossed without partition

By Allah’s decree infant spoke without prior tuition

With His might mountains climbed without frustration

To all one’s puzzles Allah alone can proffer real solution

So I open and raise my palms up here goes the supplication

Make me a servant always showing gratitude and appreciation

After every help, succour, relief with each one of Your proclamation

To Allah alone belongs honour, gratitude, appreciation and glorification

QUOTATIONS

“And your Lord inspired the bee(s), saying: "Take your habitations in the mountains and in the trees and in what they erect. Then, eat of all fruits, and follow the ways of your Lord made easy (for you)." There comes forth from their bellies, a drink of varying colour wherein is healing for men. Verily, in this is indeed a sign for people who think” [Holy Qur’an 16: 68 – 69].

“Eat honey for your abdominal pain, the man ate honey and the pain disappeared after the third dosage”. – Holy Prophet

“Indeed the words of Almighty Allah and the words of the Holy Prophet Muhammad (*SAW*) are the truth”. – Abdulwahid Ajibola

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NOMENCLATURE

AESC; Animal Ethics Screening Committee

Alb; Albumin

ALP; Alkaline phosphatase

ALT; Alanine transaminase

AMPK; Adenosine monophosphate-activated protein kinase

Amyl; amylase

AST; Aspartate transaminase

ATP; Adult Treatment Panel

AUC; Area under the curve

BGC; Blood glucose concentration

BW; Body weight

BWG; Body weight gain

Ca; calcium

CAS; Central Animal Services

CCK; cholecystokinin

Chol; Cholesterol

cm; centimetres

CON; Control

Crea; Creatinine

CRF; Commercial rat feed

CRP; C-reactive protein

CVD; cardiovascular disease

^{o}C; degree centigrade

DEXA; dual energy X-ray absorptiometry

ESR; erythrocyte sedimentation rate

FBG; Fasting blood glucose

F; Female

FFAs; Free fatty acids

g; grams

g/cm; grams per centimetre

g/L; grams per litre

GH; growth hormone

GIT; Gastrointestinal tract

Glob; Globulin

Gluts; glucose transporters

Glyc sugars; Glycaemic sugars

GS; Golden syrup

GSH; Golden syrup High

GSL; Golden syrup Low

HDL; High-density lipoprotein

H & E; Haematoxylin & Eosin

HFCS; High-fructose corn syrup

HOMA-IR; Homeostasis model assessment of insulin resistance

IGF-1; Insulin-like growth factor-1

IR; Insulin resistance

LDL; Low-density lipoprotein

LI; Large intestine

M; Male

mag; magnification

MetS; Metabolic syndrome

mg; milligram

mg/dl; milligrams per decilitre

mg/dL; milligrams per decilitre

mg/kg BW; milligrams per kilogram body weight

mg/mm; milligrams per millimetre

mmol/L; millimoles per litre

ml; millilitres

ml/kg BW; millilitres per kilogram body weight

mm; millimetres

n; number of animals

NAFLD; non-alcoholic fatty liver disease

NCEP; National Cholesterol Education Program

NFG; Non-fasting blood glucose

NFT; Non-fasting blood triglycerides

ng/ml; nanograms per millilitre

NH; Natural honey

NHH; Natural honey High

NHL; Natural honey Low

nr; normal range of values

P; phosphorus

Pb; Lead

%; percent

% BW; percentage body weight

p; probability

PNH; Pure natural honey

OGTT; Oral glucose tolerance test

RF; Rat feed

RT-PCR; Reverse transcription Polymerase Chain reaction

SD; Sprague-Dawley

SEM; standard error of mean

SI; Small intestine

SMIF; synergistic multiple ingredients factor

T bil; Total bilirubin

TBW; Terminal body weight

TGs; Fasting blood triglycerides

TP; total protein

µm; micrometres

µmol/L; micromoles per litre

µU/ml; microunits per millilitre

U/L; units per litre

UMF; Unique Manuka Factor

v/v; volume/ volume

vs; versus

w/v; weight/ volume

PREFACE

Every living being requires adequate nutrients for growth and health. Most nutritional requirements are obtained via food consumption. There is the need to maintain a balance between food consumption, nutrients intake and the body's internal environment for normal functional activities of the creature. The types of food and dietary patterns of individuals play prominent roles to influence this maintenance of normal functional internal environment within the human body or body homeostasis. Nutritional inadequacies, lack or excess of certain nutrients can affect maintenance of body homeostasis, which can invariably lead to health compromise. Carbohydrates which include artificial and natural sweeteners provide energy for body metabolism and growth. However, just as the deficiency of these substances is inimical to health, excess consumption of refined sugars can cause metabolic disorders, thus leading to ill-health. Hence, there is need for alternative source of energy to provide nutrients, drive metabolic activities for growth without any threat to human health. This search informed my current investigation of the protective mechanisms of pure natural honey and its influence on metabolism in animal models.

CHAPTER ONE

INTRODUCTION AND JUSTIFICATION

CHAPTER ONE

INTRODUCTION AND JUSTIFICATION

1.0 – Introduction

Human beings and animals require adequate nutrients for growth, and maintenance. The nutritional requirements are obtained through the consumption of essential nutrients and supplements. Lack or excess of certain nutrients can affect the body's homeostasis [Miller and Miller, 1960; Ott and Asquith, 1989; Tjaderhane and Larmas, 1998; Duro *et al*, 2002; Tylavsky, 2004; Ariefdjohan *et al*, 2008; Muellenbach *et al*, 2009], and can hinder metabolic activities within the organism. The energy that drives body metabolism comes mainly from foods rich in carbohydrates including sugars. The lifestyle of modern day man involves a high intake of 'junk' and 'fast foods', with added sugars mainly sucrose and fructose, which include high fructose corn syrup (HFCS) [Bray *et al*, 2004; Melanson *et al*, 2007]. The metabolism of these nutrients takes place within body cells and tissues and if there is an excess of the undesirable nutrients, depending on source, body homeostasis is consequently altered, and pathophysiological conditions are precipitated.

1.1 – Sugar consumption

Sugars are sweet-flavoured substances classified as carbohydrate foods and composed of carbon, hydrogen and oxygen with the molecular formula of $C_nH_{2n}O_n$ (where n is between 3 and 7) [Pigman and Horton, 1972]. There are various types of sugar: monosaccharides ($C_6H_{12}O_6$) (glucose, fructose and galactose);

disaccharides ($C_{12}H_{22}O_{11}$) (sucrose, maltose and lactose); and oligosaccharides (fructo-oligosaccharide) [Pigman and Horton, 1972; Buss and Robertson, 1976]. Other types are polysaccharides (natural polymers of sugars) which include starch, polyols (sugar alcohols), cellulose, maltodextrin, deoxyribose ($C_5H_{10}O_4$) and ribose ($C_5H_{10}O_5$) [Merck, 1989, Robinson, 2006].

The industrial sugar production from sugarcane and sugar beet dates as far back as the 12th century in Tyre, Lebanon for export to Europe [Ponting, 2000; Barber, 2004], thus supplementing natural honey as a sweetening agent [Ponting, 2000]. Other nations have since joined Lebanon in the production and export of refined sugars. According to a report from the United States Department of Agriculture, the five largest sugar producers were Brazil, India, the European Union, China and Thailand in 2011. Table 1.1 shows the sugar production in some countries of the world [USDA, 2011]. Sugar forms an important part of the human diet in most parts of the world. Its inclusion in the diet makes food more palatable and provides the required energy for metabolic activities. On the average, it provides more kilocalories per capita daily than the other food groups, except cereals and vegetable oils [FAO, 2007]. Perhaps, this makes sugar consumption inevitable in most communities, especially the developed countries with their extensive production and use of HFCS [Bray *et al*, 2004; Gaby, 2005]. According to one FAO report, an average of 24 kilograms of sugar, equivalent to over 260 food calories per day, was consumed annually per person of all ages in the world in 1999 [FAO, 2007]. Sugar consumption is increasing with the rise in human populations, and it is expected to reach 25.1 kilograms per person per year by 2015 [FAO, 2012;

USDA, 2012]. In the United States of America, the per capita consumption of refined sugar varied between 27 and 46 kilograms in the last 40 years [USDA, 2012]. The sugar intake between 2007 and 2012 in some other parts of the world also increased tremendously as shown in Table 1.2 overleaf.

Table 1.1: World sugar production (1000 metric tons)*.

Country	**2007/08**	**2008/09**	**2009/10**	**2010/11**	**2011/12**
Brazil	31,600	31,850	36,400	38,350	35,750
India	28,630	15,950	20,637	26,650	28,300
European Union	15,614	14,014	16,687	15,090	16,740
China	15,898	13,317	11,429	11,199	11,840
Thailand	7,820	7,200	6,930	9,663	10,170
United States	7,396	6,833	7,224	7,110	7,153
Mexico	5,852	5,260	5,115	5,495	5,650
Russia	3,200	3,481	3,444	2,996	4,800
Pakistan	4,163	3,512	3,420	3,920	4,220
Australia	4,939	4,814	4,700	3,700	4,150
Others	38,424	37,913	37,701	37,264	39,474
Total	**163,536**	**144,144**	**153,687**	**161,437**	**168,247**

*Source: US Department of Agriculture, 2011

Table 1.2: World sugar consumption (1000 metric tons)*.

Country	**2007/08**	**2008/09**	**2009/10**	**2010/11**	**2011/12**	**2012/13**
India	22,021	23,500	22,500	23,500	25,500	26,500
European Union	16,496	16,760	17,400	17,800	17,800	17,800
China	14,250	14,500	14,300	14,000	14,400	14,900
Brazil	11,400	11,650	11,800	12,000	11,500	11,700
United States	9,590	9,473	9,861	10,086	10,251	10,364
Others	77,098	76,604	77,915	78,717	80,751	81,750
Total	**150,855**	**152,487**	**153,776**	**156,103**	**160,202**	**163,014**

*Source: US Department of Agriculture, 2012

There has been a tremendous rise in the intake of refined sugars in recent times as shown in Table 1.2 [USDA, 2012], due to its inclusion in cooking and baking, as well as in the production of sweets, canned fruits, jams, jellies, dairy foods, carbonated beverages and other sweetened drinks [Bray *et al*, 2004; Cook *et al*, 2003; Cruz and Goran, 2004; Elliott *et al*, 2002; Gaby, 2005; Schulze *et al*, 2004; Tappy and Le, 2010]. There are also increasing numbers of reports on the feeding of artificial and natural sugars to newborn babies [Haffejee and Moosa, 1985; Jones, 2001; Ramenghi *et al*, 2001; Rivero-Urgell and Santamaria-Orleans, 2001]. These reports include the use of refined sugars in infant formula [Rivero-Urgell and Santamaria-Orleans, 2001], and the traditional rites of feeding honey to newborn babies [Bianchi, 1977; MacMillan, 1999], supported by cultural norms [Al-Bukhari, 1976; Holy Qur'an 16 (An-Nahl): 68 – 69].

The increased consumption of artificial sugars especially fructose is due to the HFCS's thorough blending and mixing properties, as well as the low cost of production relative to sucrose [Bray *et al*, 2004; Gaby, 2005]. Thus the manufacturers of beverages and other carbonated-water products sweeten their products with the HFCS in a bid to save costs [Bray *et al*, 2004; Elliott *et al*, 2002; Gaby, 2005; Schulze *et al*, 2004]. However, this reduction in production costs is not devoid of another price tag; the metabolic syndrome (MetS) as reported in several adult studies [Bray *et al*, 2004; Elliott *et al*, 2002; Gaby, 2005; Promdee *et al*, 2007; Tappy and Le, 2010]. Other previous workers also confirmed that excessive sugar intake is taking its toll on human health [Deen, 2004; Ford and Giles, 2003; Johnson *et al*, 2007]. The incidence and prevalence of MetS has also

been reported in children [Bellisle and Rolland-Cachera, 2001; Betts *et al*, 2005; Birch *et al*, 1989; Cook *et al*, 2003; Cruz and Goran, 2004; Dubois *et al*, 2007; Ludwig *et al*, 2001; Ruottinen *et al*, 2008; Schulze *et al*, 2004]. The two main sources of dietary fructose (sucrose and HFCS) are culpable in the epidemiology of kidney, cardiovascular and metabolic diseases [Betts *et al*, 2005; Cao *et al*, 2007; Cruz and Goran, 2004; Johnson *et al*, 2007; Ludwig *et al*, 2001].

1.1.1 – Sugar metabolism

The physiological demands of the body for growth and maintenance necessitate the ingestion, digestion and absorption of food. The dietary sucrose is hydrolysed to its constituent monosaccharides, glucose and fructose in the small intestine by the jejunal brush border enzyme, sucrase. Fructose is the most abundant sugar in artificial and natural sweeteners including honey [Decaix, 1976; Doner, 1977; White and Doner, 1980; Truswell, 1992; Gaby, 2005; Venn and Green, 2007]. It is absorbed in the jejunum and metabolized by the liver. The exposure of the liver to large quantities of fructose stimulates lipogenesis leading to the accumulation of triglycerides (TGs) in the liver. Dietary TGs cannot be absorbed by the intestine, but are broken down to monoglycerides and free fatty acids (FFAs) by pancreatic lipase during hydrolysis. The hepatic lipogenesis further yields TGs, FFAs and low density lipoprotein (LDL). This (LDL) transports cholesterol from the blood to the peripheral tissues, while high density lipoprotein (HDL) transports cholesterol from peripheral tissues and the intestines to the liver for metabolism and excretion [Ganong, 2012]. The elevated plasma LDL will increase the LDL/HDL ratio, reduce cholesterol elimination, and elevate blood total cholesterol (TC) concentration.

Thus, high levels of total cholesterol (TC), with high LDL and low levels of HDL are risk factors of metabolic syndrome (MetS) [Johnson *et al*, 2007; Rutledge and Adeli, 2007; Tappy and Le, 2010]. In addition, these anomalies can cause atherosclerosis, a cardiovascular disease (CVD) associated with MetS [Elwood *et al,* 1970]. The accumulation of TGs contributes to reduced insulin sensitivity and glucose intolerance, which are also risk factors of CVD and metabolic dysfunction.

Fructose is a precursor of glyceraldehyde (a 3-carbon sugar), and high fructose intake elevates the blood levels of glyceraldehyde. Glyceraldehyde is the most reactive sugar in the body and reacts directly with other molecules through glycation without the involvement of enzymes [Levi and Werman, 1998]. Glyceraldehyde can also react directly with vital control and messaging proteins within cells to cause damage leading to cellular dysfunction. The organelles worst hit by this abnormality are the mitochondria, resulting in an impaired energy production. When the hepatic cells are affected by this energy deprivation, metabolic shutdown is triggered leading to MetS [Hallfrisch, 1990; Ford and Giles, 2003; Johnson *et al*, 2007; Rutledge and Adeli, 2007; Tappy and Le, 2010]. Glyceraldehyde can be reduced to glycerol in the liver following the intestinal absorption of fructose, and glycerol is a component of triacylglycerols and other lipid compounds. Hence, dietary fructose has a direct impact on hepatic lipid metabolism, and this entails bypassing the enzyme phosphofructokinase, the regulatory step imposed on glucose metabolism [Rutledge and Adeli, 2007]. This allows unregulated flow of fructose-derived carbons into lipogenesis, thus increasing the concentrations of circulating LDL and TGs [Rutledge and Adeli, 2007]. The high blood concentrations of TGs, LDL, and decreased concentrations

of HDL potentiate MetS [Johnson *et al*, 2007; Rutledge and Adeli, 2007]. In addition to the fructose-induced lipogenesis, dietary fructose also contributes to gluconeogenesis through its metabolite, glyceraldehyde with consequent altered glucose homeostasis and insulin resistance [Tappy and Le, 2010]. These pathological changes further contribute to the development of metabolic syndrome.

1.1.2 – Adverse effects of Sugar

In addition to the global menace of MetS, excess consumption of sugar has also been associated with various health problems. A high sucrose intake induces insulin resistance and contributes to obesity and other deleterious metabolic changes [Cao *et al*, 2007]. Previous studies show an increased incidence of MetS during childhood. Out of about 25% overweight children worldwide, 30% are susceptible to developing MetS [Cook *et al*, 2003; Cruz and Goran 2004]. In addition, high sucrose diet decreases protein intake and retards growth [Ruottinen *et al*, 2008], leads to obesity [Ludwig *et al*, 2001], and diabetes mellitus [Betts *et al*, 2005] in infants and children.

The other health hazards of high sucrose diets are dental caries and dyslipidaemia, poor calcium intake [Johnson and Fray, 2001; Promdee *et al*, 2007], bone loss and fracture [Tjaderhane and Larmas 1998; Johnson and Fray, 2001], arteriosclerosis, cardiovascular diseases (CVDs) [Elwood *et al,* 1970; Johnson and Fray, 2001; Promdee *et al*, 2007], and other metabolic pathologies [Hallfrisch, 1990; Rutledge and Adeli, 2007; Tappy and Le, 2010].

1.2 – Metabolic syndrome

Metabolic syndrome is a condition characterized by specific risk factors that increase susceptibility to diabetes mellitus, renal and cardiovascular diseases [Ford and Giles 2003]. According to the World Health Organization (WHO), metabolic syndrome refers to the co-occurrence of several known cardiovascular risk factors, including insulin resistance, obesity, hypertension and atherogenic dyslipidaemia [Alberti and Zimmet, 1998]. The key features of atherogenic dyslipidaemia are high plasma TG levels, low HDL cholesterol levels and an increase in small dense LDL. Insulin resistance and visceral obesity are associated with atherogenic dyslipidemia [Semenkovich, 2006]. Some authors say that insulin resistance is the central feature of MetS [Balkau and Charles, 1999; Reaven, 2006], while others recognize central obesity as the key component of MetS pathophysiology [Zimmet *et al*, 2005]. Other authors define metabolic syndrome as incorporating endothelial dysfunction [Gimbrone *et al,* 2000; Huang, 2005; Kim *et al*, 2006]. Nonetheless, endothelial dysfunction results from insulin resistance [Huang, 2005]. However, a comprehensive definition of MetS incorporating the key features of hyperglycaemia/insulin resistance, visceral obesity, atherogenic dyslipidaemia and hypertension is in vogue [Huang, 2009]. This comprehensive MetS definition has been simplified by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) [NCEP, 2002; Grundy *et al*, 2005]. According to the NCEP ATP III definition, MetS is present if at least only three of the following five criteria are met. These are central obesity; high blood pressure; high fasting triglycerides; low fasting HDL cholesterol; and hyperglycaemia [National Cholesterol Education Program, 2002]. It does not require that any specific criterion be met, and is one of the most widely

used criteria of metabolic syndrome [Huang, 2009]. The high intake of refined sugars, mainly fructose and sucrose has been implicated in the pathogenesis of MetS in adults and children.

1.2.1 – Pathogenesis of Metabolic syndrome

When excess sucrose is consumed and metabolized into its constituent monosaccharides (fructose and glucose), there is an elevation in the plasma concentrations of FFAs and TGs formed from their glycerol metabolite [van Schaftingen, 1993]. This leads to impaired insulin-stimulated glucose uptake via the glucose transporters, Glut1 and Glut4 [Scheepers *et al*, 2004]. Glucose transporters (Gluts) are substrate-specific membrane proteins distributed in tissues and they cause the facilitated diffusion of sugars into cells [Scheepers *et al*, 2004; Douard and Ferraris, 2008]. Consequently, there is reduced insulin sensitivity [Tappy and Le, 2010], and this decreased insulin sensitivity coupled with impaired cellular glucose uptake by metabolic tissues, mainly muscles at rest can cause hyperglycaemia. The prolonged exposure to glucose intolerance, hyperglycaemia and insulin resistance increases susceptibility to diabetes mellitus [Tappy and Le, 2010], which is associated with MetS. In addition to the indirect fructose culpability in the onset of MetS, fructose also has a direct link with the pathogenesis of MetS. Fructose contributes directly to MetS pathogenesis through the non-enzymatic reaction of glyceraldehyde produced by fructose as described earlier [Levi and Werman, 1998].

1.2.2– Prevalence of Metabolic syndrome

The changes in the routine of modern man encourage a sedentary lifestyle characterised by physical inactivity, reduced physical exercise and a high intake of sugar-rich junk foods. Evidence indicates that this poor lifestyle is culpable in the epidemic of overweight and obesity around the world [Bray *et al*, 2004] including children [Cook *et al*, 2003; Cruz and Goran, 2004; Deen, 2004], due to their high sugar consumption [Birch *et al*, 1989]. According to reports, obesity is an increasing global epidemic with an estimated 1.3 billion people overweight or obese which include 22 million children under the age of five years [Rocchini, 2002; WHO, 2005]. Other studies also show that 25% children are overweight worldwide, and 30% of overweight children are susceptible to MetS [Cook *et al*, 2003; Cruz and Goran, 2004]. In one Asian cross-sectional study comprising 1178 adults (age 20 – 80 years) out of an estimated population of 307,724 in 2001, a very high MetS prevalence rate of 43.2 % (n = 509) was recorded with significantly higher prevalence in females (52.2% (n = 307)) than males (34.2% (n = 202)) [Prasad *et al*, 2010, 2012]. According to various reports, the prevalence ranges from 34.5 – 40% among the US adults, with little gender difference (females: 23.4%; males: 24.0%) [NIH, 2002; Park *et al*, 2003]. In Africa and Europe, the prevalence rate of obesity (central component of MetS) is 30 – 40% [WHO, 2005].

In South Africa, 29% men and 56% women are either obese or overweight [WHO, 2005]. The gender dichotomy in the incidence and prevalence of this MetS risk factor, obesity in South Africa is a reflection of the trend of metabolic syndrome in the Southern Africa sub-region [Walker *et al*, 2001]. There appears to be more

obese women than obese men in Southern Africa. According to the South African Demographic and Health Survey conducted in 2002, the obesity prevalence in African women was double that of Caucasian women [Puoane *et al*, 2002]. Data from other authors also supports the high prevalence rate of obesity in African women [Motala *et al*, 2003; Ntyintyane *et al*, 2006; Shaw *et al*, 2010; Erasmus *et al*, 2012; Kengne *et al*, 2012; Matsha *et al*, 2012]. In addition to obesity, many South African studies have also reported very high blood pressure and hypertension in African people [Steyn *et al*, 1997; Seedat, 1999; Van Rooyen *et al*, 2000; Walker *et al*, 2001; Schutte *et al*, 2003; Opie and Seedat, 2005]. The larger percentage of this population is mostly women [Puoane *et al*, 2002]. Thus, due to the high levels of obesity and hypertension observed in these African women, there is high tendency that they might be subjected to the risk for developing the metabolic syndrome.

The prevalence of MetS increases with age in all the different regions of the world [Ford *et al*, 2002; NIH, 2002; Cook *et al*, 2003; Park *et al*, 2003; Prasad *et al*, 2012]. In their exclusive study of metabolic syndrome in children and adolescents, Cruz and Goran (2004), opine that metabolic syndrome develops during childhood, and has a higher prevalence in overweight children than normal weight children. These authors also pointed out that MetS risk factors, obesity and insulin resistance observed at adult stage were evident in early life, but only manifest later in life when full blown MetS develops. Several other workers also document the progression from existence of one or more MetS risk factors to full blown MetS

with age [Bradshaw *et al*, 1995; Motala *et al*, 2003; Ntyintyane *et al*, 2006; Shaw *et al*, 2010; Erasmus *et al*, 2012; Kengne *et al*, 2012; Matsha *et al*, 2012].

1.2.3 – Symptoms of Metabolic Syndrome

Metabolic syndrome is characterized by overweight, insulin resistance, high blood pressure, high blood sugar, high cholesterol levels and abdominal or central obesity (belly fat) [Ford *et al*, 2002; Laaksonen *et al*, 2002], consequently, with increased risk of heart disease, pre-diabetes, diabetes, hypertension and stroke [Ford *et al*, 2002; Villegas *et al*, 2003; Havel, 2005; Johnson *et al*, 2007; Miller and Adeli, 2008]. The people who eat large quantities of pure natural honey as high as between 70 – 95 g daily, instead of refined carbohydrates do not suffer any of these pathological changes [Shambaugh *et al*, 1990; Gheldof and Engeseth, 2002; Venn and Green, 2007]. Hence, the consumption of pure natural honey seems to be beneficial and appears to be a readily available dietary substitute to refined sugars [Busserolles *et al*, 2002a] in order to curb the menace of metabolic syndrome.

1.3 – Pure natural honey

Pure natural honey (PNH) is a natural, sweet, flavourful liquid food of high nutritional value [White and Doner, 1980], and immense health benefits [Ajibola *et al*, 2007]. Honey is produced by honey-bees (*Apis mellifera* and *Apis cerana*) as blossom honey by secreting nectars of flowers, and as honeydew honey (forest

honey) when they (honey-bees) secrete the exudates of plant sucking insects (Aphids).

1.3.1 – History of PNH

Pure natural honey is a complex substance made when the nectar and sweet deposits from plants and trees are gathered, modified and stored in the honeycomb by honey bees as a food source for the bee colony. Honey production commonly known as beekeeping and scientifically known as Apiculture is the practice and management of bees in a hive in such a way that it will be observable for its developmental stages and manipulation (Ojeleye, 2003). The two common species (*Apis mellifera* and *Apis cerana*) of honey bees are often maintained, fed, and transported in hives by beekeepers moving from field to field as the crop needs pollination, [Caron, 1999; Wilson, 2004] in a bid to gather honey [Wilson, 2004]. The *Apis* species have had their honey gathered by indigenous peoples for consumption and exploited for commercial purposes. Sometimes, honey is also gathered by human beings from the nests of various stingless bees [Santos and Antonini, 2008]. Beekeeping probably began at different times in different parts of the world, and the first evidence of beekeeping appears in the painting of ancient Egypt, dating back to around 2500 BC (NHB, 2004). The authenticity of the time when the first human being became a beekeeper is doubtful, but there is a report that Reverend L. L. Langstroth developed a wooden hive known as Langstroth hive in 1862 (Caron, 1999). Thus, he is designated the Father of Modern Beekeeping. The Langstroth hive is based on the simple principle of surrounding movable frames with a “bee-space”– an area just large enough to discourage bees

from gluing their combs solidly to the walls [Wilson, 2004]. Honey production was enhanced by the vast land space travelled by honey bees. The farther an honey bee travels, the more honey it produces. In 1911, a bee culturist estimated that a quart of honey represented bees flying over an estimated 48,000 miles to gather the pollen needed for the nectar to produce honey [Caron, 1999].

PNH is a liquid spoken of by all religious books, and accepted by all generations, traditions and civilizations, both ancient and modern. The religion of Islam recommended the use of honey as food and medicine, and even named an entire chapter in the Holy Qur'an called Surah al-Nahl meaning chapter of the Honey Bee [Holy Qur'an 16 (An-Nahl)]. In the book of Hadith, Prophet Muhammad strongly advocated the use of honey for curative and healing purposes [Al-Bukhari, 1976]. Furthermore, the Holy Qur'an explicitly promotes honey as a nutritious and healthy food in chapter 16 thus: "And your Lord inspired the bee(s), saying: "Take your habitations in the mountains and in the trees and in what they erect. Then, eat of all fruits, and follow the ways of your Lord made easy (for you)." There comes forth from their bellies, a drink of varying colour wherein is healing for men. Verily, in this is indeed a sign for people who think" [Holy Qur'an 16 (An-Nahl): 68 – 69]. In Christianity there are references made to the importance of bees and honey in the Bible. These include the Books of Exodus [33:3], Mathew [3:4], Judges [14:8] and Proverbs [24:13]. According to the Bible, King Solomon said: "Eat honey my son, because it is good" [Proverbs 24:13]. In fact, it was reported in the Bible that John the Baptist actually thrived on a diet made of locusts and wild honey for a long period of time when he was in the wilderness [Mathew 3:4]. The other sets of

people, sects, traditions and civilizations that attested to the popularity of honey include Budhists, Jews, Hindus and Vedas [Crane, 1975; Jones, 2001].

1.3.2 – Nutritional Profile of PNH

Honey is composed primarily of sugars and water, and also includes small amounts of a wide array of vitamins and minerals, including B vitamins. The other constituents of honey are amino acids, antibiotic-rich inhibine, proteins, phenol antioxidants, and micronutrients [White and Doner, 1980]. The sugars in honey are sweeter and give more energy than artificial sweeteners [White and Doner, 1980; Ajibola *et al*, 2007], and fructose (sweetest natural sugar) is the most abundant sugar in honey (Table 1.3). These substances are of high nutritional and health importance. Some of the vitamins found in honey include niacin, riboflavin, and pantothenic acid, along with minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc. The vitamins, minerals, other micronutrients and trace elements found in honey are listed in Tables 1.4 and 1.5. The high nutritional profile of honey with its wide range of nutrients (although in minute quantities), encourages its use as food. Nonetheless, it is advisable to take large quantities (70 – 95 g daily) of honey to get the full desirable nutritional and health benefits [Al-Waili and Boni, 2003; Al-Waili, 2004; Inoue *et al*, 2005; Yaghoobi *et al*, 2008; Munstedt *et al*, 2009].

It is important to note that some of the chemical elements found in PNH are heavy metals as shown in Tables 1.4 and 1.5. Some of the heavy metals shown in Table 1.5 can become toxic to body cells and tissues if present in honey above permissible levels by pollution standards leading to toxicity [Bogdanov, 2006; Bibi *et al*, 2008]. The details about the contaminants of honey are further discussed under the adverse effects of eating honey in section 1.3.16 of this chapter.

Table 1.3: Nutritional composition of blossom honey and honeydew honey*.

Constituent	Blossom honey		Honeydew honey	
	Range	Mean	Range	Mean
Water	15 – 20	17.2	15 – 20	16.3
Total sugars		79.7		80.5
Monosaccharides				
fructose	30 – 45	38.2	28 – 40	31.8
glucose	24 – 40	31.3	19 – 32	26.1
Disaccharides				
sucrose	0.1 – 4.8	0.7	0.1 – 4.7	0.5
others	2.0 – 8.0	5.0	1.0 – 6.0	4.0
Trisaccharides				
oligosaccharides	3.1			10.1
erlose	0.5 – 6.0	0.8	0.1 – 6.0	0.1
melizitose		< 0.1	0.3 – 22	4.0
others	0.5 – 1.0	0.5	0.1 – 6.0	3.0
Minerals	0.1 – 0.5	0.2	0.6 – 2.0	0.9
Amino acids, proteins	0.2 – 0.4	0.3	0.4 – 0.7	0.6
Acids	0.2 – 0.8	0.5	0.8 – 1.5	1.1
pH value	3.2 – 4.5	3.9	4.5 – 6.5	5.2

*Data in g/100 g, Adapted from [White and Doner, 1980; Bogdanov *et al*, 2008]

Table 1.4: Proximate analyses showing some of the chemical elements found in honey*.

Minerals	**Amount** (mg/100g)	**Vitamins**	**Amount** (mg/100g)
Sodium (Na)	1.6 – 17	Thiamine (B_1)	0.00 – 0.01
Calcium (Ca)	3 – 31	Riboflavin (B_2)	0.01 – 0.02
Potassium (K)	40 – 3500	Niacin (B_3)	0.10 – 0.20
Magnesium (Mg)	0.7 – 13	Pantothenic acid (B_5)	0.02 – 0.11
Phosphorus (P)	2 – 15	Pyridoxine (B_6)	0.01 – 0.32
Selenium (Se)	0.002 – 0.01	Folic acid (B_9)	0.002 – 0.01
Copper (Cu)[a]	0.02 – 0.6	Ascorbic acid (C)	2.2 – 2.5
Iron (Fe)[a]	0.03 – 4	Phyllochinon (K)	0.025
Manganese (Mn)[a]	0.02 – 2		
Chromium (Cr)[a]	0.01 – 0.3		
Zinc (Zn)[a]	0.05 – 2		

*Adapted from [White and Doner, 1980; Bogdanov *et al*, 2008] [a]Heavy metals

Table 1.5: Other chemical elements found in honey*.

Element	**Amount** (mg/100g)	**Element**	**Amount** (mg/100g)
Aluminium (Al)	0.01 – 2.4	Lead (Pb)[a,b]	0.001 – 0.03
Arsenic (As)[a,b]	0.014 – 0.026	Lithium (Li)	0.225 – 1.56
Barium (Ba)	0.01 – 0.08	Molybdenum (Mo)[a]	0 – 0.004
Boron (B)	0.05 – 0.3	Nickel (Ni)[a]	0 – 0.051
Bromine (Br)	0.4 – 1.3	Rubidium (Rb)	0.040 – 3.5
Cadmium (Cd)[a,b]	0 – 0.001	Silicon (Si)	0.05 – 24
Chlorine (Cl)	0.4 – 56	Strontium (Sr)	0.04 – 0.35
Cobalt (Co)[a]	0.1 – 0.35	Sulphur (S)	0.7 – 26
Fluoride (F)	0.4 – 1.34	Vanadium (V)	0 – 0.013
Iodide (I)	10 – 100	Zirconium (Zr)	0.05 – 0.08

* Adapted from [White and Doner, 1980; Bogdanov *et al*, 2008]; [a]Heavy metals; [b]Toxic heavy metals.

1.3.3 – Uses of PNH

Honey is widely embraced by all ages, and its use transcends the barriers of culture and ethnicity. The use of natural honey as food and medicine by mankind has been in existence from the time immemorial. In fact, honey is very likely the world's most ancient sweetener and has been in use throughout the world across the millennia [Crane, 1975]. Honey's popularity and versatility is evident from the variety of uses it has enjoyed throughout history. From ancient times, PNH has not only been used as food sweetener but also as natural beauty agent and has been employed by some cultures for its medicinal attributes [Crane, 1975]. The numerous health benefits of honey made it an important aspect of traditional medicines, and researchers have also documented honey's benefits in modern medicine [Beck and Smedley, 1944; Menshikov and Feidman, 1949; Khotkina, 1955; Salem, 1981; Emarah, 1982; Haffejee and Moosa, 1985; Kandil *et al*, 1987; Ali, 1995a, 1995b; Sela *et al*, 1998; Molan, 1999a; Baltuskevicius *et al*, 2001; Gharzouli *et al*, 2001; Molan, 2001; Gharzouli *et al*, 2002; Al-Waili, 2003; Al-Waili, 2004; English, Pack and Molan, 2004; Mahawar and Jaroli, 2006; Korkmaz and Kolankaya, Yaghoobi *et al*, 2008], especially in wound treatments [Efem, 1988; Atimono *et al*, 1990; Bergman *et al*, 1993; Ajibola, 1995; Al-Waili and Saloom, 1999; Molan, 1999a]. Several different surveys have been compiled on the nutritional and the health aspects of honey [Molan, 1998; Molan, 1999a; Molan, 1999b; Molan, 2001a; Molan, 2001b; Al-Quassemi and Robinson, 2003]. However, the need for more research to discover its seemingly inexhaustible nutritional and medicinal properties becomes imperative.

1.3.4 – Influence of PNH on Growth

Honey is a complete meal with all classes of food, as shown in Tables 1.1, 1.2 and 1.3. It contains major components of a meal, and micronutrients that will enhance the digestion and absorption of dietary essentials [White and Doner, 1980], as well as non-essentials required for the metabolic activities and the proper functioning of the human body [Alvarez-Suarez *et al*, 2010]. Previous studies in Nigeria and New Zealand show enhanced body weight gain by experimental rats fed blossom honey and honeydew honey in several different studies [Molan, 2001b; Ajibola *et al*, 2007; Chepulis and Starkey, 2008]. In 2008, Chepulis and Starkey fed honeydew honey to 8-week old rats for 52 weeks to assess weight gain. These workers show that the growth influence of honey in rodents is due to increased bone growth and mineralization [Chepulis and Starkey, 2008], probably due to calcium content of honey. In his extensive review of the literature, Molan [2001b] confirmed the growth stimulating property of honey. He opined from his histological studies on wounds that stimulation of cell growth by honey gives it its wound healing properties.

1.3.5 – PNH: Source of Antioxidants

Free radicals and reactive oxygen species (ROS) have been implicated in contributing to the processes of cellular dysfunction, pathogenesis of diseases and aging. The consumption of antioxidant rich foods can protect against cellular damage and possibly prevent the development of chronic diseases. Previous researchers indicate that honey constituents include numerous compounds with antioxidant potential [Gheldof and Engeseth, 2002; Al-Waili, 2003; Schramm *et al*,

2003]. The amount and type of these antioxidants depend largely upon the floral source and/or variety of the honey, just as all other phytochemical compounds of honey [Frankel *et al*, 1998; Gheldof and Engeseth, 2002]. Generally, darker honeys have been shown to be higher in antioxidant content than lighter honeys [Frankel *et al*, 1998]. In their analysis of the phytochemical composition of monofloral Cuban honeys, Alvarez-Suarez and co-workers, agreed with this submission and concluded that Cuban honeys contain important phenolic, flavonoid and carotenoid concentrations with high antioxidant capacity [Alvarez-Suarez *et al*, 2010]. Some researchers in California also submitted that human beings can be protected from the damaging effects of free radicals and ROS, in part, by consuming foods rich in antioxidants such as honey [Schramm *et al*, 2003]. These workers reported from their study in which two buckwheat honey treatments were administered to 37 healthy human adults at 1.5 g/kg body weight, with corn syrup as control, showed increased ($p < 0.05$) plasma total-phenolic content and plasma antioxidant. Thus, demonstrating bioavailability of antioxidants in processed honey, and that they increase antioxidant activity of plasma. They advocated substituting honey for processed sugars in some foods as the sweetener for enhanced antioxidant defence system in adults [Schramm *et al*, 2003].

1.3.6 – Influence of PNH on Exercise and Athletic Performance

The consumption of energy giving substances before, during and after any form of physical exercise improves the individual's performance and increases the rejuvenation of muscles [Kreider *et al*, 2002; Earnest *et al*, 2004]. Dietary

supplementation with NH, which provides up to 17 mg of carbohydrates for every tablespoon consumed [White and Doner, 1980], is associated with improved physical performance and gives the much needed energy, thus serving as an inexpensive substitute to commercially available endurance enhancers. The data obtained from the Sports Nutrition and Exercise Laboratory of one University show that honey can be used effectively instead of glucose for energy replenishment during physical exercise [Kreider *et al*, 2002]. The physiological actions of NH observed during this performance included an increase in heart rate and a fairly constant blood glucose level. This suggests honey to be a better source of energy than pure glucose. Earnest and co-workers [2004] improved on this preliminary investigation in another trial by administering low (honey) or high (glucose) glycaemic index (GI) carbohydrate gels to athletes, and testing them on the performance of cyclists travelling a distance of about 65 km. The results of the cycling event show that both the low (honey) and the high (glucose) GI substances caused increases in performance. However, the effect produced by eating NH surpassed that observed in the athletes fed with glucose. This aligns with other previous studies that NH consumption does not compromise metabolic and physical activities [Al-Waili, 2003; Ajibola *et al*, 2007; Chepulis and Starkey, 2008]. NH has been shown to decrease blood glucose level in hyperglycaemia as is seen in diabetic subjects [Al-Waili, 2003, 2004; Yaghoobi *et al*, 2008; Bahrami *et al*, 2009; Cortés *et al*, 2011; Erejuwa *et al*, 2010, 2011a, b; 2012a, b; Erejuwa, 2012], plausibly due to the beneficial effects of fructose [Al-Waili, 2003; Bahrami *et al*, 2009; Erejuwa *et al*, 2010, 2011a, b; 2012a, b; Erejuwa, 2012], and more importantly honey's several phytochemical constituents [White and Doner, 1980; Cortés *et al*, 2011]. A very recent review of the hypoglycaemic effect of honey by

some workers concludes that, the synergistic effect of fructose and oligosaccharides constituents of honey might contribute to the low glycaemic response after a honey meal [Erejuwa *et al*, 2012a]. These experimental and clinical trials show that honey is a well-tolerated liquid food.

Furthermore, honey can be an effective carbohydrate source and a better substitute of glucose for exercise and athletic performance, due to its constituent of various classes of sugars. People favour slow-burning sugars for sustenance as energy source during physical exercise. Honey is beneficial in this regard as it releases fructose slowly into the blood stream to produce a sustained energy boost and maintain homeostasis. The other major component of NH apart from fructose is glucose (Table1.3). Fructose and glucose are ketose and aldose sugars respectively with chemical structural differences, and consequently different patterns of metabolism, despite both being monosaccharides with quick burning tendency. It is important to note that glucose is rapidly metabolized for absorption into the blood system for energy provision. On the other hand, fructose absorption is slow, and will continue to sustain the individual with energy, while the glucose moiety burns out. The various phytochemical constituents of honey [White and Doner, 1980] also contribute to the progressive slow rate of fructose metabolism [Herman and Zakim, 1968]. In addition, honey contains disaccharides such as sucrose and oligosaccharides as well as other trisaccharides (Table 1.3) that are slow burning sugars. These could facilitate energy replenishment, muscle recuperation and enhancement of performance in athletes nourished with honey, while those relying on glucose for an energy boost might have been exhausted.

1.3.7 – PNH's role in Digestion and Absorption

The enzymes present in honey aid in digestion of food especially raw sugars and starch. The beneficial aspect of eating natural honey as a source of energy [Kreider *et al*, 2002; Earnest *et al*, 2004], over the commonly used refined sugars is that, the sugars contained in honey are in a very simple pre-digested form [White and Doner, 1980], and thus are directly absorbed by our body. Whereas, the refined sugar (sucrose) has to be broken down in our body into simpler forms to be absorbed. As a sweetener, honey has nutritional advantages over sugar, providing some micro nutrients [White & Doner, 1980], which act to aid digestive processes in the body. The gastrointestinal tract (GIT) is full of bacteria. These bacteria are essential for life and good health. One group of bacteria that has been shown to be particularly important to the health and proper functioning of the GIT is called bifidobacteria. One way to increase the bifidobacteria populations in the gut is by consuming foods containing prebiotics, substances that increase the growth and activity of good bacteria. The important effects of honey on the human digestion have been linked to oligosaccharides [Busserolles *et al*, 2002a]. These honey constituents have prebiotic effects, similar to that of fructooligosaccharides [Yun, 1996; Sanz *et al*, 2005], and the oligosaccharide, panose was the most active oligosaccharide in natural honey. The oligosaccharides cause an increase of bifidobacteria and lactobacilli and exert the prebiotic effect in a synergistic mode of action [Ustunol and Gandhi, 2001]. According to the results from an *in vitro* study on five bifidobacteria strains, honey had a growth promoting effect similar to that of fructose and glucose oligosaccharides [Kajiwara *et al*, 2002]. The monofloral honeys of alfalfa, sage and sour-wood stimulated the growth of five human intestinal bifidobacteria [Shin and Ustunol, 2005]. In another study honey

increased both *in vivo* (within the small and large intestines of rats) and *in vitro* the growth of *Lactobacillus acidophilus* and *Lactobacillus plantarum,* while sucrose had no effect [Shamala *et al*, 2000]. In certain cases, consumption of relatively large amounts of honey (70 to about 95 g) can lead to a mild laxative effect in individuals with insufficient capacity for absorption of honey fructose [Ladas *et al*, 1995; Ladas and Raptis, 1999]. Honey has a laxative effect on the digestive system. Another nutraceutical function of honey is provision of calcium. Honey consumption provides calcium, which is readily absorbable and strengthens bone. This can help reduce the risk of osteoporosis or low bone weight (both risk factors of bone fragility) in young and old individuals. Research in animal models showed that calcium absorption increased correspondingly with increased honey intake [Ariefdjohan *et al*, 2008].

1.3.8 – Influence of PNH on Children's nutrition

There is anecdotal evidence encouraging the feeding of honey to new born babies in some customs and traditions [Menshikov and Feidman, 1949; Khotkina, 1955; Slobodianiuk and Slobodianiuk, 1969; Al-Bukhari, 1976; Salem, 1981; MacMillan, 1999]. It has been shown in some studies that feeding honey to infants can improve memory and growth, reduce anxiety and enhance children's performance in later life [Bianchi, 1977; Ramenghi *et al*, 2001]. In 2009, Chepulis and co-workers partly gave scientific credence to this beneficial practice in their New Zealand behavioural study in animals. They fed eight weeks old experimental rats diets supplemented with either honeydew honey or sucrose, and their control group was given a sugar-free diet (all diets patterned after typical New Zealand

human diet). These workers noted improved spatial memory and reduced anxiety in the honey-fed rodents compared to the other groups over the 12 months trial period. The workers concluded that early introduction of honey in the diet was beneficial and reduced memory loss and cognitive decline associated with ageing [Chepulis *et al*, 2009].

The application of honey in human infant nutrition also revealed some interesting and beneficial observations. The palatability of honey for infants was investigated by Ramenghi and other workers in 2001, and these workers reported that honey was well tolerated and significantly reduced the crying phases of babies than sterile water [Ramenghi *et al*, 2001]. In a review on the importance of honey relative to sucrose in children's nutrition, honey fed infants were found to have improved haematological profiles and calcium uptake, no digestion problems, lighter and thinner faeces, better skin colour, less susceptibility to diseases, and steady weight gain [Bianchi, 1977]. These positive effects of honey when incorporated in infants' diet were attributed to its effects on the digestion process, and the effect of prebiotic honey constituents, (oligosaccharides) on intestinal flora of these children [Rivero-Urgell and Santamaria-Orleans, 2001]. These and other experimental studies involving PNH feeding to neonates suggest that feeding honey to children could be beneficial instead of sweets and other sugary substances that they are often inclined to eat.

1.3.9 – Effects of PNH in Haematology and Immunity

Honey has been found to be beneficial to people suffering from anaemia. In collaboration with other workers, I investigated honey's influence on health in 2007, and reported enhanced blood profiles in the blossom honey-fed adult rats [Ajibola *et al*, 2007]. The study recorded improved haemoglobin concentration (iron constituent of NH played an important role in this), increased erythrocyte count and elevated haematocrit in the honey eaters. In another laboratory in the same year [2007], Chepulis also documented enhanced haematology and immune response in rats fed a 10% honeydew honey supplemented diet. The author noted higher lymphocyte count and increased neutrophil phagocytosis in NH-fed rats than control. This aligned with previous studies that prebiotics can enhance immune function [Yamada *et al*, 1999; Schley and Field, 2002]. In one Californian study, some workers also give credence to the immune property of honey by proposing that honey has an impact on plasma oxidant defense systems in human subjects [Schramm *et al*, 2003]. In addition, the researchers observed that the aqueous portion of the blood (plasma) is protected by honey that is honey consumption is haematoprotective (boosts immunity by assisting the immune components of the plasma through antioxidants)**.** This is in agreement with the fact that most of the antioxidant components in processed honey are water soluble. In summarizing the facts that honey can be considered to be a satisfactory immuno-nutrient, some workers opine that honey, taken orally, stimulates and increases antibody production during primary and secondary immune responses against thymus-dependent and thymus independent antigens [Al-Waili and Haq, 2004].

1.3.10 – PNH promotes Oral health

The use of natural honey can promote oral health and wellness. In 2001, Molan concluded that honey with high level of antibacterial activity has the potential to reduce the risk of dental caries [Molan, 2001b]. In addition to the carioprotective effect of New Zealand Manuka honey (a very potent antimicrobial honey), Molan and co-workers have shown from their extensive research on the influence of honey on oral health that honey prevents dental plaque, gingivitis, periodontitis [English *et al*, 2004]. Other workers in different laboratories have also shown that honey is non-cariogenic or less cariogenic than the refined sugar, sucrose [Edgar and Jenkins, 1974; Decaix, 1976; Steinberg *et al*, 1996; Sela *et al*, 1998]. The carioprotective effect of honey has been adduced to its antibacterial activity, which inhibits the growth of caries-causing bacteria [Steinberg *et al*, 1996; Molan, 2001b]. In one electron microscopy study, honey consumption was found to be safer and less inimical to oral health than drinking fruit juice [Grobler *et al*, 1994]. There was erosion of tooth enamel ten minutes after drinking fruit juice, while honey ingestion delayed this observation till 30 minutes after honey meal and the tooth erosion was even very weak. The plausible explanation for the less cariogenic effect of honey is the protective role of honey constituents which include calcium, fluoride, phosphorous and other colloidal honey components. In summary, it can be concluded that honey has constituents with carioprotective effect.

1.3.11 – Effects of PNH in GIT disorders

Anecdotal evidence advocates the use of natural honey in the treatment of ailments of the GIT in the past [Menshikov and Feidman, 1949; Khotkina, 1955; Slobodianiuk and Slobodianiuk, 1969; Al-Bukhari, 1976; Salem, 1981]. These ancient practices are presently being supported by the global application of honey for the prevention and treatment of gastrointestinal disorders such as gastritis, gastroenteritis and peptic ulcers [Kandil *et al*, 1987; Ali, 1995a, 1995b, 1997; Al-Swayeh and Ali, 1998; Gharzouli *et al*, 2001, 2002; Nasuti *et al*, 2006]. Honey has been shown to be a gastroprotective agent and potent inhibitor of *Helicobacter pylori,* the causative agent of gastritis and peptic ulcers [Ali *et al*, 1991; Al-Somal *et al*, 1994; Osato *et al*, 1999]. In rats honey ameliorated gastric ulcers experimentally induced by alcohol, ammonia, aspirin and indomethacin [Ali, 1995a, 1995b; Gharzouli *et al*, 2001, 2002]. Two mechanisms for this action have been proposed. The first suggests that this effect is due to the antioxidant properties of honey. Honey was found to maintain or enhance the level of non-protein sulfhydryl substances (such as glutathione) in gastric tissue subjected to factors inducing ulceration [Ali, 1995a, 1995b, 1997; Al-Swayeh and Ali, 1998]. A similar observation was made when Anzer honey pre-treatment was used to prevent N-ethylmaleimide (NEM) -induced liver damage in rats [Korkmaz and Kolankaya, 2009]. The findings imply that depletion of glutathione concentration plays a causal role in NEM-induced liver injury, and that the hepatoprotective effect of Anzer honey may be mediated through sulfhydryl- sensitive processes.

A second mechanism of action being proposed shows that honey is not involved in prostaglandin production, but has a stimulatory effect on the sensory nerves in the stomach that respond to capsaicin [Ali, 1995a; Al-Swayeh and Ali, 1998]. Honey intake prevented indomethacin induced gastric lesions in rats by reducing the ulcer index, microvascular permeability, and myeloperoxidase activity of the stomach [Nasuti *et al*, 2006]. The ingestion of dandelion honey reduced gastric juice acidity by more than 50% [Baltuskevicius *et al*, 2001]. The gastric emptying of saccharides after ingestion of honey was slower than that after ingestion of a mixture of glucose and fructose [Pokorn and Vukmirovic, 1978], and thus, mitigating diarrhoea. The clinical uses of honey in infants and children revealed a shorter duration of bacterial diarrhoea and did not prolong the duration of viral diarrhoea unlike that associated with the use of conventional antibacterial therapy [Haffejee and Moosa, 1985]. In honey, there is little water available to promote the growth of bacteria and yeast. In addition, honey's natural acidity (pH 3.2 to 4.5, Table 1.3) inhibits some pathogens, as it also contains a small amount of hydrogen peroxide. Other substances contributing to honey’s antibacterial potency are inhibine and lysozyme as well as its sugars through their osmotic effect [Bogdanov, 1997; Molan, 2001a].

1.3.12 – Use of PNH in Ophthalmic disorders

The use of honey in the treatment of eye diseases is well documented. The ancient people used honey from Attica [Beck and Smedley, 1944], and lotus honey from India [Fotidar and Fotidar, 1945] as curative substances for eye disorders. The Indian locals still use honey as eye drops to cure eye disease [Ranjit -Singh

and Padmalatha, 2004; Solovan *et al*, 2004; Mahawar and Jaroli, 2006]. According to a report from Imperato and Traore, 1969 documented by Molan, 1999b, honey is also a traditional therapy in Mali for measles, as was put in the eyes to prevent scarring of the cornea which occurs as a complication of this infection [Imperato and Traore, 1969; Molan, 1999b]. There was an astounding success reported from honey application in clinical trials of 102 patients with different ophthalmological disorders such as blepharitis, conjunctivitis and keratitis, not responding to conventional treatment [Emarah, 1982]. After the honey application under the lower eyelid like an eye ointment, improvement was seen in 85% of the cases, with no deterioration seen in any of the other 15%. However, a transient stinging sensation and redness of the eye was observed soon after putting honey in the eye, but it was not debilitating so as to halt the treatment in the 102 cases in the trial. The use of honey in ophthalmic conditions in Asia and Eastern Europe has also been reported by Molan in a review on the use of honey in ophthalmology [Molan, 1999b]. Ophthalmic surgeons at Rangaraya Medical College, India, treated corneal ulcers caused by bacteria with honey [Meier and Freitag, 1955]. In addition, honey has also been used for the treatment of eyes discharging pus [Meier and Freitag, 1955]. In one review conducted in 1999, Molan also cited Russian authors, Mozherenkov and Prokof'eva that observed antibacterial, antifungal and anti-inflammatory actions with application of the honey under the lower eyelid [Mozherenkov, 1984]. It has been used for the treatment of chemical and thermal burns to the eye, conjunctivitis, and infections of the cornea, when applied undiluted or as a 20-50% solution in water [Molan, 1999b].

1.3.13 – Metabolic and Cardiovascular effects of PNH

The health profiles obtained from several experimental studies and clinical trials extensively showed that natural honey has high metabolic and cardiovascular health significance [Busserolles *et al*, 2002a; Gheldof and Engeseth, 2002; Al-Waili, 2004; Chepulis and Starkey, 2008; Yaghoobi *et al*, 2008]. It has been shown that honey intake ameliorates risk factors for metabolic and cardiovascular diseases in patients and healthy individuals at risk. Diabetic patients can within limits safely eat this natural and sweet sugar-containing liquid food, honey instead of refined sugars. According to Costa-Neto [1999] reported by Santos and Antonini, the Pankararé tribe of Brazil even recommended the use of PNH for the treatment of diabetes, bronchitis, mycosis, and throat aches amidst other ailments [Costa-Neto, 1999; Santos and Antonini, 2008].

Earlier researchers from other laboratories and clinical trials further affirmed the metabolic and cardiovascular health significance of eating honey by recording some health profiles. These were reduction in the plasma levels of risk factors which include total cholesterol [Al-Waili, 2004; Yaghoobi *et al*, 2008]; LDL [Al-Waili, 2004; Yaghoobi *et al*, 2008; Gheldof and Engeseth, 2002]; TGs [Al-Waili, 2004; Busserolles *et al*, 2002a]; glucose in normal and diabetic patients [Al-Waili, 2004; Yaghoobi *et al*, 2008]; C-reactive protein [Al-Waili, 2004; Yaghoobi *et al*, 2008]; while the health indices elevated in the blood were HDL [Al-Waili, 2004; Yaghoobi *et al*, 2008; Chepulis and Starkey, 2008; Busserolles *et al*, 2002a]. In addition, other workers recorded higher plasma α-tocopherol level and α-tocopherol/triacylglycerol ratio, lower plasma nitrite and nitrate levels in honey-fed

relative to fructose-fed rats, and consequently low susceptibility of the heart to lipid peroxidation in these subjects [Busserolles *et al*, 2002a].

1.3.14 – Uses of PNH in Chemotherapy and Wound management

Honey has antiseptic properties. It is good for treating burns, infected surgical wounds and ulcers. Glenys Round, a cancer specialist and Julie Betts in Waikato Hospital, New Zealand reported excellent results of the therapy from patients with fungating wounds, recalcitrant leg ulcers and pressure sores using the Unique Manuka Factor (UMF)-containing honey known as manuka honey [Knox, 2004]. The UMF is a mystery ingredient of this Australian jellybush honey identified by Prof Peter Molan as reported in the BBC news by Angie Knox on Tuesday 8 June, 2004, and also documented in a book entitled: "*Allah's Miracles in the Qur'an"* [Knox, 2004; Yahya, 2005]. According to the cancer specialist, the application of honey dressings was used in these patients (including those with cancer broken through the skin) after failure of healing from conventional treatments which include radiation therapy. In a previous study, I successfully treated experimental surgical wounds in Nigerian Dwarf goats with blossom honey. The study observed epithelialization and significantly greater contraction of the honey treated wounds relative to untreated wounds [Ajibola, 1995]. Several other workers had also used honey successfully for the same purpose in human surgical wards and on experimental animals [Bergman *et al*, 1983; Wadi *et al*, 1987; Efem, 1988; Hejase *et al*, 1996; Oryan and Zaker, 1998; Al-Waili and Saloom, 1999].

1.3.15 – Antimicrobial activity of PNH

Pure natural honey is a very potent broad spectrum antibiotic which most multi-resistant bacteria are found sensitive to (Table 1.4). Alvarez-Suarez and co-workers confirm this in their report of the analysis of different types of honey and opine that antimicrobial activity is present in all varieties of honey [Alvarez-Suarez *et al*, 2010]. The workers suggest that hydrogen peroxide formation may play an important role as natural antibacterial product for minimizing the invasive effects of bacteria in the native monofloral Cuban honeys analyzed. Although, the Cuban honeys varied in their chemical constituents' values, all still exhibit antimicrobial activity against both Gram-positive and Gram-negative bacteria which include *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli* [Alvarez-Suarez *et al*, 2010]. In their various studies with about 200 New Zealand (manuka and non-manuka) honeys, Molan and co-workers also agreed that differences exist in the antibacterial and antifungal activities, although all exhibit potency [Molan, 1992; Cooper *et al*, 2002; Brady *et al*, 2004; Lin *et al*, 2008]. The bacteria and other infectious micro-organisms responsive to NH treatment are listed in Table 1.6.

Table 1.6: List of Bacteria and other Organisms found to be sensitive to honey*.

Actinomyces pyogenes	*Proteus species*
Bacillus anthracis	*Pseudomonas aeruginosa*
Campylobacter coli	*Rubella virus*
Campylobacter jejuni	*Salmonella cholerae-suis*
Candida albicans	*Salmonella typhi*
Corynebacterium diphtheria	*Salmonella typhimurium*
Echinococcus parasite	*Serrata marcescens*
Enterococcus avium	*Shigella species*
Enterococcus faecalis	*Staphylococcus aureus*
Enterococcus faecium	*Streptococcus agalactiae*
Enterococcus raffinosus	*Streptococcus dysgalactiae*
Epidermophyton floccosum	*Streptococcus faecalis uberis*
Escherichia coli	*Streptococcus mutans*
Haemophilus influenza	*Streptococcus pneumonia*
Helicobacter pylori	*Streptococcus pyogenes*
Klebsiella pneumonia	*Streptococcus uberis*

Table 1.6 Continued

Leishmania parasite	*Trichophyton mentagrophytes*
Microsporum canis	*Trichophyton mentagrophytes* var.
Microsporum gypseum	*Trichophyton tonsurans*
Mycobacterium tuberculosis	*Trichophyton rubrum*
Nocardia asteroids	*Vibrio choleriae*

*Adapted from [Molan, 2001b; Cooper *et al*, 2002; Lin *et al*, 2008].

1.3.16 – Adverse effects of eating honey

PNH like any other natural foods can also be exposed to contamination by antibiotics, pesticides, heavy metals and other toxic compounds [Bogdanov, 2006]. These poisonous substances can result from disease control, accidental exposure, environmental hazards and inimical human practices [Bibi *et al*, 2008; Schneider, 2011]. It was reported that European health authorities found lead (Pb) in honey bought from India in early 2010 [Schneider, 2011]. The presence of contaminants in Indian honeys was confirmed a year later in a test by the Indian Export Inspection Council. The findings showed the presence of lead and at least two antibiotics in almost 23 percent of the 362 test samples of honey meant for export [Schneider, 2011]. The antibiotics could have been the residual effect of treatment and control of infection in honey-bees. A link to this hypothesis is the almost simultaneous, coincidental control of the bacterial epidemic of foulbrood disease in bee hives by Chinese beekeepers, with the use of several antibiotics manufactured in India for animal use, including chloramphenicol [Schneider, 2011]. Medical researchers had shown that treatment of children with chloramphenicol as an antibiotic can cause susceptibility to DNA damage and carcinogenicity. The amount of chloramphenicol found in NH although minute, according to public health experts, could cause a severe, fatal reaction such as aplastic anaemia in about one out of 30,000 people [Schneider, 2011]. This led to the ban placed on honeys originating from China by the United States of America agency, Food and Drug Administration (FDA).

The high concentration of heavy metals in honey can be a source of illness to human beings. Heavy metals are chemical elements with a specific gravity that is at least five times the specific gravity of water. The heavy metals that are of concern in NH production and apicultural practices are listed in Tables 1.4 and 1.5. In small quantities, some heavy metals are nutritionally essential for a healthy life. These are referred to as the trace elements, and are listed under minerals in Table 1.4. The non-essential elements associated with NH are often absent or present in very minute and insignificant levels. These heavy metals are of no biological and chemical significance. They are listed in Table 1.5. However, there are certain heavy metals present in honey above permissible levels by pollution standards leading to toxicity [Bibi *et al*, 2008]. The toxicity is due to accumulation of these heavy metals within the human's or animal's soft tissues which cannot degrade or destroy and eliminate them. It has been reported that Pb can cause brain, kidney, nervous system and red blood cell damage. The other health problems caused by heavy metals toxicity include headache, metabolic abnormalities, respiratory disorders, nausea and vomiting [Garcia-Fernadez *et al*, 1996].

The use of agrochemicals in growing of flowers causes contamination of nectar with heavy metals [White and Doner, 1980; Bogdanov, 2006], such as arsenic (As), cadmium (Cd) and Pb. These three heavy metals (As, Cd, Pb) have been identified in the priority list of top 20 hazardous substances compiled by an agency of the United States of America Department of Health and Human Services, known as The Agency for Toxic Substances and Disease Registry (ATSDR) in 2001 [Bogdanov, 2006]. According to ATSDR, arsenic is the most hazardous and toxic substance, being the first on the Agency's priority list, closely followed by Pb

as second, while Cd ranked seventh on the list. Arsenic poisoning could result from environmental contamination through the use of pesticides and paints manufacturing [Bogdanov, 2006]. The limited sources of As pollution might be the reason for lack of As contaminated honey cases being reported, despite the severity of its hazard. Cd contaminated honey could be as a result of its (Cd) use in Pb and zinc (Zn) mining; use of pesticides; and improper handling and disposal of old, used Cd batteries. Nonetheless, Cd concentration in NH is 0 to 0.001 mg/100g honey (Table 1.5), probably due to its similar limited sources of environmental pollution like arsenic, and legislation on protection against occupational health [Bogdanov, 2006]. These showed that this natural food (NH) can be said to be safe from two of the three most hazardous contaminants. However, it is known that heavy metal poisoning results from long term low level exposure to contaminants [Bogdanov, 2006]. Hence, no effort should be spared at ensuring the availability of wholesome NH devoid of toxic heavy metals especially Pb, which is the most commonly encountered highly hazardous substance of the three identified by ATSDR as being associated with NH.

The other factors causing metal contamination of NH are the methods of honey harvesting, processing and storage. Most small scale local beekeepers use low cost metallic containers due to low purchasing power [Schneider, 2011]. Thus, the acidic nature (pH 3.2 – 4.5) of NH (Table 1.3) corrodes the metal containers [Anklam, 1998; Schneider, 2011]. Lead is the widely reported of all heavy metals causing honey contamination [Bogdanov, 2006; Bibi *et al*, 2008; Schneider, 2011], due to the many health problems it causes. The possible reason for its presence in different types of honey from several geographical locations could be as a result of

the high concentration of Pb in the air due to oil extraction and automobile exhaust emissions [Bibi *et al*, 2008]. Another environmental factor causing high lead presence in honey could be due to poor waste disposal of paints, printing materials such as ink, as well as used dry batteries in some places [Bogdanov, 2006]. The other heavy metals of public health importance found in NH apart from As, Cd and Pb are chromium (Cr) and zinc (Zn) (Table 1.3). The contact of honey with stainless steel surfaces during honey production can also generate a high Cr content, due to the corrosive effect of honey acidity. It has also been documented that NH storage in galvanized containers can be a source of Zn contamination [González-Paramas *et al*, 2000; Bogdanov, 2006]. Therefore it is important to take into account the type and quality of equipment used to produce and store honey after harvesting as the possible sources of honey contamination with heavy metals. In addition, the increasing overwhelming demand of this natural product necessitates the promotion of all feasible activities towards ensuring quality [Schneider, 2011]. This would increase the production of residue-free and wholesome honey for domestic and international consumption [McKee, 2003]. The safety of consumers and the high global demand for quality honey make international legislation on the food imperative.

There is also a public health concern for infants regarding the presence of *Clostridium* (*Cl.*) *botulinum* in honey. Since this bacterium is ubiquitous and may be present in natural foods, and honey mostly being a non-sterilized packaged food, the risk of contamination cannot be ruled out. In order to avoid the exposure of infants with immature GIT to the risk of contamination with *Cl. botulinum* and its toxin-producing spores, it is advisable not to feed infants that are below one year

old with raw honey. However, in order to avoid the risk of introducing Clostridial infection through this natural product, honey can be sterilized by gamma irradiation without any loss of NH's properties [English *et al*, 2004]; thus, preventing contamination without reducing its nutraceutical value and potency.

1.3.17 – By-products of PNH

In addition to PNH, apicultural practices also produce natural by-products alongside honey which include wax, propolis, pollens, cerume (a mixture of wax and propolis) and jelly [Crane, 1990; Wilson, 2004]. Propolis or bee glue is created from resins, balsams and tree saps. Some species of honey bees called dwarf honey-bees nest in tree cavities, and use propolis to seal cracks in the hive. These dwarf bees use propolis to defend against ants by coating the branch from which their nest is suspended to create a sticky moat [NHB, 2004; Wilson, 2004]. Propolis is consumed by humans as a health supplement in various ways and also used in some cosmetics [Steinberg *et al*, 1996; Caron, 1999; NHB, 2004]. Honey-bees also collect pollen in the pollen basket and carry it back to the hive, where it is used as a protein source necessary during brood-rearing [Wilson, 2004]. In certain environments, excess pollen can be collected from the hives of *A. mellifera* and *A. cerana,* and it is often eaten as a health supplement [Steinberg *et al*, 1996; Caron, 1999; NHB, 2004; Santos and Antonini, 2008].

1.4 – Justification

The search for a healthy substitute to refined sugars becomes imperative to curb the menace of metabolic syndrome. PNH appears to be a good source of natural sugars as substitute to the refined sugars. NH was reported by some workers to be protective against the hypertriglyceridemic and pro-oxidative effects of fructose [Busserolles *et al*, 2002a]. Although honey is mainly consumed by adults, some traditional rites include the feeding of honey to infants at birth [Al-Bukhari, 1976; Haffejee and Moosa, 1985; MacMillan, 1999; Jones, 2001; Santos and Antonini, 2008]. However, there is a dearth of scientific information on the effect of feeding honey to neonates [Bianchi, 1977; Ramenghi *et al*, 2001]. Despite the recommendation of six months exclusive breastfeeding of human babies, some mothers especially the working class still nourish their infants with infant formulae [WHO, 2001; WHO, 2011]. The immediate and remote beneficial effects of exclusive breastfeeding have been extensively discussed by some authors in their recent studies [Kramer and Kakuma, 2012; Horta and Victora, 2013; Jesri *et al*, 2013]. These benefits have been previously highlighted in various studies across the globe [Kallio *et al*, 1992; WHO, 2000; Kramer *et al*, 2001; Bhandari *et al*, 2003; Arenz *et al*, 2004; Khadivzadeh and Parsal, 2004; Onayade *et al*, 2004; Weyermann *et al*, 2006; Quigley *et al*, 2007; Baker *et al*, 2008; Kramer *et al*, 2009; Fewtrell *et al*, 2011]. The advantages of prolonged breastfeeding of four to six months devoid of complementary feeding have been shown to outweigh any consideration that might necessitate the use of refined formulations as complementary infant nourishment [WHO, 2000; Kramer *et al*, 2002; Bhandari *et al*, 2003; Kramer *et al*, 2003; Arenz *et al*, 2004; Chantry *et al*, 2006, 2007; Baker *et al*, 2008; Duijts *et al*, 2010; Fewtrell *et al*, 2011; Kramer *et al*, 2011; WHO, 2011].

The inclusion of sugars in these infant dietary formulations has been documented [Rivero-Urgell and Santamaria-Orleans, 2001]. The need to evaluate the safety of honey consumption vis-à-vis refined sugars in children becomes imperative.

The gastrointestinal tract (GIT) is the first contact point for carbohydrate foods and other orally administered substances. When food materials are ingested, the structural integrity of the GIT can be affected, and consequently influence the GIT's functional aspects. The structure of the GIT can also change in response to different dietary constituents, which can enhance functional or physiological changes in intestinal and visceral organs such as cellular growth and functions of intestinal transporters [Pacha, 2000], with consequent alterations in size [Pacha *et al*, 2003]. The GIT is a potent source of regulatory peptides [Strader and Woods, 2005], such as cholecystokinin, secretin, gastrin, ghrelin and motilin [Konturek *et al*, 2003]. The presence of food in the duodenum stimulates production of the intestinal hormones, such as cholecystokinin and secretin which stimulate exocrine pancreatic activity, leading to the secretion of an array of hydrolytic enzymes. Dietary manipulations during the peri-natal suckling period have long lasting and apparently irreversible effects on some transport mechanisms in the GIT [Pacha, 2000]. These established facts informed the decision to feed suckling Sprague Dawley (SD) rats as animal models of neonatal babies with natural honey and cane syrup to compare their metabolic health effects on long and short terms. In addition, most previous studies use fructose in form of HFCS, hence the present study was to establish the effects of cane syrup on the development of metabolic dysfunction when included in the diet from an early age, and since cane syrup is more commonly consumed in this part (developing countries) of the world.

1.5 – Statements of objectives

The inclusion of PNH in the diets of adult and infant human beings is beneficial and devoid of health hazards [Bianchi, 1977; Ramenghi *et al*, 2001; Al-Waili and Boni, 2003; Schramm *et al*, 2003; Al-Waili, 2004; Al-Waili and Haq, 2004; Al-Waili *et al*, 2006]. The study was to establish through the use of rat models that the consumption of natural honey neither provokes metabolic syndrome nor potentiate any metabolic risk factors in adults and children. In addition, the investigation of the effects of PNH intake on the animal models would contribute to the prevention and management of MetS and other metabolic diseases.

1.5.1 – Broad objective

The study aimed to use Sprague-Dawley rats as animal models to assess the effects of early introduction of pure natural honey as a dietary substitute for refined sugars on pre-disposition to development of metabolic syndrome starting at the neonatal stage, and then observe the acute and the chronic effects of the interventions.

1.5.2 – Specific objectives

The specific objectives of the study were to:

a. Measure the growth of rats through the body weight gain (BWG), linear growth determined from tibia and femur length, and key hormones regulating metabolism and associated with obesity (leptin and insulin);

b. Assay the concentrations of metabolic substrate (glucose, TGs, FFAs) in circulation; and in storage (hepatic glycogen, liver lipid content);

c. Determine the morphology and morphometry of the gastrointestinal tract and metabolic tissues by gross and microscopic assessment of the intestines, liver, heart, kidney and adipose tissues;

d. Observe the gender differences in the above mentioned parameters in response to acute and long term dietary supplementation with either natural honey or cane syrup to rats.

1.6 – Hypotheses

i. Dietary supplementation with natural honey would promote body weight gain and linear growth in SD rats.

ii. Dietary supplementation with natural honey will not increase the concentrations of metabolic substrates in circulation and storage.

iii. Dietary supplementation with natural honey has no adverse influence on the morphology and morphometry of the gastrointestinal tract and metabolic tissues.

iv. The feeding of natural honey as a dietary supplement to rats does not have any gender associated effects on predisposition to the development of metabolic syndrome.

CHAPTER TWO

STUDY DESIGN AND

EFFECTS OF LONG TERM FEEDING

CHAPTER TWO

STUDY DESIGN AND EFFECTS OF LONG TERM FEEDING

2.0 – Introduction

The study was approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, Johannesburg, South Africa. The Ethical clearance certificate number was AESC 2010/29/2B. The experimental procedures were performed according to the humane handling rules contained in the "Guidelines for the use and care of animals in Experimental, Education and other Scientific Procedures" of the University of the Witwatersrand, Johannesburg, South Africa.

The study was conducted at the Animal Unit of Central Animal Services (CAS), and the School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. The study was done in two phases – long and short terms. In the long term study (Phase 1), 59 suckling Sprague-Dawley (SD) rats were fed with either NH- or GS-supplemented diets from age 7 (neonate) to 91 (adulthood) days. For the short term study (Phase 2) to investigate the effects of NH in neonates, 69 SD pups were gavaged with NH or GS twice daily at 12-hour intervals and allowed to nurse freely in between from age 7 to 20 days.

2.0.1 – Phase One: Effects of honey and cane syrup on adult rats

The first phase involving 12 weeks of dietary treatments investigated the long term effects of NH and GS on metabolism in the rats. This long term study used six nursing dams with their litters of between 8 to 12 (average 10) pups each, summing up to a total of 59 pups.

2.1 – Materials and Methods

The fifty-nine pups consisting of 30 males and 29 females with their respective dams were provided by the Animal Unit of the CAS in batches based on availability of animals during the research period. The 59 suckling (7-day old male and female Sprague-Dawley) rats were randomly divided into five groups, based on dietary treatments using the completely randomized design. The dietary treatments were replicated on the basis of sex. Each dam had pups belonging to all the groups for fair representation. The pups had colour codes marked on their tails with a non-toxic, non-invasive, superficial permanent ink marker for identification purposes.

2.1.1 – Animals

The Sprague-Dawley (SD) male and female rats were weighed at the commencement of the study (age 7 days old). The weight of the rats was obtained by putting each rat in a plastic container of known weight, then placing the plastic container on a Precisa 310M digital balance (Precisa®, Vadodara, Switzerland). The mean body weight (BW) was 17.9 g (SEM 0.28), (range 14 – 22 g). The mean

BW of male pups was 18.1 (SEM 0.78), (range 14 – 22 g); and not significantly different from that of females (17.7 (SEM 0.63), (range 15 – 21 g). The pups were allowed to adapt to the housing conditions and experimental interventions (handling, weighing and gavaging) for three days before the commencement of the experimental protocol. The familiarization to experimental procedures included oral treatment with 0.1 ml of 50 % solution of their requisite dietary supplementation given to the pups for the first three days (day 7 – day 9). The oral gavaging was done using a plastic orogastric gavaging needle and 1ml hypodermic syringe. The pups in the control group were gavaged with distilled water in a similar manner, such that they were also exposed to same the handling as the treatment groups.

2.1.2 – Housing

During the pre-weaning period, the littermate pups with their respective dams were housed as a family in standard rat cages of 425 x 270 x 140 mm in dimension provided by the CAS, University of the Witwatersrand, South Africa. The rat cages were made of polycarbon plastic with a solid bottom, and had a top covering of stainless steel grid with provisions to hold rat chow and water drinking bottles on these lids. The cages were lined with bedding of clean, hard wood shavings mixed with shredded pieces of paper for environmental enrichment. The bedding provided for the rats was changed twice a week. The pups were allowed to remain with their dams and allowed to suckle *ad libitum* in the same cages until weaned at 21 days as in normal standard operating protocol in the animal unit. On weaning, the dams were returned to the stock population of the animal unit. The pups were then housed in pairs in the same type of cages as described earlier in this section

(425 x 270 x 140 mm cages supplied by the CAS). The environmental temperature was set at 22 ± 2 ^{o}C and the lighting on a 12 hour light-dark cycle, lights on at 0700 hours and switched off at 1900 hours.

2.1.3 – Treatments and feeding

The pups in each litter were treated for the first 3 days (age 7 – 9 days) with 0.1 ml of either cane syrup (GS) or NH or distilled water according to their expected dietary treatments, in order to familiarise them to the experimental procedures which included handling, weighing and gavaging. Thereafter, the rats were gavaged with either distilled water, diluted GS or NH (50 % concentration) during the neonatal stage (pre-weaning), and then commercial rat feed (CRF), or diets supplemented with either GS or NH after weaning. The cane syrup was supplied in form of the commercially available golden syrup, GS (golden syrup®, Illovo Sugar Ltd, Natal, South Africa). The natural honey used was raw monofloral sunflower honey from a commercial source (Willy's B's Farm, Eikenhoff 1872, South Africa). The dilution of the golden syrup and honey was done so as to avoid gavaging the young rats with thick substances that could clog their respiratory tracts.

2.1.3.1 – Pre-weaning control and treatment diets (7 – 21 days)

The suckling rats were given either distilled water (control group) or one of the four treatment diets during the pre-weaning phase. The four treatment groups were gavaged with either a low dose (10 ml/kg BW) or with a high dose (20 ml/kg BW) of a 50 % solution (volume/ volume) of either GS or NH (Table 2.1). The four

treatment diets were named GS low (GSL), NH low (NHL), GS high (GSH) and NH high (NHH). The male and female pups in each litter were randomly assigned to the four diets and control. Gender-based replication was achieved for each of the treatment diets and the control (n = 6 for each diet except one (NHH) which had 5 females). The pups in each litter were administered for the first 3 days (age 7 – 9 days) with 0.1ml 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 which included handling, weighing and gavaging. The rats were gavaged with the above diets from 7 to 21 days using a plastic orogastric gavaging needle and 1 ml hypodermic syringe. The dietary treatments given once a day every morning (0700 – 0800 hours) was administered to the pups in either low or high dose, according to their groups (Table 2.1). All the rats were weighed daily before weaning to monitor growth. Table 2.2 shows the nutritional composition of the undiluted honey and golden syrup (on a dry matter basis) as determined by the South African National Accreditation Systems (SANAS) accredited Analytical laboratory [Irene Analytical Services, Agricultural Research Council (ARC), Irene, South Africa].

Table 2.1: Pre-weaning dietary treatments of experimental and control rats

Table 2.1: Pre-weaning dietary treatments of experimental and control rats (from age 10 to 21days).

Group 1: Control = 10 ml/kg distilled water.
Group 2: Cane syrup Low dose (GSL) = 10 ml/kg BW of 50 % golden syrup (GS) solution.
Group 3: Natural honey Low dose (NHL) = 10 ml/kg BW of 50 % natural honey (NH) solution.
Group 4: Cane syrup High dose (GSH) = 20 ml/kg BW of 50 % GS solution.
Group 5: Natural honey High dose (NHH) = 20 ml/kg BW of 50 % NH solution.

Table 2.2: 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 – 21) and to formulate the adult diets.

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
Glucose (g/100g)	43.35 ± 0.02	37.64 ± 0.01
Fructose (g/100g)	52.37 ± 0.03	36.46 ± 0.02
Sucrose (g/100g)	2.03 ± 0.05	26.03 ± 0.00$^{\beta}$
Maltose (g/100g)	2.75 ± 0.02	not detected
Glycaemic sugars (%)	76.71 ± 1.55	77.03 ± 0.91

Table 2.2 Continued

Proximate analyses	**Natural honey (NH)**	**Golden syrup (GS)**
Protein*(%)	0.42 ± 0.06	0.25 ± 0.05
Fat (%)	0.53 ± 0.01	0.62 ± 0.00
Others (%)	0.53 ± 0.00	0.17 ± 0.00

Analyses of data with Student's *t*-test showed that both dietary supplements (NH and GS) were iso-caloric and that their components were not significantly different ($p > 0.05$) except sucrose$^{\beta}$ that is significantly higher ($p < 0.0001$) in GS than NH: *Obtained by multiplying nitrogen content by the factor of 6.25.

2.1.3.2 – Post-weaning control and treatment diets (22 – 91 days)

Five post-weaning diets were prepared for the experimental rats. 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 % for the GSH and NHH (also v/w). The control diet was the commercially available RF to which 20 % tap water was added (v/w). The details of the dietary treatments are listed in Table 2.3. The pups were weaned at 21 days of age and put on to their post-weaning diets from day 22. The diets were prepared daily, weighed and freshly served in clean bowls. The nutritional composition of the adult diets as determined by SANAS accredited Analytical laboratory (Irene Analytical Services, Agricultural Research Council (ARC), Irene, South Africa) is given in Table 2.4. The rats were supplied with tap water *ad libitum*. During this period (post-weaning), the rats were weighed twice weekly to monitor body weight gain. The detailed dietary treatments and interventions on the experimental rats are shown in Figure 2.1.

Table 2.3: Post-weaning dietary treatments and control of the experimental rats (age 22 – 91 days).

Group 1: Control (CRF); tap water was added at the rate of 20 % of the diet (volume / weight, v/w) to commercial rat feed.
Group 2: Cane syrup Low dose (GSL); GS was added as 20 % of the diet (v/w) to rat feed.
Group 3: Natural honey Low dose (NHL); NH was added as 20 % of the diet (v/w) to rat feed.
Group 4: Cane syrup High dose (GSH); GS was added as 50 % of the diet (v/w) to the rat feed.
Group 5: Natural honey High dose (NHH); NH was added as 50 % of the diet (v/w) to rat feed.

Table 2.4: Nutritional composition (dry matter basis) of treatment diets and control.

Proximate analyses	**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]
Glucose (g/100g)	2.12 ± 0.02[a]	10.19 ± 0.01[b]	14.71 ± 0.02[c]	23.62 ± 0.02[d]	12.72 ± 0.07[c]
Fructose (g/100g)	1.31 ± 0.02[a]	10.15 ± 0.02[b]	16.53 ± 0.00[c]	23.77 ± 0.02[d]	17.33 ± 0.02[c]
Sucrose (g/100g)	0.56 ± 0.02[a]	6.90 ± 0.02[b]	2.65 ± 0.02[c]	13.45 ± 0.02[d]	2.46 ± 0.02[c]
Glyc sugars (%)	32.81 ± 0.61[a]	44.62 ± 0.19[b]	44.44 ± 0.86[b]	43.22 ± 0.50[b]	51.06 ± 1.50[c]

Table 2.4 Continued

Proximate analyses	**CRF**	**GSL**	**NHL**	**GSH**	**NHH**
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]
ADF (%)	7.33 ± 0.10[a]	6.89 ± 0.11[a]	5.07 ± 0.30[b]	7.52 ± 0.62[a]	5.15 ± 0.42[b]
NDF (%)	20.14 ± 0.15[a]	18.82 ± 0.08[b]	13.00 ± 0.13[c]	20.12 ± 0.01[a]	17.76 ± 0.19[b]
Fat (%)	3.71 ± 0.01[a]	3.83 ± 0.03[a]	3.51 ± 0.02[a]	2.08 ± 0.02[b]	2.45 ± 0.01[c]
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]

Data in the same row with the same superscript are statistically significant ($p < 0.05$) compared with those having different superscripts along the row; Glyc sugars = Glycaemic sugars (sweeteners having lower tendency to increase the blood sugar levels after consumption [Truswell, 1992]). All diets were iso-caloric; dose-matched diets were iso-nitrogenous; control diet, CRF was also iso-nitrogenous with low-dose diets. However, CRF had a significantly lower glycaemic sugar content than the other diets. *Obtained by multiplying nitrogen content by the factor of 6.25.

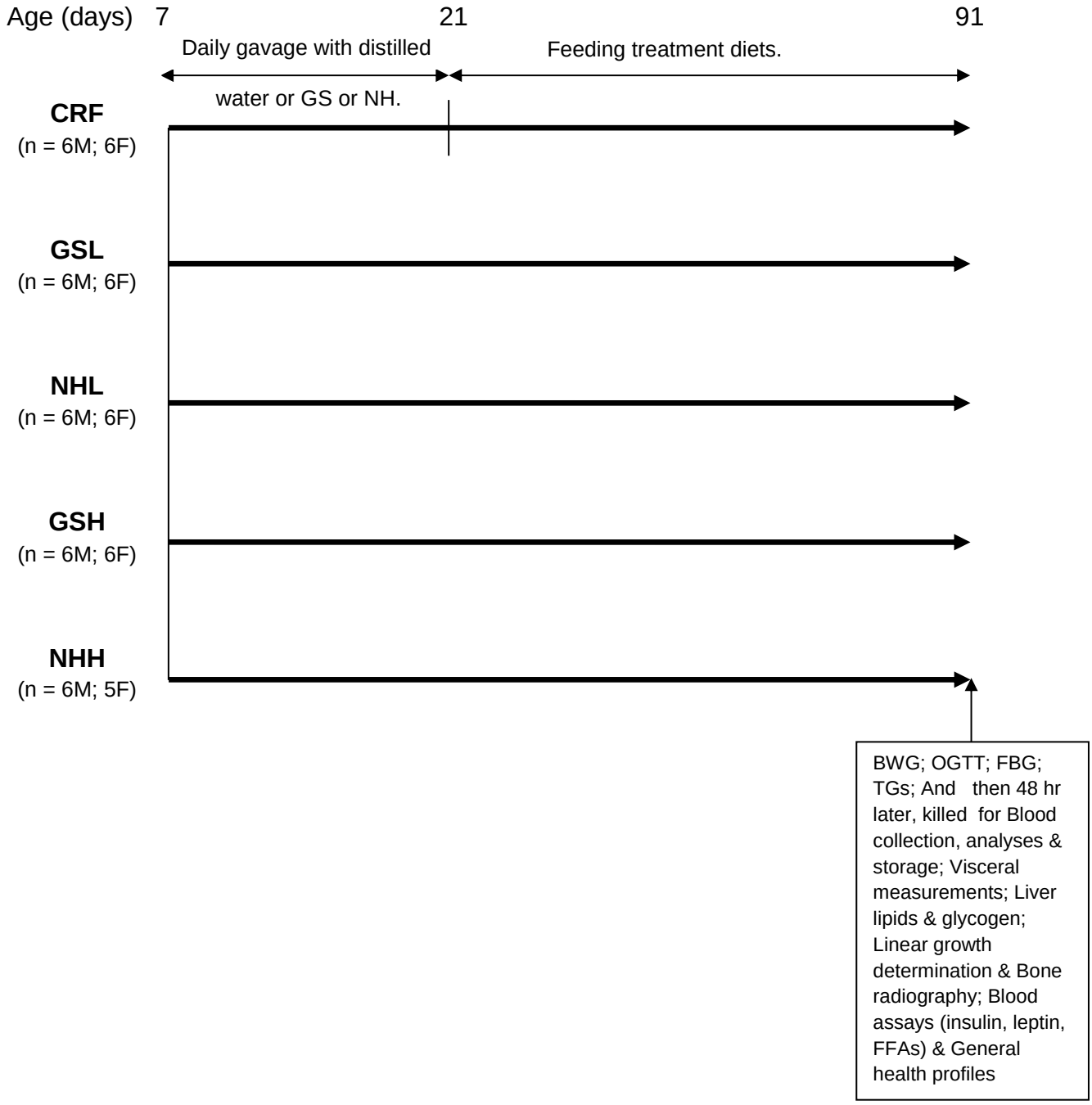


Figure 2.1: Flow chart showing the groupings, treatments and interventions.

KEY

BWG: Body weight gain
FBG: Fasting blood glucose
TGs: Fasting blood triglycerides
FFAs: Free fatty acids
OGTT: Oral glucose tolerance test
CRF: 10 ml/kg distilled water pre-weaning and rat feed mixed with 20 % tap water post weaning
GSL: Golden syrup Low (10 ml/kg golden syrup pre-weaning and 20 % post weaning)
NHL: Natural honey Low (10 ml/kg natural honey pre-weaning and 20 % post weaning)
GSH: Golden syrup High (20 ml/kg golden syrup pre-weaning and 50 % post weaning)
NHH: Natural honey High (20 ml/kg natural honey pre-weaning and 50 % post weaning)

2.1.3.3 – Feed intake of experimental rats

The feed left overnight in the food bowls was removed before supplying new feed in the bowls the following morning. The details of the estimated daily feed consumption of the experimental rats during the post weaning period were as shown in the Results section (see section 2.2.1).

2.1.3.4 – Management of dams

The dams were fed *ad libitum* with commercially available rat feed (Epol®, Johannesburg, South Africa). They were also supplied with tap water provided in 500ml water bottles with nipple tips for drinking *ad libitum*. The dams did not receive any treatment during the study, other than being weighed twice every week as part of routine husbandry to monitor their growth performance and general health status. They were returned to stock immediately after the pups were weaned.

The doses of all the dietary treatments were within the ranges of previous experimental trials in rats [Al-Waili, 2003; 2004; Al-Waili and Boni, 2003; Al-Waili and Haq, 2004; Al-Waili *et al*, 2006; Busserolles *et al*, 2002a; Ajibola *et al,* 2007; Kilicoglu *et al,* 2008; Chepulis *et al*, 2009; Prakash *et al,* 2008; Alagwu *et al,* 2009, 2011]. This allowed for proper comparison with previous studies, and ensured that the experimental rats were not exposed to nutritionally deficient diets.

2.1.4 – Growth

2.1.4.1 – Body weight gain

The growth of the experimental animals was monitored by measuring their body weight using an Ohaus Scout™ Pro digital balance (Ohaus Corporation, New York, USA) daily during the neonatal stage of the study (age 7 – 21 days); whilst the body weight measurements after weaning were done twice weekly from age 22 to 91 days. The body weight measurement of the rats was done daily between age 7 to 21 days because that was a more active growing phase when there was rapid body weight changes on daily basis and the gavaging volume had to be adjusted according to the body weight to ensure a constant dosing rate. The growth pattern of the experimental rats and a comparison of BWG on gender basis (male and female) were assessed.

2.1.4.2 – Linear growth

The lengths of the long bones (femurs and tibias) from the right hind limb of the rats were measured as indicators of linear growth. The detailed technique of assessing the long bones for measurements is described as part of the terminal procedures in subsection 2.1.10.1 in the later part of this chapter.

2.1.5 – Circulating Metabolic Substrates

The circulating metabolic substrates measured two days before the end of the adult phase of the study (13 weeks old rats) were fasting blood glucose (FBG) and fasting triglycerides (TGs). The animals were fasted overnight for 12 hours (but they still had access to drinking water), prior to the measurement of the metabolic substrates. The FBG and TGs were determined using a calibrated Glucometer (Glucometer Elite®, Kyoto, Japan) and a calibrated TGs meter (Accutrend Cobas®, Roche, Mannhein, Germany) on glucose test strips (Ascensia Elite®, Bayer (PTY) Ltd, Healthcare Division, Isando 1600, South Africa) and TGs test strips (Roche Diagnostics, Germany) respectively according to the manufacturer's instructions.

The FBG and TGs were determined two days before the end of the study (after 12 weeks) in the overnight fasted (12 hours) animals. The rats were individually placed in perspex restrainers and allowed to settle down and adapt to the restrainers for one hour. Two drops of blood were collected from the tail vein of each rat via pin prick with a sterile 22G needle after disinfecting the tail with alcohol swab (Patient Care®, Johannesburg, South Africa). The blood was used to measure FBG using a calibrated glucometer (Glucometer Elite, Kyoto, Japan) and TGs using a calibrated TGs meter (Accutrend Plus Cobas, Roche, Mannhein, Germany) on glucose and TGs test strips (Roche Diagnostics, Germany) respectively. Thereafter, the rats were immediately subjected to an oral glucose tolerance test.

2.1.6 – Oral glucose tolerance test (OGTT)

This was done according to the method of Loxham *et al* [2007], each rat was given a 50% glucose solution (2g/kg BW) by oral gavage [Klimes *et al*, 1998]. A drop of blood was collected from the tail vein with sterile 22G needles after disinfection by swabbing with an alcohol infused swab (Patient Care®, Johannesburg, South Africa) immediately before (0), and at 15, 30, 60, as well as 120 min after glucose administration [Loxham *et al*, 2007; Muellenbach *et al*, 2009]. The glucose in the blood samples was determined as described above according to the manufacturer's instructions. The rats were then returned to their respective cages.

The total area under the curves (AUC) was calculated from the OGTT results according to the trapezoidal method of Yeh and Kwan [1978] with modifications by Purves [1992] thus:

$$AUC_{0-\infty} = AUC_{0-1} + AUC_{1-last} + AUC_{last-\infty}$$

$$= \frac{Cp_0 + Cp_1}{2}.\ t_1 + \frac{Cp_1 + Cp_2}{2}.\ (t_2 - t_1) + \frac{Cp_2 + Cp_3}{2}.\ (t_3 - t_2) + \ldots + \frac{Cp_{last}}{kel}$$

where $AUC_{0-\infty}$ = Total area under the curve of the trapezoid;

AUC_{0-1} = Area under the curve of the 1st trapezoid;

AUC_{1-last} = Area under the curve between the 1st and last trapezoids;

$AUC_{last-\infty}$ = Area under the curve between the last and infinite trapezoids;

Cp_0 = Initial plasma concentration of glucose;

Cp_1=Plasma concentration of glucose at the beginning of 1st trapezoidal segment;

Cp_2=Plasma concentration of glucose at the beginning of 2nd trapezoidal segment;

Cp_3=Plasma concentration of glucose at the beginning of 3rd trapezoidal segment;

Cp_{last}=Plasma concentration of glucose at the end of the last trapezoidal segment;

t_1 = Time at the beginning of 1st trapezoidal segment;

t_2 = Time at the beginning of 2nd trapezoidal segment;

t_3 = Time at the beginning of 3rd trapezoidal segment;

kel = Elimination rate constant.

2.1.7 – Terminal blood samples collection and storage

The feeding regimens were continued for 48 hours, and then the animals were re-fasted for 12 hours overnight prior to terminal intervention. The rats were euthanized with sodium pentobarbitone (150 mg/kg BW intraperitoneally) (Euthanaze, Centaur labs, Johannesburg, South Africa), and before the heart completely stopped beating, 10mls of blood was collected by cardiac puncture using 10 ml syringes and 21 G 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 ^{o}C and 5000 x G for 15 minutes. The serum and plasma collected were

frozen at –20 ^{o}C for later analyses of hormones, metabolic substrates and clinical biochemistry.

2.1.8 – Viscera, Morphometric measurements and preservation

The rats were meticulously dissected and the intestines were collected, emptied and laid out on a cooled board to measure the length using a metric ruler attached to the board [Leopold, 1953]. The weight of the small and large intestines; and the other abdominal visceral organs which include caecum; stomach; liver, spleen; pancreas; kidneys as well as the visceral fat was obtained using a Precisa 310 M digital balance (Precisa®, Vadodara, Switzerland). The sections of the middle part of the small intestine (jejunum), 1cm long were collected together with sections of the liver (caudate lobe) and preserved in 10 % phosphate buffered formalin for histological evaluation. After weighing the liver sample of each rat was stored at –20 ^{o}C for later analysis of lipid and glycogen contents. The remaining carcasses were also frozen at –20 ^{o}C for later measurement of the lengths and weights of the long bones.

2.1.9 – Liver lipid and glycogen content

2.1.9.1 – Liver lipid

The liver lipid was extracted by standard procedures described by Folch *et al* [1957] with modification by Bligh and Dyer [1959]. The extraction commenced with steeping of 5 – 10 g of homogenised liver into 150 ml of a 2:1 mixture of chloroform and methanol at 4 ^{o}C. The mixture was filtered through a filter paper

(Albet Filtration and Separation Technology filter paper with pore size 7 – 11 µm, size 185 mm), into a 250 ml separating funnel. Then, 30 ml physiologic (0.9 %) saline was added and mixed thoroughly. The mixture was allowed to separate into aqueous and organic layers. Afterwards, an organic layer which contained the lipid extract was separated out into a round bottomed flask and evaporated under vacuum at 37^{o}C in a rotary evaporator (Labex®, vervaardigdeur, Krugersdorp, Transvaal, South Africa). The lipid extract was then dissolved in 20 ml chloroform and 2 ml of the solution was placed in dried, pre-weighed vials, and re-dried at 50 °C for 30 minutes in a Salvis vacucenter oven (Oakton®, USA), allowed to cool down and re-weighed using a Precisa 310 M digital balance (Precisa®, Vadodara, Switzerland) to determine the residual oil weight. The weight was then used to determine the total amount of lipid in a liver thus:

$$\text{Extracted lipid} = \frac{W_2 - W_1}{W_0} \times 100\%$$

where W_2 = Weight (grams) of vial with lipid extract;

W_1 = Weight (grams) of empty vial;

W_0 = Initial weight of liver sample.

2.1.9.2 – Liver glycogen

The glycogen content of the liver was determined indirectly by acid hydrolysis to glucose as described by Passoneau and Lauderdale [1974]. In brief the liver glycogen was hydrolysed into glucose and the glucose concentration was reported as an equivalent of glycogen stored in the liver. 1 ml of 0.03 M HCl was added to 0.1 g of liver and homogenised with an ultra turrax homogeniser for 20 seconds (Ultra-Turrax®, Siehe, Shanghai, China). Then, 1 ml of 1 M HCl was added and reaction mixture was sealed and allowed to stand in a boiling water bath for 2 hours. Thereafter, 1 ml NaOH was added to neutralise the acid before glucose determination. Glucose concentration was then determined with an Accu-Chek Active glucose meter (Roche, Germany) using Accu-Chek Active glucose test strips (Roche Diagnostics GmbH, Mannheim, Germany.

2.1.10 – Linear growth determination and radiography

2.1.10.1 – Bone length and weight

The right femoral head was removed gently from the acetabulum at the hip joint, the muscles and soft tissues were removed from the long bones (tibia and femur) and the length of the bones was measured with a ruler. The bones were then dried in a Salvis vacucenter oven (Oakton®, USA) at 40 °C for 7 days (until constant weight was obtained), and then weighed using a Precisa 310 M digital balance (Precisa®, Vadodara, Switzerland) to determine 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).

2.1.10.2 – Bone radiography

The bone density was also obtained through radiographs using a Fuji film X-ray machine (Industrial X-ray film FR; Fuji Photo Film Co., Ltd, Tokyo, Japan). The bones were scanned on the same area of the scanner table in an identical position at 1 metre height from the X-ray light using a scan speed of 48 kVp, 0.71 mA, and exposure times of 10 seconds per plate.

2.1.11 – Histomorphology

The small intestine and liver sections earlier stored in 10 % phosphate buffered formal saline were collected, fixed and embedded with paraffin for histological evaluation. The sections of the small intestinal samples were fixed and then stained with haematoxylin and eosin (H & E) stain to prepare histological sections (8 µm); whilst the liver sections also fixed and stained with H & E stain to prepare 5 µm thick histological sections.

2.1.11.1 – Small Intestine histology

The portions of the small intestine (from the middle segment) known as the jejunum, 1 cm long were used for the histological examinations. The jejunum was used for histology due to its absorptive importance. Morphometric measurements were done with an eye piece micrometer mounted on a light microscope (LM) (Reichert®, Austria) at 100 x magnification. Mean villus height per segment was estimated as the mean of the measurements (of 3 – 5 sections per rat) according to the procedures of Guth *et al*, (1984) and Ali *et al*, (1990). In an attempt to

minimise under-estimation error due to angle and position of section planes, only the tallest profiles in each segment were measured [McAnuff *et al*, 2003].

2.1.11.2 – Liver histology

The portions of the caudate lobe of the liver were cut from each liver sample for the histological assessment of the liver. The caudate lobe of the liver was used for consistency of observation because the histology of the liver is distinct from that of other organs in that every 3 – 4 cells (lobules) of it have their own vessels and their own drainage systems. The sections were examined and assessed under light microscope (magnification 400 x) for cellular damage and fatty degeneration. A minimum of 10 fields for each liver slide were observed for severity of changes according to Korkmaz and Kolankaya [2008], and mean value of observations recorded. Severity of hepatic changes was scored based on a scale of 0 to 5, where 0 indicates absence of pathological changes and 5 indicates chronic changes [Bacon *et al,* 1984; Reimer *et al,* 2011].

2.1.12 – Blood analyses: Hormones, Clinical biochemistry and Health profile

2.1.12.1 – Insulin and insulin sensitivity

The plasma insulin was determined by enzyme linked immunosorbent assay (ELISA) using a DRG Rat Insulin High Range kit (DRG® International Inc., USA) according to the manufacturer's instructions. The High Range Rat Insulin ELISA was a solid phase two-site enzyme immunoassay. The principle of the procedure involved in the quantitative determination of insulin levels in the rat plasma was

based on the direct sandwich technique in which two monoclonal antibodies were directed against separate antigenic determinants on the insulin molecule. There were 96 precoated wells in this kit, into which the standards, control, and the plasma samples were pipetted to determine the rat insulin levels. During incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microtitration well. A simple washing step removed unbound enzyme labelled antibody. The bound conjugate was detected by reaction with 3,3',5,5'-tetramethylbenzidine. The reaction was stopped by adding an acid which was the Stop Solution of the reaction (0.5 M H_2SO_4) to give a colorimetric endpoint. The sample values were then read spectrophotometrically on a plate reader machine (Multiskan Ascent, Lab system, model no 354, Helsinki, Finland) at 540 nm wavelength. A standard curve was constructed and the concentrations of the plasma insulin in the samples were determined with reference to the standard curve.

The plasma insulin levels in the samples and glucose values were used to estimate the fasting whole-body insulin sensitivity by the homeostasis model assessment of insulin resistance (HOMA-IR) formula according to the protocol of Mathews *et al* [1985] thus:

$$\text{HOMA-IR} = \frac{[\text{fasting plasma glucose (mg/dl)} \times \text{fasting plasma insulin (µU/ml)}]}{405}$$

2.1.12.2 – Leptin

The serum leptin was determined by enzyme linked immunosorbent assay (ELISA) using a Mouse Leptin Quantikine kit (Quantikine®, R&D Systems, Inc., Minneapolis, MN 55413 USA), according to the manufacturer's instructions. According to the manufacturers, the kit also recognizes rat leptin, and thus has been validated for the determination of rat leptin. All the reagents and plates used were supplied in the kit. The assay employed the quantitative sandwich enzyme immunoassay technique. Principally, the method utilized a monoclonal antibody specific for rat leptin which was pre-coated into 96-well polystyrene microplates.

The standards, control, and samples were pipetted into the wells coated with monoclonal antibody specific for rat leptin present that could bind by the immobilization of the antibody. After washing away any unbound substances with buffer, an enzyme-linked polyclonal antibody against rat leptin was added to the wells. Following a wash with buffer concentrate to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. The enzyme reaction yielded a blue coloured product that turned yellow when the Stop Solution was added. The intensity of the colour measured was in proportion to the amount of leptin bound in the initial step. The sample values were then read on a plate reader machine (Multiskan Ascent, Lab system, model no 354, Helsinki, Finland) at an optical density of 450 nm, with a second correction wavelength measurement at 540 nm. A standard curve was constructed and the concentrations of the leptin in the serum samples were determined with reference to the standard curve.

2.1.12.3 – Free fatty acids

The free fatty acids (FFAs) in the plasma samples of the experimental rats were determined colorimetrically using FFAs Half-micro test kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. The procedural principle involved the metabolic conversion of FFAs into acyl-CoA by ATP and CoA in a reaction catalyzed by Acyl-CoA-synthetase (Acyl CS), yielding AMP and pyrophosphate. Further, acyl-CoA reacted with oxygen in a reaction catalyzed by acyl-CoA oxidase (ACOD), and produced 2,3-enoyl-CoA. The resulting hydrogen peroxide converted 2,4,6-tribromo-3-hydroxy-benzoic acid (TBHB) and 4-aminoantipyrine (4-AA) into a red dye in the presence of peroxidase (POD). The dye was then measured in the visible range light at 540 nm wavelength on a plate reader machine (Multiskan Ascent, Lab system, model no 354, Helsinki, Finland). The plasma levels of the FFAs in the experimental rats were then calculated relative to both the standard and the blank curves.

2.1.12.4 – Aspartate transaminase

The enzyme activity of Aspartate transaminase (AST) was determined using the Reflotron machine (Reflotron®, Roche diagnostics LTD, Burgess Hill west Sussex, RH159RY, United Kingdom). The machine was calibrated according to the manufacturer's instructions. Afterwards, two drops of serum sample were placed on Reflotron AST test strips (Reflotron®, Roche Diagnostics GmbH, Mannheim, Germany) which were then placed into the Reflotron machine which subsequently performed the assays and gave a print out of results.

This liver function test started with a flow of the sample into the reaction zone. In the presence of AST, α-ketoglutarate and alanine sulfinate were converted to pyruvate and glutamate. This was followed by a second reaction step catalysed by pyruvate oxidase producing more pyruvate, which was cleaved into acetyl phosphate, carbon dioxide and hydrogen peroxide. The hydrogen peroxide then converted a red indicator into its oxidised blue form in the presence of POD. The blue dye formed was then measured kinetically at a wavelength of 567 nm by the Reflotron machine at room temperature (between 25 ^{o}C to 37 ^{o}C) as a measure of the enzyme activity. The whole process took about two minutes, after which the result was then displayed and printed.

2.1.12.5 – Other Liver enzymes

The activities of the other liver enzymes, alanine transaminase (ALT); and alkaline phosphatase (ALP) were determined colorimetrically from the plasma using the ALT and ALP health profile discs on the Vet Test Chemistry Analyzer (Idexx Laboratories Inc, Diamond Diagnostics Holliston MA 01746, USA).

2.1.12.6 – Health profiles

The other clinical biochemical and health profile tests were performed on the plasma using Vet Test Chemistry Analyzer (Idexx Laboratories Inc, Diamond Diagnostics Holliston MA 01746, USA). These include total protein (TP); albumin; globulin and total bilirubin (T bil) which were done colorimetrically from 300µl plasma using health profile discs on the Vet Test Chemistry Analyzer (Idexx

Laboratories Inc, Diamond Diagnostics Holliston MA 01746, USA). The other clinical parameters obtained from assaying the plasma sample on the Idexx Chemistry Analyzer were blood levels of creatinine; urea; calcium (Ca); phosphorus (Phos); cholesterol and amylase using their respective discs.

2.1.13 – Data Analyses

Results 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 undiluted NH and GS shown in Table 2.2.

The data from OGTT and 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. A p value of less than 0.05 ($p < 0.05$) was considered significant for any observation.

2.2 – Results

2.2.1 – Feed Intake

The estimated daily feed consumption of the experimental rats during the post weaning period was as shown in the table below (Table 2. 5). As shown in the table, from 5 weeks of age to termination, the male rats fed with low dose-diets (GSL and NHL) and the control (CRF) group consumed significantly higher ($p < 0.05$) amount of feed than their high dose-diets litter mates (GSH and NHH). In the females, the disparity in feed intake was observed starting from 6 weeks of age to termination (Table 2.5). The male groups were also observed to have consumed more feed than their corresponding female litter mates from age 6 weeks to end of the study at 13 weeks.

Table 2.5: Estimate of daily feed intake in the experimental rats fed NH and GS supplemented diets for 12 weeks in grams per day (g/day).

Age (weeks)	**Sex**	**CRF**	**GSL**	**NHL**	**GSH**	**NHH**
3 - 4	M	6.37 ± 0.98	6.53 ± 1.34	6.85 ± 1.64	5.97 ± 1.23	5.60 ± 0.65
	F	6.52 ± 1.08	6.23 ± 1.19	6.93 ± 1.46	5.20 ± 1.33	5.68 ± 0.89
4 - 5	M	9.39 ± 1.81	9.68 ± 1.65	10.07 ± 2.01	8.06 ± 1.57	8.13 ± 1.76
	F	9.48 ± 1.86	9.43 ± 1.07	9.61 ± 0.99	8.28 ± 1.75	8.52 ± 2.02
5 - 6	M	15.60 ± 2.05	16.12 ± 1.67	17.33 ± 1.21	12.58 ± 2.01*	13.18 ± 1.98*
	F	12.83 ± 1.29§	13.98 ± 0.89§	12.42 ± 1.87§	11.27 ± 1.66	11.92 ± 1.68§
6 - 7	M	21.27 ± 0.68	21.56 ± 1.89	22.25 ± 2.04	15.95 ± 1.27*	15.71 ± 1.33*
	F	16.67 ± 1.84§	17.13 ± 1.93§	15.93 ± 1.77§	13.72 ± 1.23*§	13.24 ± 1.05*
7 - 8	M	26.01 ± 1.79	26.20 ± 0.36	26.07 ± 1.67	18.65 ± 1.97*	18.01 ± 2.48*
	F	19.77 ± 1.41§	19.73 ± 1.73§	19.46 ± 1.96§	15.28 ± 1.79*§	15.86 ± 1.06*§
8 - 9	M	29.16 ± 2.14	29.75 ± 1.33	29.59 ± 1.57	20.67 ± 2.35*	20.43 ± 1.84*
	F	20.29 ± 1.58§	20.97 ± 1.67§	21.39 ± 1.93§	16.37 ± 1.98*§	17.04 ± 1.22*§
9 - 10	M	29.56 ± 2.38	32.02 ± 1.17	32.87 ± 2.12	22.83 ± 2.03*	22.91 ± 2.41*
	F	20.88 ± 2.15§	23.12 ± 1.51§	24.21 ± 1.43§	18.08 ± 0.97*§	19.29 ± 1.86*§

Table 2.5 Continued

Age (weeks)	**Sex**	**CRF**	**GSL**	**NHL**	**GSH**	**NHH**
10 - 11	M	30.36 ± 2.36	34.84 ± 0.88	34.25 ± 1.93	24.49 ± 2.04*	23.97 ± 1.96*
	F	22.73 ± 1.27§	24.65 ± 1.97§	26.02 ± 1.82§	20.08 ± 0.97*§	21.19 ± 1.86*§
11 - 12	M	31.53 ± 1.76	35.71 ± 2.51	35.78 ± 1.34	26.26 ± 0.98*	26.09 ± 1.04*
	F	24.07 ± 1.49§	26.97 ± 1.96§	27.50 ± 1.42§	21.63 ± 0.19*§	21.84 ± 1.47*§
12 - 13	M	33.57 ± 1.84	37.17 ± 2.21	38.12 ± 1.87	27.67 ± 1.55*	27.14 ± 1.88*
	F	27.35 ± 2.09§	28.03 ± 2.19§	29.06 ± 2.51§	23.29 ± 1.81*§	23.73 ± 1.75*§

* significantly different ($p < 0.05$) along the row; § significantly different ($p < 0.05$) from the corresponding value of male group.

2.2.2 – Growth Performance

The growth patterns of the male and female experimental animals from day 7 to 91 are shown in Figures 2.2 and 2.3.

2.2.2.1 – Body weight gain (BWG)

All the dietary treatments induced BWG in both sexes. At weaning (21days old), there was no significant difference in the body weight among all the groups as shown in Figures 2.2 and 2.3.

All the experimental rats gained significantly over the 12-week study period, with the highest BWG in the NHL male subgroup. The growth pattern of the male experimental animals showed that honey-fed rats had significantly higher ($p < 0.01$) terminal BWG of 17.0 % relative to GS-fed rats (NHL versus GSL); and marginal (5.0 %) relative to control rats (NHL versus CRF) in males at low dose dietary treatment (Figure 2.4). At high dose, GSH gained more body weight (2.2 %), albeit insignificant than NHH in males.

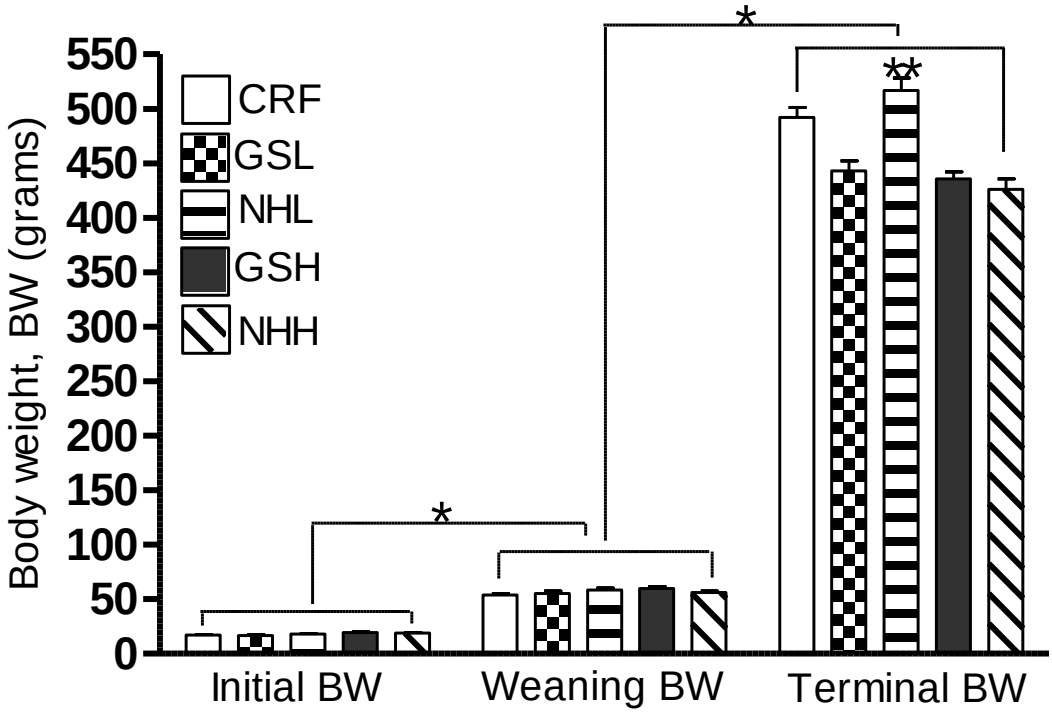


Figure 2.2: Growth pattern of male experimental rats fed NH and GS supplemented diets from 7 – 91 days. Initial body weight (BW) at d 7; Weaning BW (d 21); and Terminal BW at 91 days old: *BW significantly higher than BW of previous phase; **BW significantly higher ($p < 0.01$) than BW of other groups.

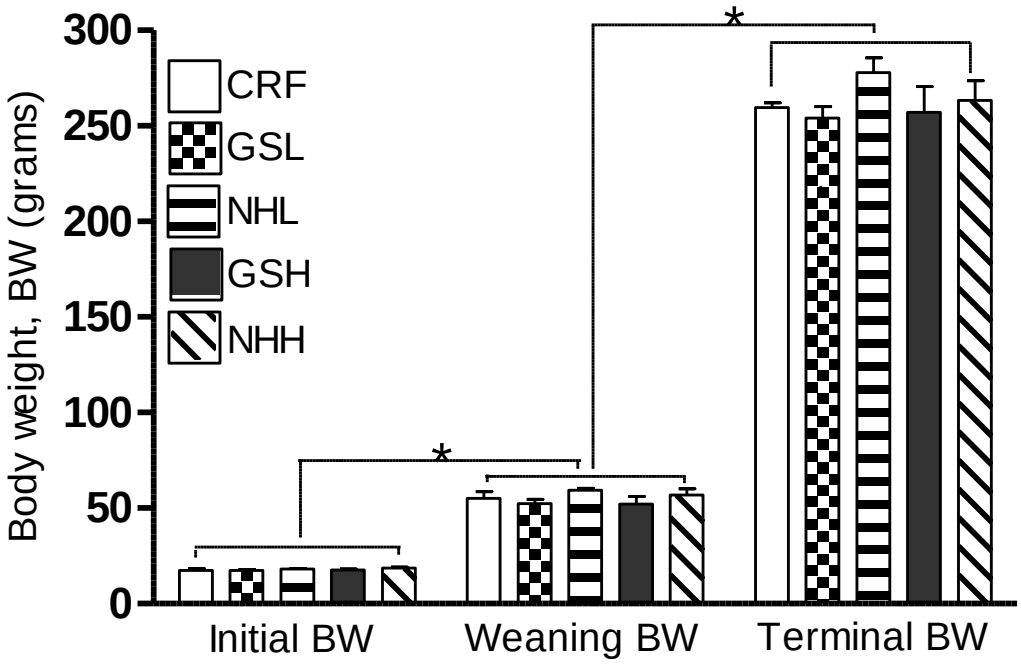


Figure 2.3: Growth pattern of female experimental rats fed NH and GS supplemented diets from 7 – 91 days. Initial body weight (BW) at d 7; Weaning BW (d 21); and Terminal BW at 91 days old: *BW significantly higher than BW of previous phase.

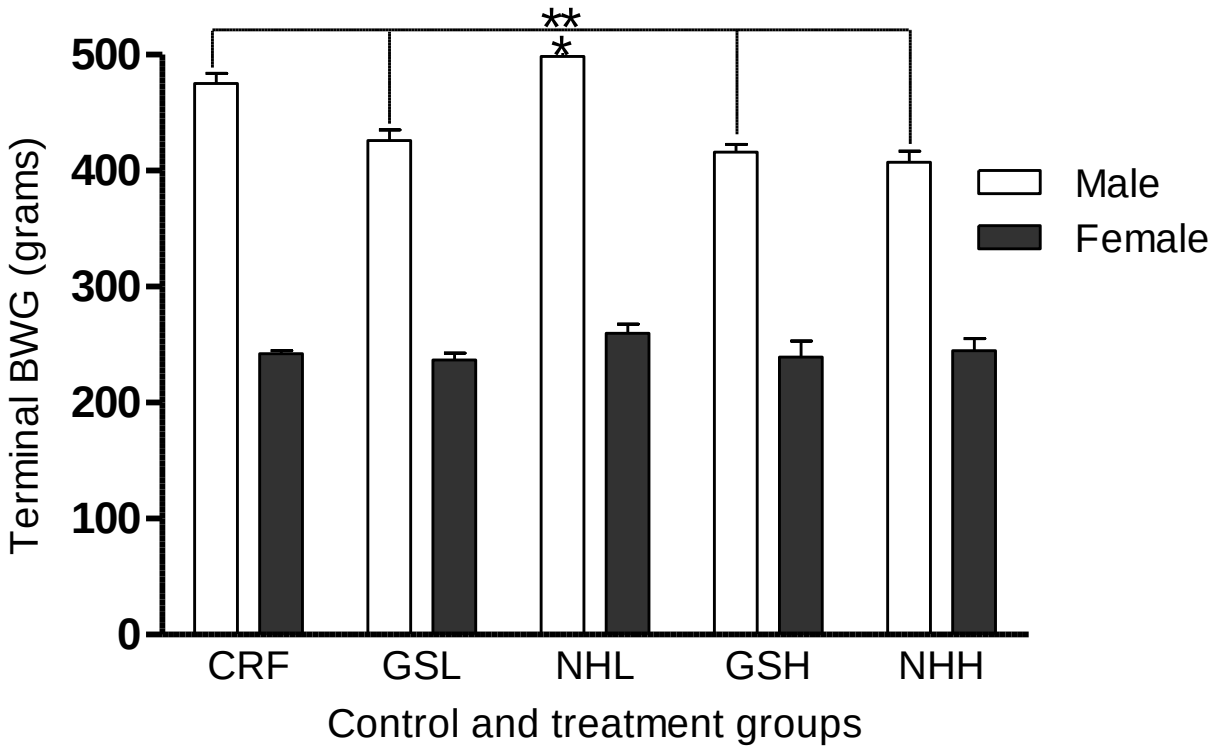


Figure 2.4: Terminal body weight gain (TBWG) of male and female rats fed NH and GS supplemented diets from 7 – 91 days. * significantly higher ($p < 0.01$) than the other male groups; ****** significantly higher ($p < 0.0001$) than that of corresponding female group.

The results of terminal BWG in the females (Figure 2.4) showed that honey-fed females gained 9.0% BW more than those fed cane syrup supplemented diet (NHL *versus* GSL) at low dose; and 6.9% than control (NHL *versus* CRF) at the end of the study. There were no differences in terminal BWG among the groups at high doses. The terminal BWG of the male rats was significantly higher ($p < 0.0001$) than the terminal BWG of the corresponding female groups on the same dietary treatments.

2.2.2.2 – Linear growth

The results for linear growth are detailed in Table 2.6. Feeding NH induced significant elongation and greater weight of the femur and tibia ($p < 0.05$) in males at low dose (NHL rats); whilst at high dose (NHH), only the elongation ($p < 0.05$) of the femur was observed. The relative densities of both long bones calculated as mg/mm showed that in males, NHL femurs were marginally 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 NHL were also marginjally 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 relative density of the NHL femur was calculated from Table 2.6 to be marginally higher than those of GSL and CRF by 4.3 % and 9.4 % respectively, while GSL femur was slightly denser than that of control by 4.9 %. The marginal differences in densities calculated as percentage increase, though not statistically significant could be better appreciated from the radiological assessment of the bones shown as graphical representations in Figures 2.5 and 2.6. There were no

differences in all the three parameters of linear growth (weight, length and density) measured in the tibias of all the female rats (Table 2.6).

The radiographs that were taken to assess the radio-opacity of the bones showed that there were also no significant differences in the bone relative densities of all the rats fed the high doses of NH and GS. However the NH-fed rats had denser bones than the other groups (Figures 2.5 and 2.6). Table 2.6 also showed that the male bones (femur and tibia) were significantly heavier than their female counterparts and the tibia was longer in male rodents than the females amongst all groups. The relative densities of the femur in males were also observed to be significantly higher than those of the female rats.

Table 2.6: Weight, length and density of femur and tibia in experimental rats fed NH and GS supplemented diets 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$^{\alpha}$

*significantly higher ($p < 0.05$) than data across the columns; $^{\alpha}$ significantly different ($p < 0.05$) from the corresponding value of male group.

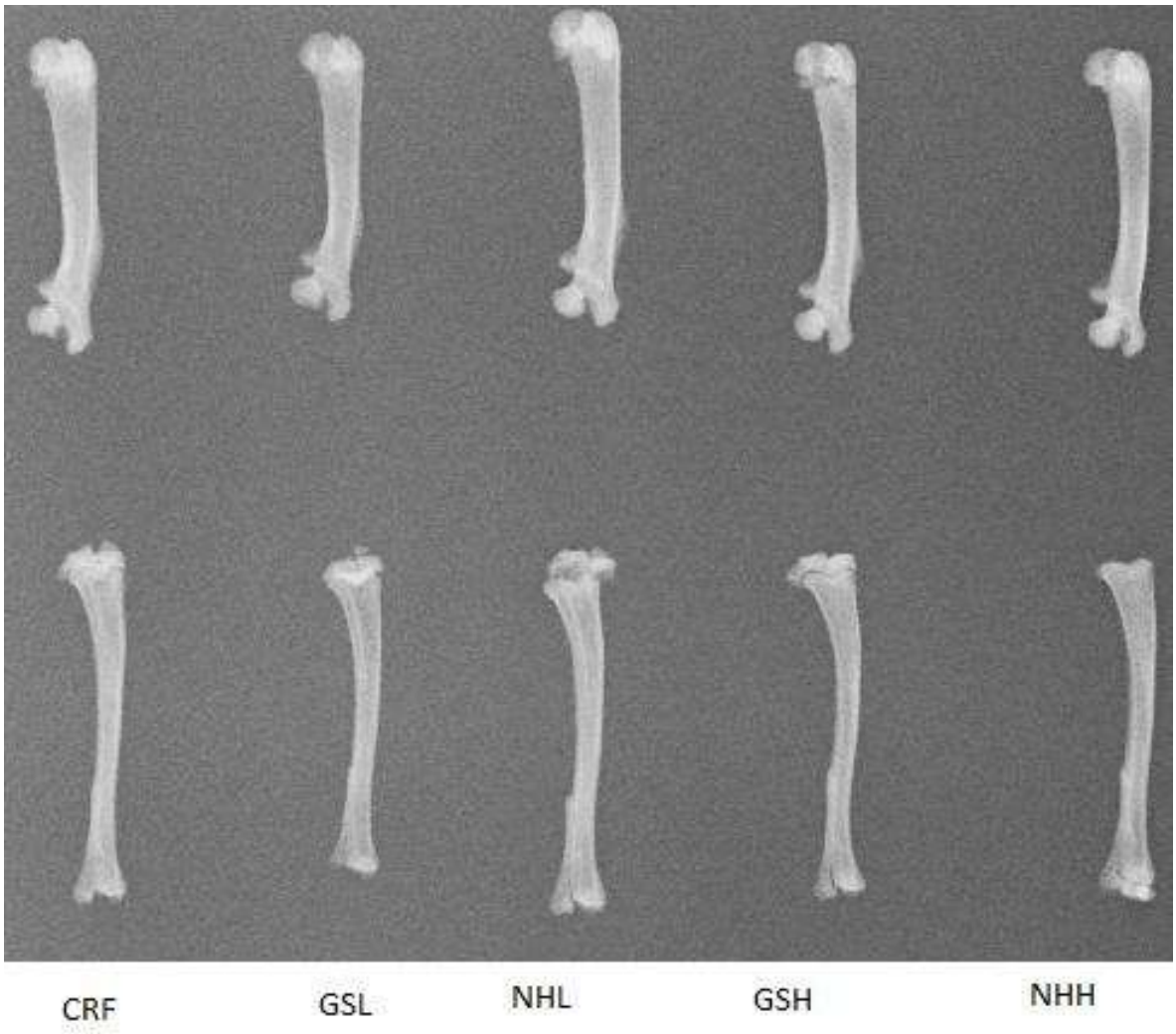


Figure 2.5: Long bones of male experimental rats at 13 weeks old. The top row shows the femurs whilst the bottom row shows the tibias from representative rats of the different groups. NHL (Natural honey Low) bones higher density than GSL (Golden syrup Low) and the control (CRF, commercial rat feed); NHH (Natural honey High) bones density higher than those of GSH (Golden syrup High).

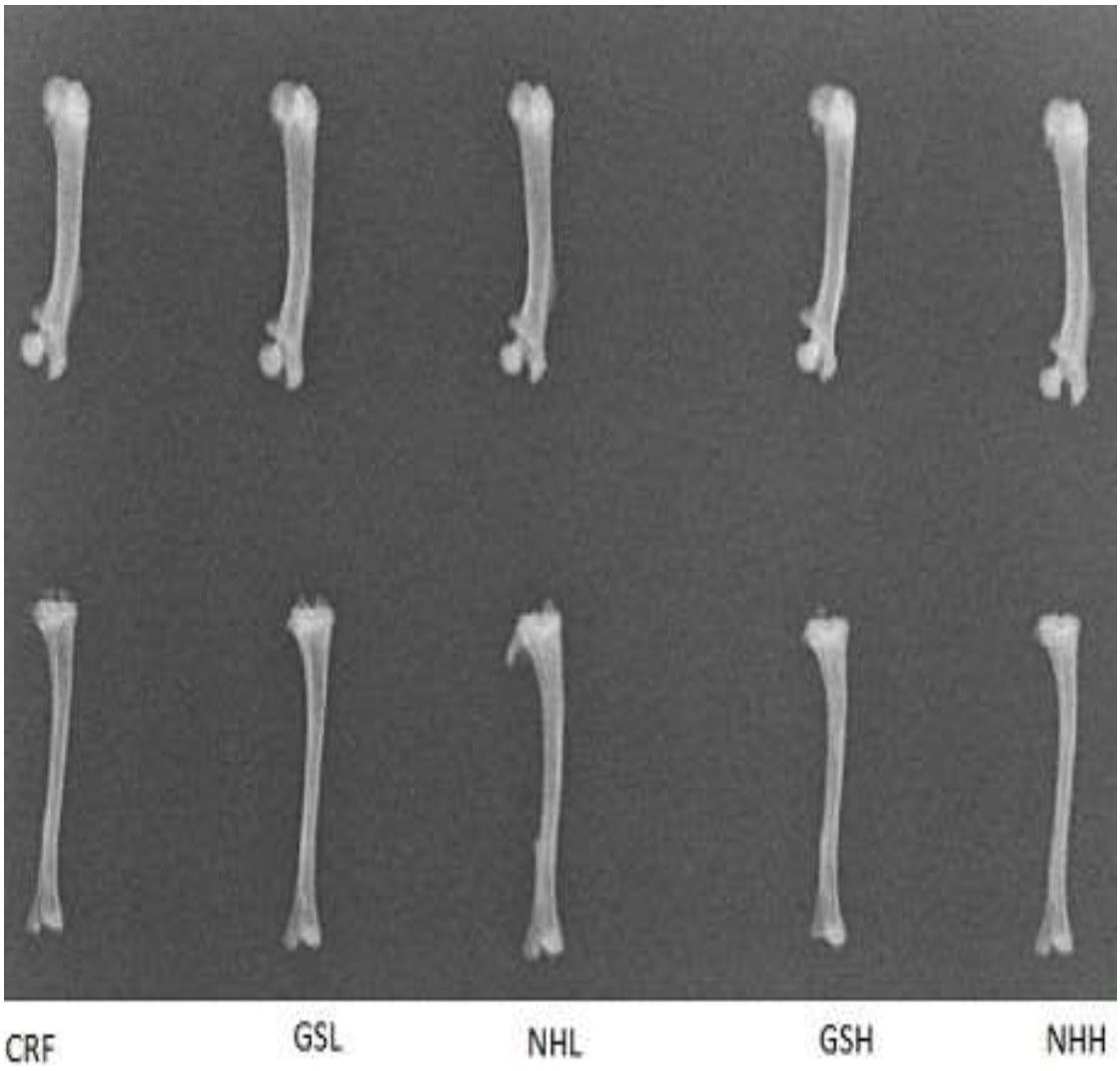


Figure 2.6: Long bones of female experimental rats at 13 weeks old. The top row shows the femurs whilst the bottom row shows the tibias from representative rats of the different groups. NHL (Natural honey Low) bones higher density than GSL (Golden syrup Low) and the control (CRF, commercial rat feed); NHH (Natural honey High) bones density higher than those of GSH (Golden syrup High).

2.2.3 – Oral Glucose Tolerance Test

The results of the OGTT in all the experimental animals (Figures 2.7 and 2.8) showed that the blood glucose concentration (BGC) increased significantly ($p < 0.05$) after 15 minutes and reached a peak 30 minutes after the glucose administration. The BGC returned to basal levels by 120 minutes in all the groups, except in the GS-fed (GSL and GSH) male rats. In GS-fed male rats, the difference in BGC was significant at all the time points recorded, except between 60 and 120 minutes (Figure 2.7). The peak blood glucose level in the cane syrup fed male rats (GSL and GSH) at 30 minutes was significantly higher than BGC recorded for NHL-fed male rats ($p < 0.05$), and NHH and control groups ($p < 0.001$).

In the female rats those nurtured with natural honey showed a similar pattern of response to oral glucose dosing as both (NHL and NHH) groups recorded significant differences in BGC at all the time points, returning to basal values by 120 minutes (Figure 2.8). The female GSL and control rats had a difference in BGC only at 15 minutes after oral glucose dosing, with return to basal level at 120 minutes. The GSH female rats had differences in blood glucose level at all the time points recorded except between 60 and 120 minutes, and values also returned to basal level at 120 minutes.

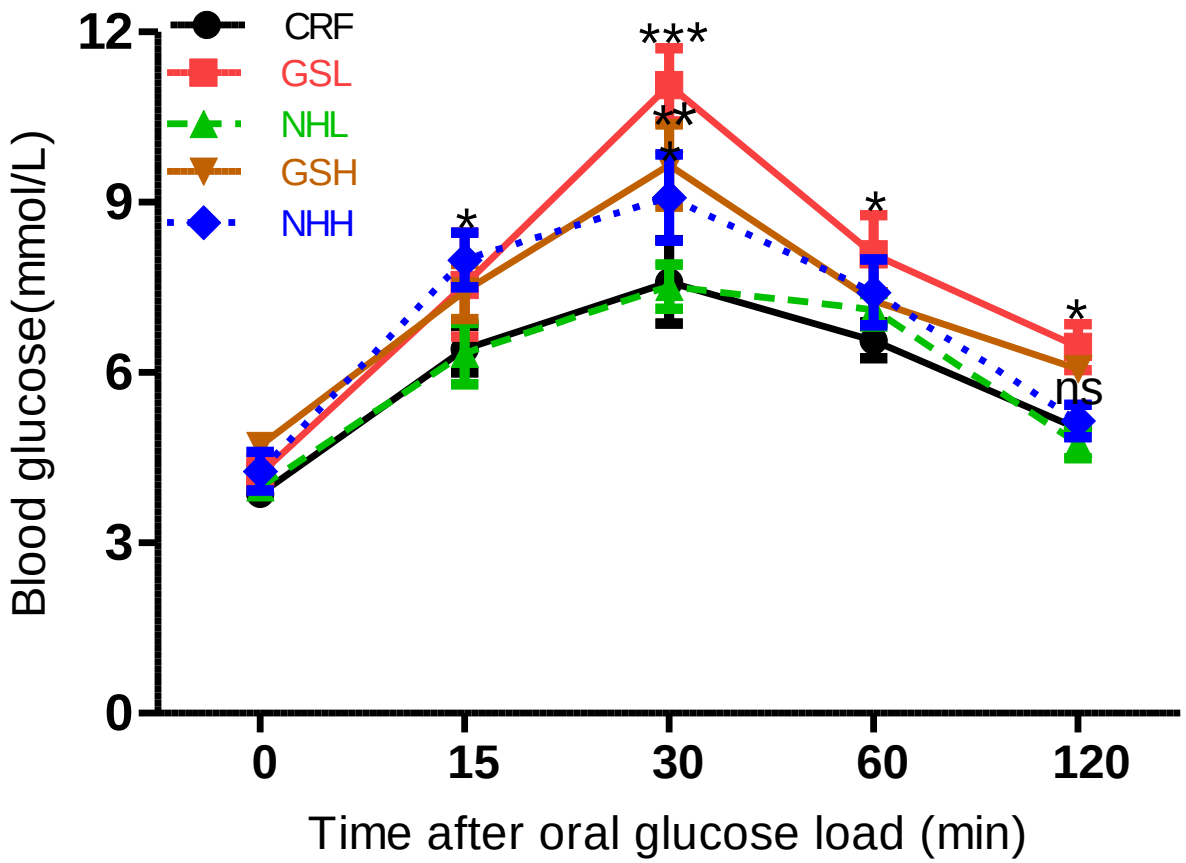


Figure 2.7: Blood glucose concentration (BGC) profile during each 2-hour OGTT in male experimental rats. Error bars represent SEM. *BGC greater ($p < 0.05$) than the basal values; **BGC of GSH greater ($p < 0.05$) than values from NH-fed and control (CRF) rats at peak (30 minutes); ***BGC of GSL greater ($p < 0.001$) than NHL, NHH and CRF at peak; ns – BGC of NHL, NHH and CRF not significantly different ($p > 0.05$) between 0 and 120 minutes.

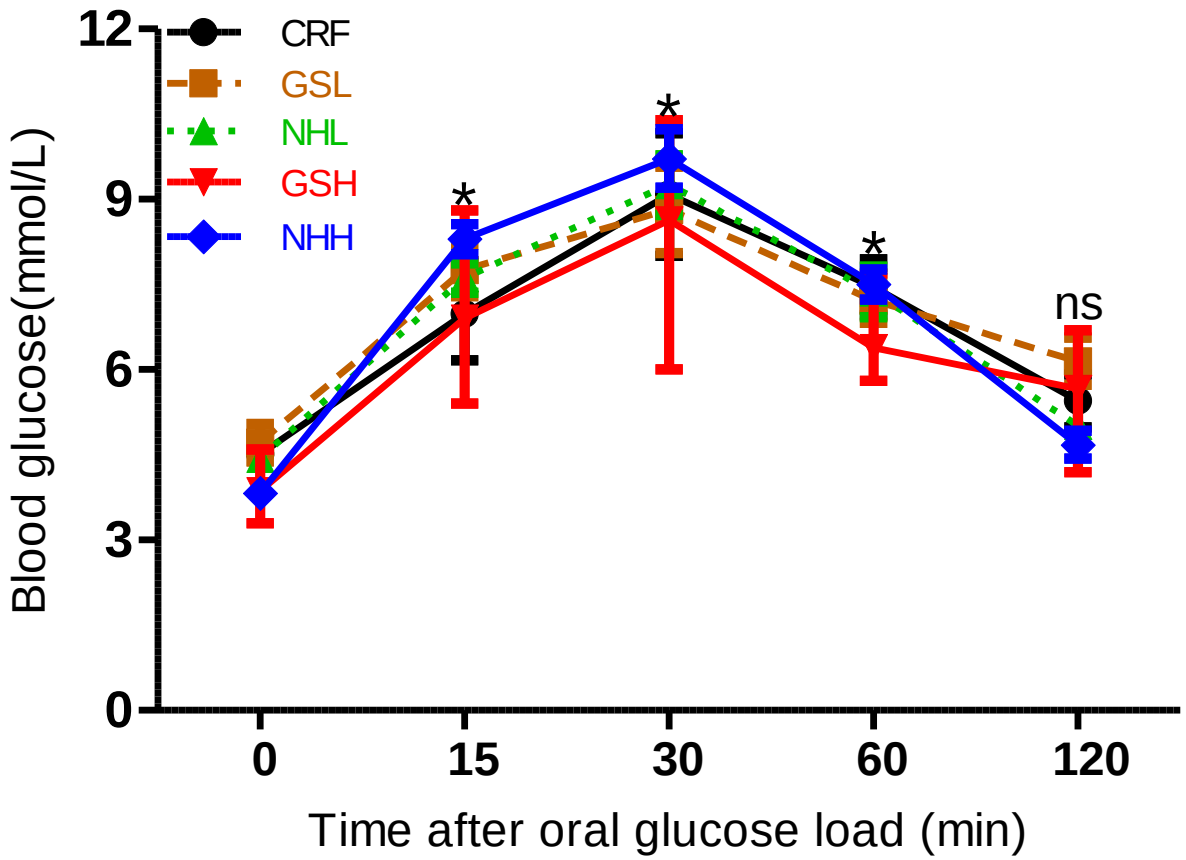


Figure 2.8: Blood glucose concentration profile during each 2-h OGTT in female experimental rats. Error bars represent SEM. *BGC greater ($p < 0.05$) than the basal values; ns – not significantly different ($p > 0.05$), as all returned to basal values by 120 minutes.

The total area under the curves (AUC) calculated from the OGTT results are shown in Figures 2.9 and 2.10. The AUC of the GSL-fed male rats were significantly ($p < 0.05$) higher than those of the other male groups (Figure 2.9). The difference in the AUC of the female groups did not attain any significance (Figure 2.10).

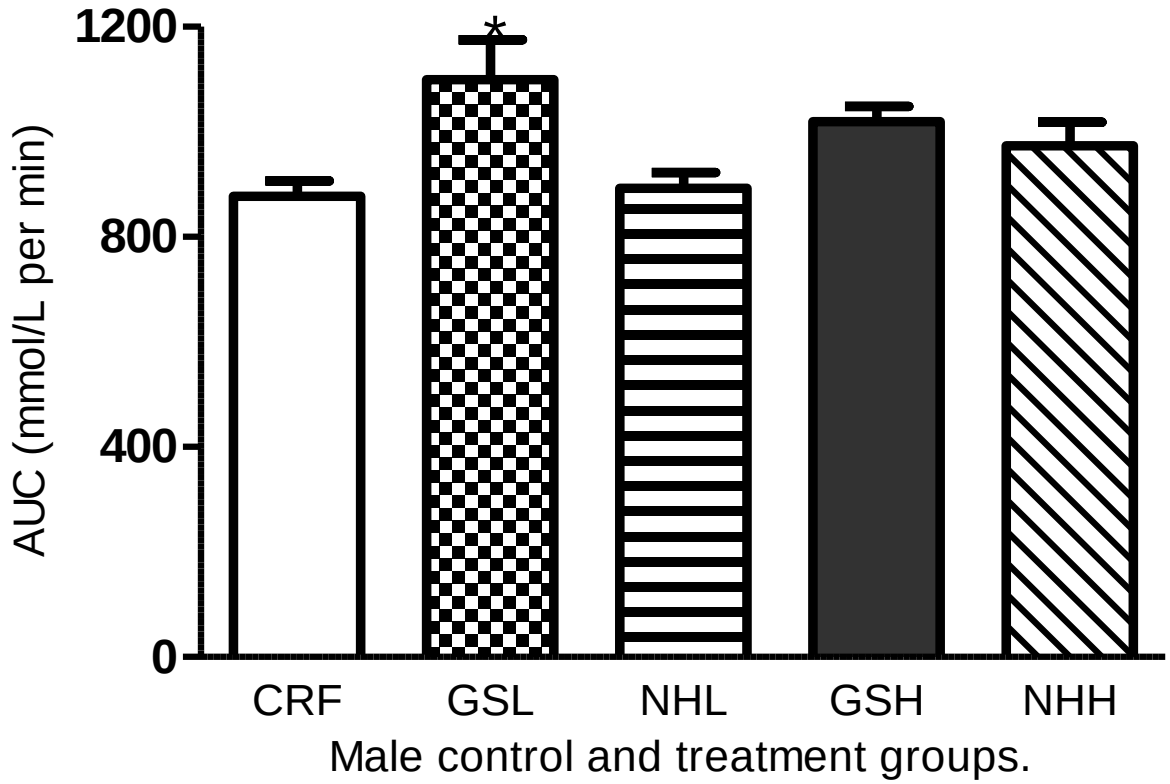


Figure 2.9: Effects of 12-week NH and GS dietary supplementation on total area under the curves (AUC) of oral glucose tolerance test (OGTT) in male experimental rats. Error bars represent the SEM. * $p < 0.05$ compared with NHL; NHH and control, CRF.

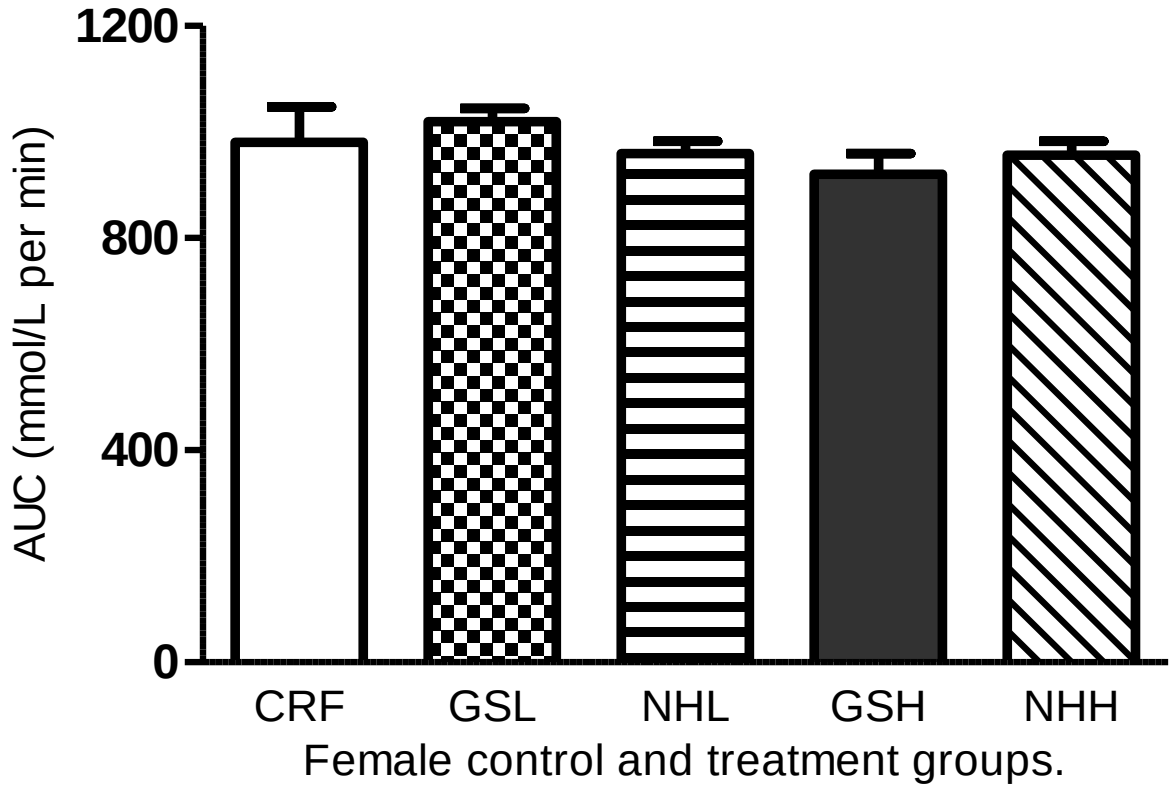


Figure 2.10: Effects of 12-week NH and GS dietary supplementation on total AUC of OGTT in female experimental rats. Error bars represent SEM. AUCs not significantly different ($p \geq 0.05$) showed that all the female rats were normoglycaemic, as there was no main effect of the trial unlike recorded with males.

2.2.4 – Circulating Metabolic Substrates and Hormones

The measured concentrations of the circulating metabolic substrates (FBG, TGs and FFAs) of GS-fed male rats were significantly higher ($p < 0.05$) than those recorded for NH-fed in male animals (Table 2.7), whilst the blood concentrations of FBG in GSL female rats; and TGs in GS female rats were observed to be significantly higher than those of other female animals. The female rats also recorded significantly lower ($p < 0.05$) values of FBG except NHH female rats with marginal (3.34 %) lower value relative to the corresponding (NHH) male rats (68.8 ± 3.92 *vs* 71.1 ± 3.38 mg/dL). In TGs and FFAs, gender differences were noticeable only among the GS-fed rats. The other substrate measured, cholesterol also had higher ($p < 0.05$) blood values induced by GS intake at low dose in both sexes than other treatment groups and control, whilst at high dose only males on the high cane syrup diet (GSH) had significantly elevated blood cholesterol concentration (Table 2.7). The circulating cholesterol concentrations of NHL-fed and the control (CRF) rats were significantly lower ($p < 0.05$) in females compared to their male counterparts; whilst the other groups (GSL, GSH and NHH) showed no gender differences at the end of the 12-week dietary treatments.

Table 2.7 also showed the effects of the dietary interventions on the hormonal profiles (leptin and insulin), and describes the insulin sensitivity of the male and female experimental animals. It was found that the differences in the blood levels of leptin among the treatment and control groups neither attained any significance ($p > 0.05$), nor showed gender difference. The results of the other hormone measured in the plasma, insulin showed higher values ($p < 0.05$) at both low and

high doses in male rats fed with cane syrup, but the insulin values obtained from the female groups were not significantly different ($p > 0.05$) (Table 2.7).

Table 2.7: Metabolic substrates and hormones in male and female experimental rats fed NH and GS supplemented diets for 12 weeks.

Diet	**Sex**	**FBG** (mmol/L)	**TGs** (mmol/L)	**FFAs** (mmol/L)	**Chol** (mmol/L)	**Leptin** (ng/ml)	**Insulin** (µU/ml)	**HOMA-IR**
CRF	M	4.47 ± 0.17	1.15 ± 0.06	0.36 ± 0.04	1.15 ± 0.20	0.22 ± 0.15	118.2 ± 17.3	20.9 ± 2.8
	F	3.72 ± 0.11$^{\alpha}$	1.16 ± 0.09	0.26 ± 0.01	0.71 ± 0.04$^{\alpha}$	0.20 ± 0.14	105.1 ± 13.7	19.8 ± 3.35
GSL	M	4.72 ± 0.33*	1.75 ± 0.13*	0.48 ± 0.06*	1.28 ± 0.14*	0.99 ± 0.40	230.7 ± 32.5*	46.1 ± 6.92*
	F	4.43 ± 0.22$^{\beta\alpha}$	1.18 ± 0.10$^{\alpha}$	0.32 ± 0.03$^{\alpha}$	1.19 ± 0.12*$^{\beta}$	0.70 ± 0.11	132.6 ± 19.7$^{\alpha}$	28.8 ± 6.27$^{\alpha}$
NHL	M	3.77 ± 0.13	1.27 ± 0.05	0.29 ± 0.04	1.04 ± 0.04	0.34 ± 0.11	129.5 ± 13.3	21.8 ± 2.53
	F	3.32 ± 0.17$^{\alpha}$	1.13 ± 0.02	0.25 ± 0.03	0.73 ± 0.05$^{\alpha}$	0.13 ± 0.11	104.3 ± 16.6$^{\alpha}$	20.3 ± 2.75
GSH	M	5.03 ± 0.19*	2.02 ± 0.15*	0.46 ± 0.02*	1.18 ± 0.07*	1.31 ± 0.58	326.4 ± 76.2*	72.7 ± 16.8*
	F	3.87 ± 0.20$^{\alpha}$	1.65 ± 0.11$^{\beta\alpha}$	0.33 ± 0.02$^{\alpha}$	0.92 ± 0.06	0.89 ± 0.12	143.7 ± 21.7$^{\alpha}$	25.2 ± 4.67$^{\alpha}$
NHH	M	3.95 ± 0.19	1.32 ± 0.07	0.33 ± 0.02	0.85 ± 0.08	0.64 ± 0.15	131.3 ± 19.6	23.2 ± 3.71
	F	3.82 ± 0.22	1.33 ± 0.19	0.27 ± 0.03	0.98 ± 0.10	0.89 ± 0.38	129.9 ± 9.8	22.2 ± 2.34

* significantly different ($p < 0.05$) across the column; $^{\alpha}$ significantly different ($p < 0.05$) from corresponding male values; $^{\beta}$ significantly different ($p < 0.05$) among females; FBG = fasting blood glucose; TGs = fasting blood triglycerides; FFAs = free fatty acids; Chol = cholesterol; HOMA-IR = homeostasis model assessment of insulin resistance.

The insulin values of the male GS-fed (GSL and GSH), and NHL groups were also significantly higher than the corresponding values obtained from the female rats. The HOMA-IR values given in Table 2.7 showed that the cane syrup induced insulin resistance (IR) at both doses in males, whilst the females did not exhibit any significant difference in IR values. Gender differences in IR values were also observed amongst GS-fed (GSL and GSH) rats, as the females had lower HOMA-IR values than their male counterparts.

2.2.5 – Gross visceral measurements

The different treatments did not result in any significant differences ($p > 0.05$) in the morphometric values of small intestine and of the large intestine as shown in Table 2.8.

The weights of the other abdominal visceral organs were recorded in Table 2.9. For the males, there were significant differences recorded in the absolute (g) and relative (% BW) weights of the male visceral organs namely liver, pancreas, caecum and visceral fat. The male GS-fed rats had higher absolute liver weight (g) at both (low and high) dietary treatments, but relative to BW, only GSL rats had heavier liver (% BW) than the other male groups. There were significant increases ($p < 0.05$) in the weights (g and % BW) of the pancreas and caecum in the rats fed with the NH supplemented diets than the GS-fed rats (Figure 2.8). Cane syrup induced highly significant ($p < 0.0001$) visceral fat weight in males relative to the control and the treatment animals fed natural honey supplement.

Interestingly, following the 12 weeks of dietary supplementation with NH and GS, there were no records of significant differences ($p > 0.05$) in the gross measurements of abdominal organs amongst the female groups, except for the absolute weight of visceral fat in the control group which was significant lower ($p < 0.05$) than the others, but showed no significant difference when expressed relative to body weight (% BW) (Table 2.9).

Table 2.8: Gross measurements of small intestine (SI) and large intestine (LI) in male and female experimental rats fed NH and GS supplemented diets for 12 weeks.

Organ	Unit	Sex	CRF	GSL	NHL	GSH	NHH
SI	g	M	8.09 ± 0.30[a,c]	9.41 ± 0.57[b]	9.05 ± 0.20[b,c]	8.26 ± 0.13[a,c]	7.84 ± 0.30[a]
		F	6.42 ± 0.16*	6.58 ± 0.24*	6.70 ± 0.29*	6.50 ± 0.33*	6.19 ± 0.14*
	cm	M	131.75 ± 0.98[a,c]	140.75 ± 4.00[b]	138.33 ± 0.67[b,c]	128.00 ± 1.37[a]	130.50 ± 2.43[a,c]
		F	123.00 ± 2.22*	130.40 ± 3.84*	124.00 ± 2.06*	124.60 ± 2.90*	113.40 ± 4.86*
	g/cm	M	0.06 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00
		F	0.05 ± 0.00	0.05 ± 0.00*	0.05 ± 0.00*	0.05 ± 0.00*	0.06 ± 0.00
	% BW	M	1.86 ± 0.04	2.01 ± 0.05	1.96 ± 0.09	1.96 ± 0.05	1.92 ± 0.05
		F	2.45 ± 0.09*	2.27 ± 0.17	2.41 ± 0.07*	2.38 ± 0.06*	2.27 ± 0.08
LI	g	M	2.29 ± 0.11[a,c]	2.41 ± 0.11[b,c]	2.18 ± 0.11[a,c]	1.98 ± 0.07[a]	2.03 ± 0.06[a]
		F	1.60 ± 0.10	1.73 ± 0.08	1.80 ± 0.09	1.82 ± 0.11	1.67 ± 0.05
	cm	M	24.00 ± 0.33	23.92 ± 1.03	23.33 ± 0.69	24.33 ± 0.71	24.33 ± 0.42
		F	20.70 ± 0.73	20.60 ± 0.55	20.20 ± 0.53	20.90 ± 0.77	21.60 ± 0.86

Table 2.8 Continued

Organ	Unit	Sex	CRF	GSL	NHL	GSH	NHH
LI	g/cm	M	0.10 ± 0.01	0.10 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.08 ± 0.00
		F	0.08 ± 0.00	0.08 ± 0.00	0.09 ± 0.01	0.09 ± 0.00	0.08 ± 0.00
	% BW	M	0.53 ± 0.02	0.51 ± 0.02	0.47 ± 0.03	0.47 ± 0.02	0.50 ± 0.01
		F	0.61 ± 0.04	0.60 ± 0.05	0.65 ± 0.02	0.67 ± 0.03	0.61 ± 0.03

Length (cm), absolute (g) and relative (%BW) weights, and weight : length ratio calculated as g/cm of SI and LI in male and female experimental animals; data in the same row with different superscripts are significantly different ($p < 0.05$); * significantly different at $p < 0.05$ from male values.

Table 2.9: Absolute (g) and relative (%BW) weights of the abdominal visceral organs in male and female experimental rats fed NH and GS supplemented diets for 12 weeks.

Organ	Unit	Sex	CRF	GSL	NHL	GSH	NHH
Liver	g	M	11.16 ± 0.34	14.23 ± 0.56*	10.92 ± 0.32	12.57 ± 0.30*	10.24 ± 0.40
		F	7.02 ± 0.33$^{\beta}$	7.44 ± 0.34$^{\beta}$	7.49 ± 0.30$^{\beta}$	7.74 ± 0.41$^{\beta}$	7.20 ± 0.28$^{\beta}$
	% BW	M	2.57 ± 0.04	3.03 ± 0.15*	2.72 ± 0.13	2.57 ± 0.04	2.51 ± 0.06
		F	2.67 ± 0.10	2.55 ± 0.18$^{\beta}$	2.70 ± 0.06	2.83 ± 0.05	2.63 ± 0.04
Caecum	g	M	1.62 ± 0.06	1.45 ± 0.07	1.73 ± 0.05	1.38 ± 0.06	1.49 ± 0.09
		F	1.23 ± 0.08	1.15 ± 0.06	1.19 ± 0.04$^{\beta}$	1.18 ± 0.10	1.05 ± 0.04
	% BW	M	0.37 ± 0.01*	0.31 ± 0.01	0.38 ± 0.02*	0.33 ± 0.01	0.37 ± 0.02*
		F	0.46 ± 0.02	0.40 ± 0.04	0.43 ± 0.02	0.43 ± 0.03	0.39 ± 0.02
Stomach	g	M	2.31 ± 0.10	2.12 ± 0.08	2.46 ± 0.13	2.23 ± 0.09	2.33 ± 0.06
		F	1.85 ± 0.07	1.72 ± 0.04	1.77 ± 0.05	1.76 ± 0.10	1.61 ± 0.12
	% BW	M	0.54 ± 0.03	0.46 ± 0.02*	0.55 ± 0.02	0.52 ± 0.01	0.55 ± 0.02
		F	0.71 ± 0.03	0.60 ± 0.05	0.64 ± 0.02	0.64 ± 0.02	0.60 ± 0.06
Pancreas	g	M	1.80 ± 0.10	2.20 ± 0.16*	2.52 ± 0.11*	1.73 ± 0.12	2.25 ± 0.09*
		F	1.41 ± 0.08	1.66 ± 0.07	1.68 ± 0.13	1.51 ± 0.22	1.41 ± 0.10$^{\beta}$
	% BW	M	0.42 ± 0.03	0.47 ± 0.04	0.55 ± 0.03*	0.41 ± 0.03	0.56 ± 0.04*
		F	0.54 ± 0.03	0.57 ± 0.05	0.61 ± 0.04	0.54 ± 0.06	0.52 ± 0.04$^{\beta}$

Table 2.9 Continued

Organ	Unit	Sex	CRF	GSL	NHL	GSH	NHH
Spleen	g	M	1.16 ± 0.04	1.23 ± 0.06	1.25 ± 0.06	0.94 ± 0.04*	0.96 ± 0.04*
		F	0.93 ± 0.03	0.87 ± 0.01	0.91 ± 0.02	0.88 ± 0.07	0.78 ± 0.02
	% BW	M	0.27 ± 0.01	0.26 ± 0.01	0.27 ± 0.02	0.22 ± 0.01*	0.23 ± 0.01
		F	0.36 ± 0.02	0.30 ± 0.02	0.33 ± 0.01	0.32 ± 0.02β	0.29 ± 0.01
Kidneys	g	M	2.99 ± 0.07	3.21 ± 0.11	3.11 ± 0.13	2.56 ± 0.08*****	2.53 ± 0.07*****
		F	1.96 ± 0.07$^{\beta}$	1.91 ± 0.05$^{\beta}$	1.95 ± 0.08$^{\beta}$	1.95 ± 0.10$^{\beta}$	1.82 ± 0.05$^{\beta}$
	% BW	M	0.69 ± 0.01	0.68 ± 0.02	0.68 ± 0.05	0.60 ± 0.01	0.62 ± 0.01
		F	0.75 ± 0.03	0.66 ± 0.05	0.70 ± 0.00	0.72 ± 0.03	0.67 ± 0.03
Visceral fat	g	M	8.56 ± 0.50	17.70 ± 0.73*	11.11 ± 0.37	15.48 ± 0.85*	10.21 ± 1.04
		F	6.68 ± 0.66*	10.25 ± 1.00$^{\beta}$	8.17 ± 0.66	10.09 ± 1.32$^{\beta}$	9.20 ± 0.92
	% BW	M	1.97 ± 0.13	3.75 ± 0.14*	2.39 ± 0.07	3.64 ± 0.14*	2.48 ± 0.21
		F	2.56 ± 0.28	3.48 ± 0.34	2.96 ± 0.26	3.65 ± 0.36	3.39 ± 0.38

* significantly different ($p < 0.05$) along the row; $^{\beta}$ significantly different at $p < 0.05$ from male values.

2.2.6 – Histological assessment of the small intestine (jejunum) and the liver

Microscopic examinations of the small intestinal villi and crypts showed reduced height and width of the villi, and crypt depth, as well as the SI villus height : crypt depth ratio in the male rats fed cane syrup (GSL and GSH), relative to the NHL group (Table 2.10). However, these parameters did not differ ($p > 0.05$) between the GS-fed male rats and control animals, except the SI villus width which was reduced.

The female rats on the low dose diets (GSL and NHL) had increased ($p < 0.05$) SI villus height and crypt depth compared to those on the high dose-diets (GSH and NHH) and control rats (Table 2.10). The honey fed female rats had similar SI villus width compared to the control (CRF) rats, but higher SI villus width ($p < 0.05$) compared to the GS-fed female rats; whilst the SI villus height : crypt depth ratio did not differ ($p > 0.05$) amongst the female rats.

The hepatic changes observed under the light microscope were also recorded in Table 2.10. The representative photomicrographs of the histological sections showing the histopathology and the normal hepatic cytology of the experimental animals were presented graphically in Figures 2.11 – 2.14. The rats fed CRF and those fed NH had liver sections showing normal cytology however, there were various degrees of fatty degeneration in hepatic sections of GS-fed animals (Table 2.10). The histological sections also showed aggregation or accumulation of Kupffer cells in the livers of cane syrup fed rats (Figures 2.13 and 2.14).

Table 2.10: Histological characteristics of the small intestine (SI) and the liver in male and female experimental rats fed NH and GS supplemented diets for 12 weeks.

Measurements	Sex	CRF	GSL	NHL	GSH	NHH
SI villus height (µm)	M	63.5 ± 3.6[a]	65.0 ± 5.4[a]	84.0 ± 4.0[b]	60.2 ± 0.7[a]	64.8 ± 1.1[a]
	F	45.5 ± 1.5[a]*	54.2 ± 1.0[b]	56.8 ± 0.8[b]*	45.8 ± 1.2[a]*	47.2 ± 2.0[a]*
SI villus width (µm)	M	16.8 ± 0.3[a]	12.3 ± 1.0[b]	25.2 ± 1.5[c]	11.7 ± 0.8[b]	16.8 ± 0.9[a]
	F	16.0 ± 0.9[a]	11.5 ± 0.6[b]	18.8 ± 0.6[c]*	12.0 ± 0.6[b]	17.2 ± 0.8[a,c]
SI crypt depth (µm)	M	20.2 ± 1.0[a]	20.5 ± 1.5[a]	24.0 ± 1.0[b]	19.8 ± 0.5[a]	20.8 ± 0.5[a]
	F	21.8 ± 1.4[a]	26.0 ± 1.2[b]*	27.5 ± 1.1[b]	20.0 ± 1.1[a]	22.2 ± 0.6[a]
SI villus height/ crypt depth	M	3.2 ± 0.1[a]	3.2 ± 0.2[a]	3.5 ± 0.1[b]	3.1 ± 0.1[a]	3.1 ± 0.1[a]
	F	2.1 ± 0.1*	2.1 ± 0.1*	2.1 ± 0.1*	2.3 ± 0.1*	2.1 ± 0.1*

Table 2.10 Continued

Measurements	Sex	CRF	GSL	NHL	GSH	NHH
Liver histo-morphology	M	0.7 ± 0.2^{a}	3.7 ± 0.3^{b}	1.2 ± 0.3^{a}	3.3 ± 0.3^{b}	1.3 ± 0.4^{a}
	F	0.7 ± 0.2^{a}	3.8 ± 0.2^{b}	1.2 ± 0.3^{a}	3.9 ± 0.2^{b}	1.2 ± 0.3^{a}

Data in the same row with different superscripts are significantly different ($p < 0.05$); * significantly different at $p < 0.05$ from male values; CRF = commercial rat feed; GSL = golden syrup low; NHL = natural honey low; GSH = golden syrup high; NHH = natural honey high.

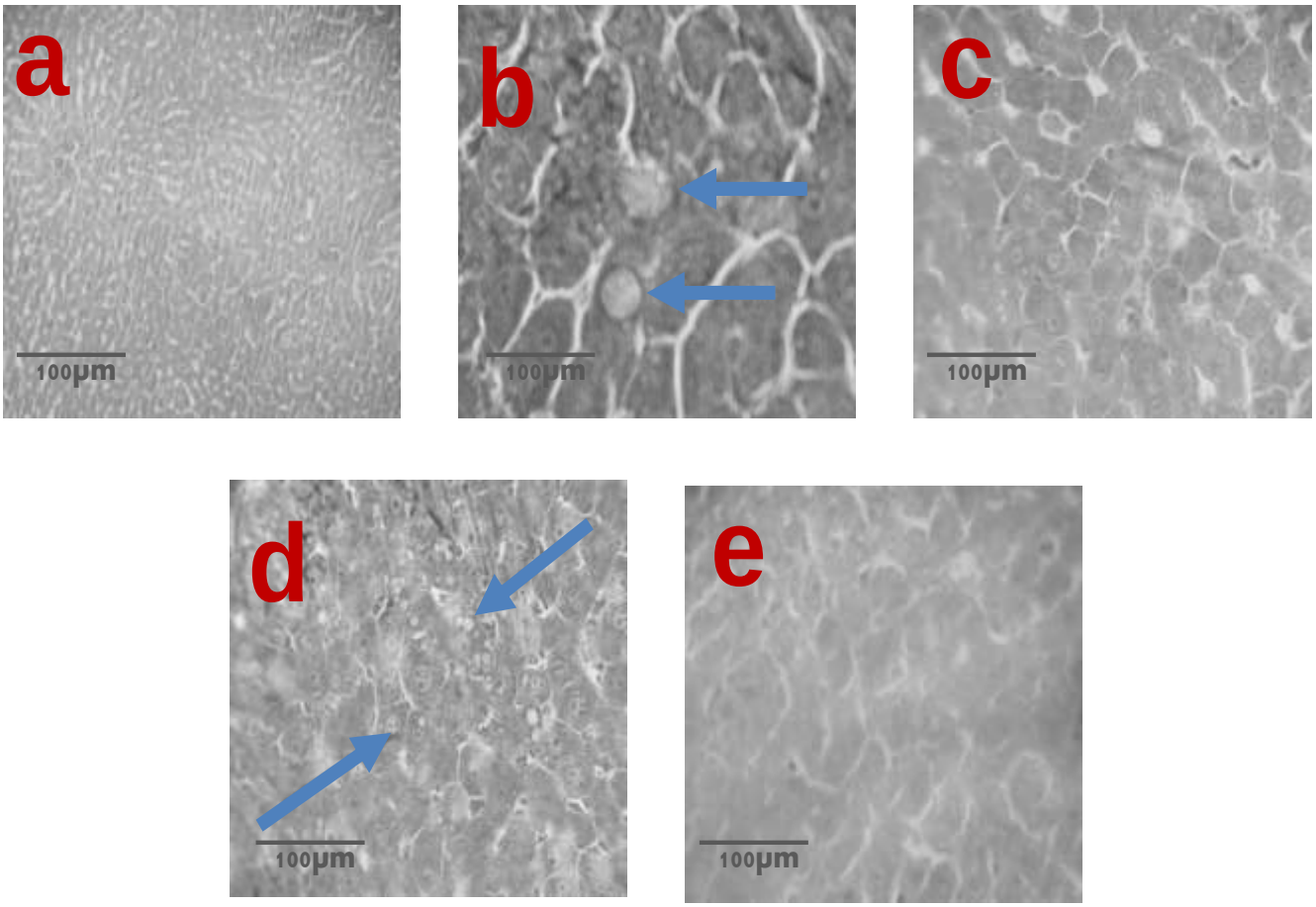


Figure 2.11 Photomicrographs of the livers (Haematoxylin & Eosin stain) from male rats fed NH and GS supplemented diets for 12 weeks, Scale bar is 100µm: (a) CRF, Control, 10 ml/kg Distilled water pre-weaning and Commercial rat feed mixed with 20 % tap water post weaning; (b) GSL, Golden syrup Low, 10 ml/kg golden syrup (GS) pre-weaning and 20 % GS mixed with rat feed post weaning; (c) NHL, Natural honey Low, 10 ml/kg natural honey (NH) pre-weaning and 20 % NH mixed with rat feed post weaning; (d) GSH, Golden syrup High, 20 ml/kg GS pre-weaning and 50 % GS mixed with rat feed post weaning; (e) NHH, Natural honey High, 20 ml/kg NH pre-weaning and 50 % NH mixed with rat feed post weaning; magnification (mag) of control liver is 100 x, while treatment groups' livers mag is 400 x; Arrows showed fat droplets in GSL liver and fatty degeneration in GSH liver.

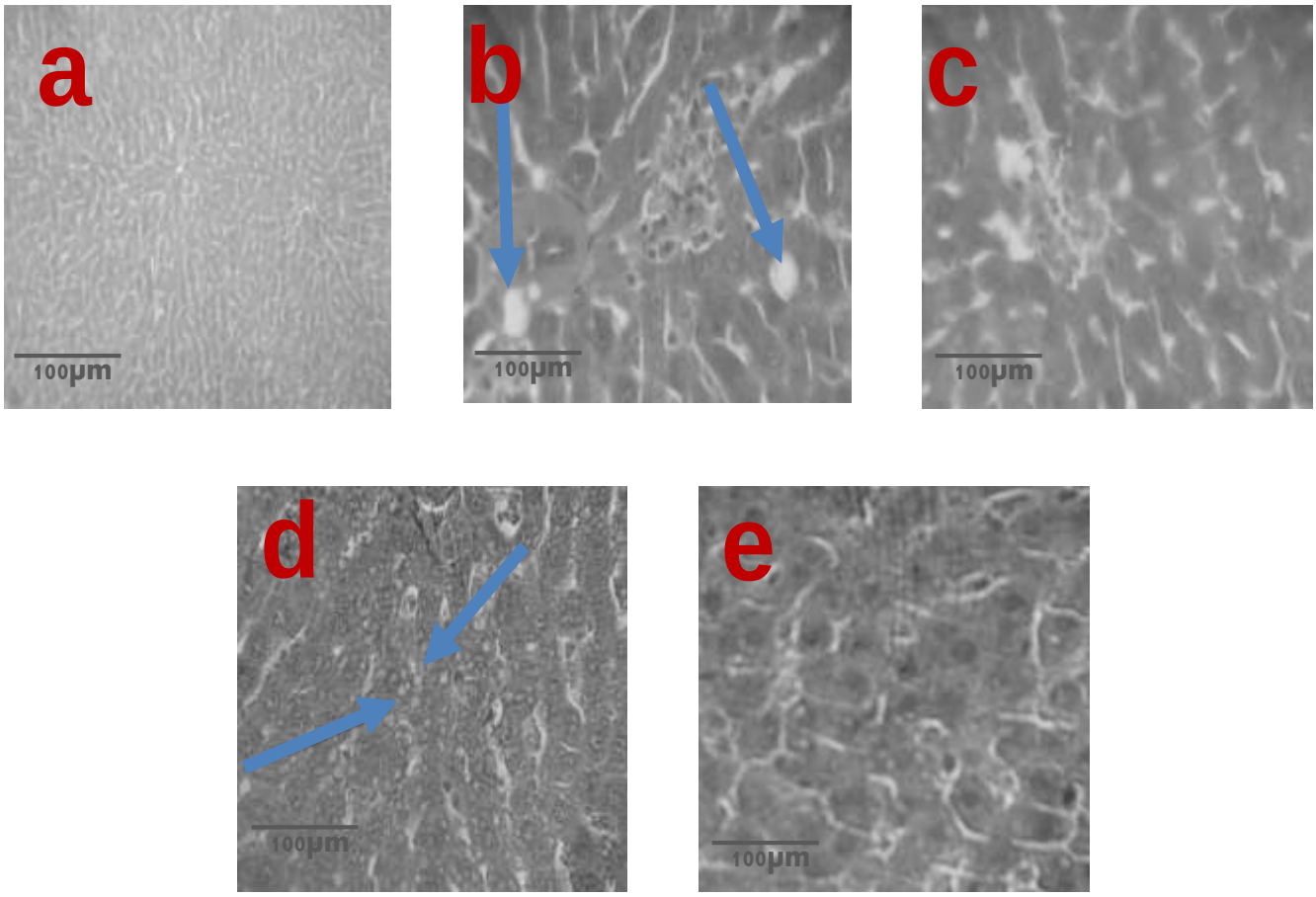


Figure 2.12 Photomicrographs of the livers (Haematoxylin & Eosin stain) from female rats fed NH and GS supplemented diets for 12 weeks, Scale bar is 100µm: (a) CRF, Control, 10 ml/kg Distilled water pre-weaning and Commercial rat feed mixed with 20 % tap water post weaning; (b) GSL, Golden syrup Low, 10 ml/kg golden syrup (GS) pre-weaning and 20 % GS mixed with rat feed post weaning; (c) NHL, Natural honey Low, 10 ml/kg natural honey (NH) pre-weaning and 20 % NH mixed with rat feed post weaning; (d) GSH, Golden syrup High, 20 ml/kg GS pre-weaning and 50 % GS mixed with rat feed post weaning; (e) NHH, Natural honey High, 20 ml/kg NH pre-weaning and 50 % NH mixed with rat feed post weaning; magnification (mag) of control liver is 100 x, while treatment groups' livers mag is 400 x; Arrows showed fat droplets in GSL liver and fatty degeneration in GSH liver.

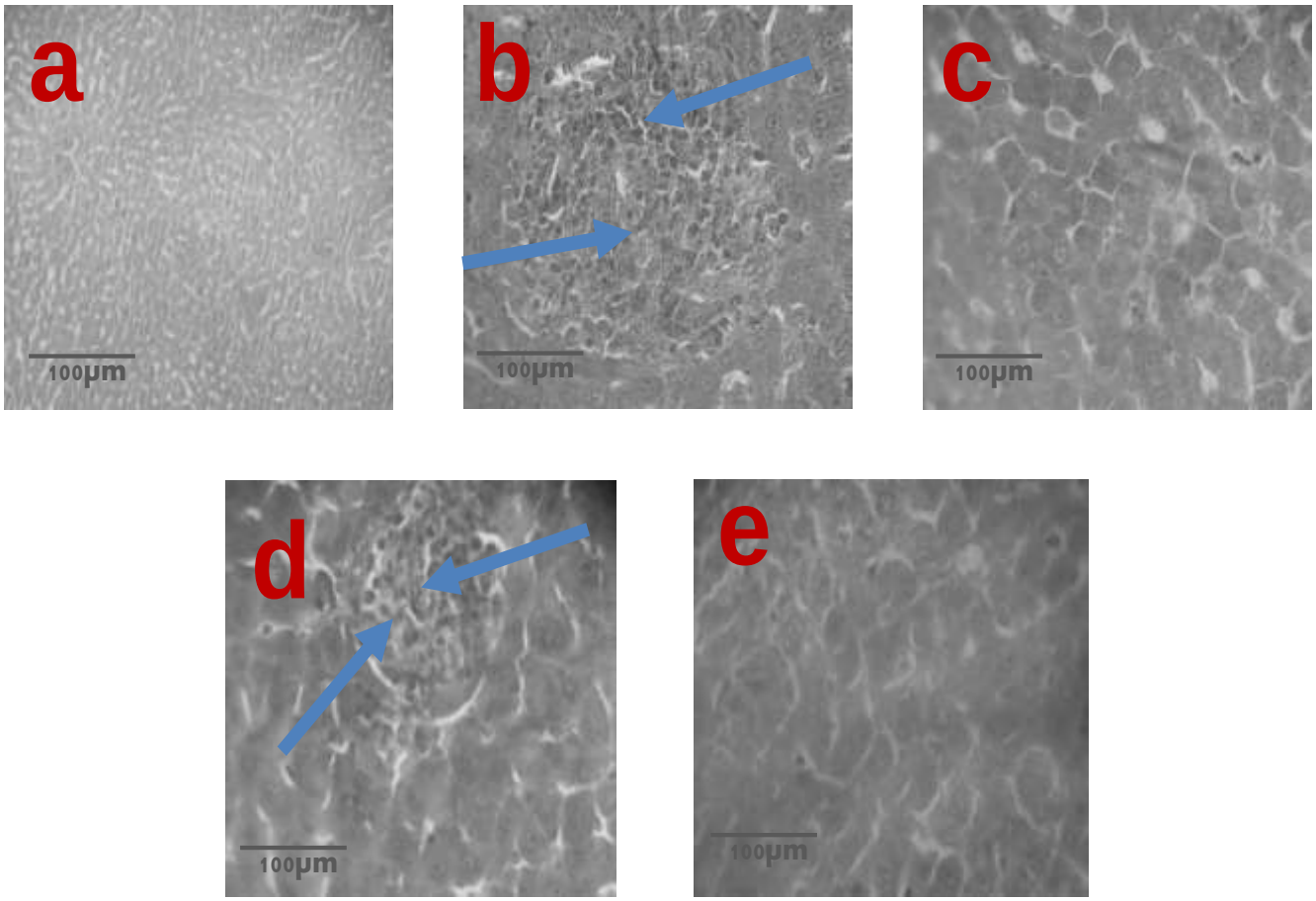


Figure 2.13 Photomicrographs of the livers (Haematoxylin & Eosin stain) from male rats fed NH and GS supplemented diets for 12 weeks showing the liver architecture, Scale bar is 100µm: (a) CRF, Control, 10 ml/kg Distilled water pre-weaning and Commercial rat feed mixed with 20 % tap water post weaning; (b) GSL, Golden syrup Low, 10 ml/kg golden syrup (GS) pre-weaning and 20 % GS mixed with rat feed post weaning; (c) NHL, Natural honey Low, 10 ml/kg natural honey (NH) pre-weaning and 20 % NH mixed with rat feed post weaning; (d) GSH, Golden syrup High, 20 ml/kg GS pre-weaning and 50 % GS mixed with rat feed post weaning; (e) NHH, Natural honey High, 20 ml/kg NH pre-weaning and 50 % NH mixed with rat feed post weaning; magnification (mag) of control liver is 100 x, while treatment groups' livers mag is 400 x; Arrows showed the Kupffer cells accumulation around the central vein due to inflammation in GSL and GSH livers.

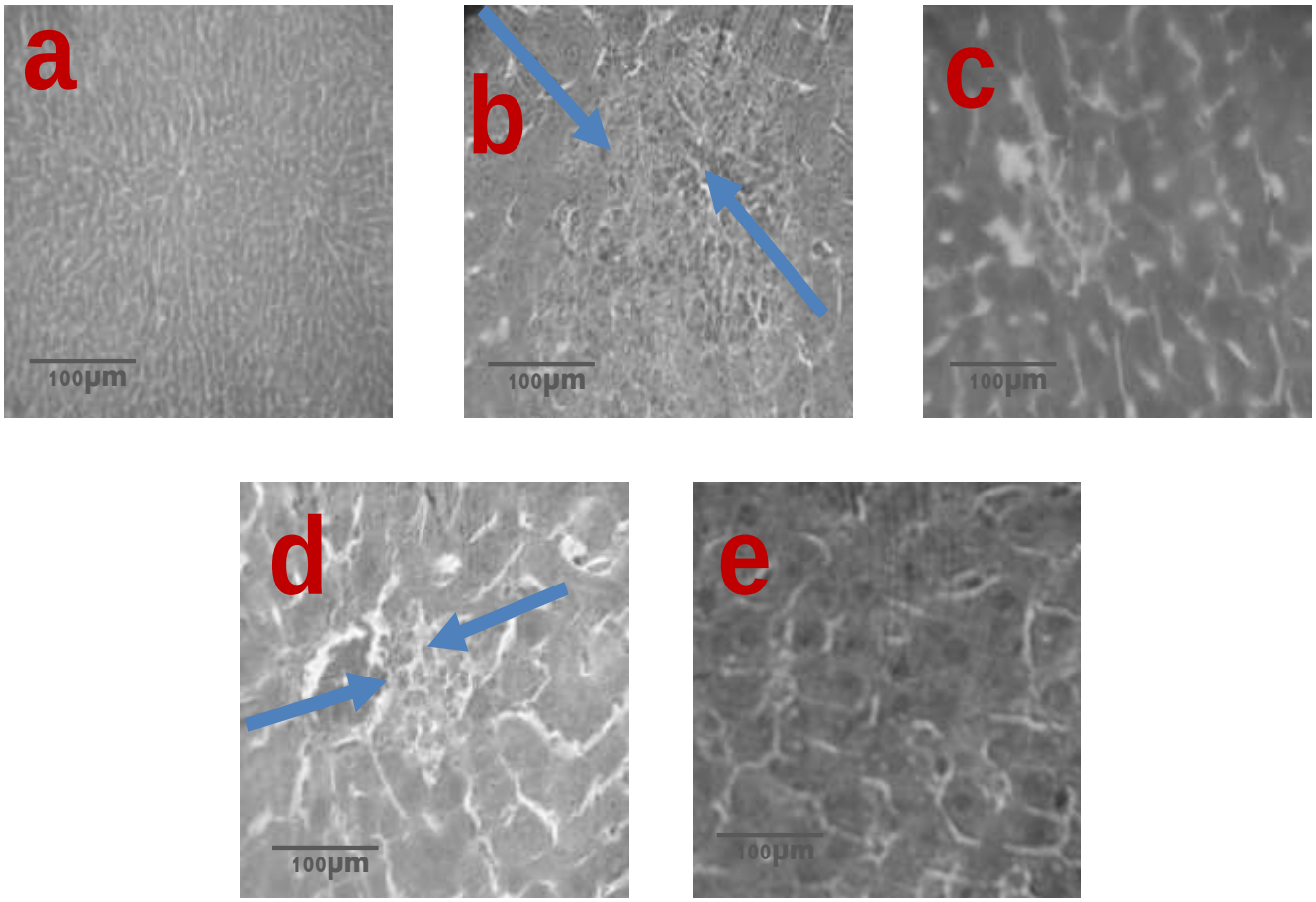


Figure 2.14 Photomicrographs of the livers (Haematoxylin & Eosin stain) from female rats fed NH and GS supplemented diets for 12 weeks showing the liver architecture, Scale bar is 100µm: (a) CRF, Control, 10 ml/kg Distilled water pre-weaning and Commercial rat feed mixed with 20 % tap water post weaning; (b) GSL, Golden syrup Low, 10 ml/kg golden syrup (GS) pre-weaning and 20 % GS mixed with rat feed post weaning; (c) NHL, Natural honey Low, 10 ml/kg natural honey (NH) pre-weaning and 20 % NH mixed with rat feed post weaning; (d) GSH, Golden syrup High, 20 ml/kg GS pre-weaning and 50 % GS mixed with rat feed post weaning; (e) NHH, Natural honey High, 20 ml/kg NH pre-weaning and 50 % NH mixed with rat feed post weaning; magnification (mag) of control liver is 100 x, while treatment groups' livers mag is 400 x; Arrows showed the Kupffer cells accumulation around the central vein due to inflammation in GSL and GSH livers.

2.2.7 – Lipid and glycogen content of the liver

The values of the metabolic substrates stored in the liver (lipid and glycogen) were recorded in Table 2.11. The lipid content of the liver in GS-fed male rats was higher ($p < 0.05$) at both dietary levels (low and high-diet doses) than in other male rats, whilst no significant difference ($p > 0.05$) was recorded amongst the females. There was no significant difference ($p > 0.05$) in the hepatic glycogen content amongst the male and female rats and similarly no gender differences manifested (Table 2.11).

Table 2.11: Liver lipid and glycogen content (as glucose equivalent) in male and female rats fed NH and GS supplemented diets for 12 weeks.

Diet	**Sex**	**Liver lipid** (% Liver weight)	**Liver glycogen** (as glucose equivalents) (mmol/L)
CRF	M	4.58 ± 0.33	4.55 ± 0.64
	F	4.36 ± 0.21	3.90 ± 0.23
GSL	M	6.32 ± 0.14*	5.32 ± 0.27
	F	5.11 ± 0.13$^{\beta}$	3.35 ± 0.28
NHL	M	4.59 ± 0.27	4.85 ± 1.10
	F	4.37 ± 0.14	3.75 ± 0.39
GSH	M	5.94 ± 0.23*	4.85 ± 0.56
	F	5.11 ± 0.25$^{\beta}$	3.35 ± 0.21

Table 2.11 Continued

Diet	**Sex**	**Liver lipid** (% Liver weight)	**Liver glycogen** (as glucose equivalents) (mmol/L)
NHH	M	4.93 ± 0.14	4.15 ± 1.03
	F	4.79 ± 0.23	3.96 ± 0.40

* significantly different ($p < 0.05$) across the column; $^{\beta}$ significantly different ($p < 0.05$) from corresponding male values; CRF = commercial rat feed; GSL = golden syrup low; NHL = natural honey low; GSH = golden syrup high; NHH = natural honey high.

2.2.8 – General health profiles

The data obtained from surrogate plasma markers of the liver function are summarised in Table 2.12 whilst the other clinical biochemistry parameters showing the general health profiles of the animals are documented in Table 2.13. The plasma concentrations of the key liver marker enzymes (AST and ALT) of the cane syrup fed, especially GS male rats were higher ($p < 0.05$) than the values obtained from the control (CRF) rats (Table 2.12). In addition, the values of alanine transaminase of the GS-fed rats were even higher compared to the upper limits of the normal range (Table 2.12). The values of the other liver marker enzymes (ALP and T bil) of the cane syrup fed, and all liver enzymes parameters obtained from the NH-fed rats were within the normal ranges (Table 2.12). 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 (Tables 2.12 – 2.14). These were total bilirubin, calcium, phosphorus, total protein, globulin and creatinine.

Table 2.12: Plasma concentrations /activities of surrogate markers of liver function in male and female rats fed natural honey and cane syrup supplemented diets for 12 weeks.

Diet	**Sex**	**AST** (U/L)	**ALT** (U/L)	**ALP** (U/L)	**T bil** (µmol/L)
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

Table 2.12 Continued

Diet	**Sex**	**AST** (U/L)	**ALT** (U/L)	**ALP** (U/L)	**T bil** (µmol/L)
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
nr§	*M*	*55 – 96*	*23 – 32*	*92 – 265*	*6.7 – 12*
	F	*58 – 99*	*21 – 28*	*56 – 152*	*6.7 – 12*

* significantly different ($p < 0.05$) in males across the column; $^{\beta}$ significantly different ($p < 0.05$) among females; $^{\alpha}$ significantly different ($p < 0.05$) from male values; AST = aspartate transaminase; ALT = alanine transaminase; ALP = alkaline phosphatase; T bil = total bilirubin; CRF = commercial rat feed; GSL = golden syrup low; NHL = natural honey low; GSH = golden syrup high; NHH = natural honey high; nr = normal range of values; § adapted from Kurata *et al*, 2002.

Table 2.13: Surrogate markers of general health assayed in the plasma of the male and female rats fed NH and GS supplemented diets for 12 weeks.

Diet	**Sex**	**Ca** (mmol/L)	**Phos** (mmol/L)	**TP** (g/L)	**Albumin** (g/L)	**Glob** (g/L)	**Amylase** (U/L)
CRF	M	2.4 ± 1.00	2.4 ± 0.04	58.8 ± 1.11	27.7 ± 0.67	31.2 ± 0.87	1024.5 ± 81.2
	F	2.5 ± 0.17	2.2 ± 0.22	64.0 ± 3.65	29.2 ± 4.39	34.8 ± 2.82$^{\alpha}$	672.3 ± 69.0$^{\alpha}$
GSL	M	2.1 ± 0.09	2.6 ± 0.16	62.5 ± 1.57	31.0 ± 2.73	31.0 ± 1.46	1021.5 ± 139.4
	F	2.3 ± 0.12	2.2 ± 0.21	62.8 ± 2.64	29.2 ± 1.78	33.8 ± 2.2$^{\alpha}$	774.0 ± 50.6$^{\alpha}$
NHL	M	2.8 ± 0.04	2.4 ± 1.00	59.7 ± 0.67	29.0 ± 0.37	30.8 ± 0.70	1083.0 ± 57.6
	F	2.5 ± 0.05	2.0 ± 0.08	60.3 ± 1.26	27.8 ± 1.76	32.3 ± 0.99$^{\alpha}$	867.3 ± 60.1
GSH	M	2.2 ± 0.06	2.5 ± 0.07	58.5 ± 0.56	27.3 ± 0.49	31.2 ± 0.60	1354.8 ± 85.4
	F	2.4 ± 0.03	2.2 ± 0.09	61.3 ± 2.20	29.0 ± 3.30	32.2 ± 1.40$^{\alpha}$	1016.8 ± 31.6$^{\alpha}$

Table 2.13 Continued

Diet	**Sex**	**Ca** (mmol/L)	**Phos** (mmol/L)	**TP** (g/L)	**Albumin** (g/L)	**Glob** (g/L)	**Amylase** (U/L)
NHH	M	2.5 ± 0.05	2.4 ± 0.05	57.7 ± 1.09	26.5 ± 0.89	31.2 ± 0.60	1267.7 ± 68.2
	F	2.5 ± 0.09	2.0 ± 0.13	62.6 ± 2.42	27.4 ± 3.49	35.2 ± 3.07$^{\alpha}$	894.4 ± 18.6$^{\alpha}$
nr[§]	*M*	*2.4 – 2.9*	*1.7 – 2.7*	*52 – 63*	*26 – 35*	*26 – 32*	*1003 – 1406*
	F	*2.4 – 2.8*	*1.7 – 2.7*	*55 – 65*	*26 – 31*	*32 – 36*	*653 – 1027*

$^{\alpha}$ significantly different ($p < 0.05$) from corresponding male values; Ca = calcium; Phos = phosphorus; TP = total protein; Glob = globulin; CRF = commercial rat feed; GSL = golden syrup low; NHL = natural honey low; GSH = golden syrup high; NHH = natural honey high; nr = normal range of values; [§] adapted from Kurata *et al*, 2002.

Table 2.14: Plasma concentrations of urea, creatinine and urea/creatinine ratio in the male and female rats fed NH and GS supplemented diets for 12 weeks.

Diet	**Sex**	**Urea** (mg/dL)	**Creatinine** (mg/dL)	**Urea/creatinine ratio**
CRF	M	16.25 ± 0.83	0.54 ± 0.05	30.1 ± 3.02
	F	18.65 ± 1.03$^{\beta\alpha}$	0.63 ± 0.03$^{\alpha}$	29.6 ± 1.51
GSL	M	16.18 ± 0.94	0.56 ± 0.02	28.9 ± 1.55
	F	14.50 ± 1.00	0.58 ± 0.03	25.0 ± 1.33
NHL	M	15.01 ± 0.69	0.56 ± 0.01	26.8 ± 1.57
	F	14.15 ± 0.67	0.53 ± 0.04	26.7 ± 2.64
GSH	M	11.96 ± 1.28*	0.46 ± 0.02	26.0 ± 1.91
	F	14.95 ± 0.64$^{\alpha}$	0.61 ± 0.05	24.5 ± 2.11

Table 2.14 Continued

Diet	**Sex**	**Urea** (mmol/L)	**Creatinine** (µmol/L)	**Urea/creatinine ratio**
NHH	M	12.00 ± 0.56*	0.48 ± 0.03	25.0 ± 2.10
	F	14.79 ± 0.94$^{\alpha}$	0.55 ± 0.04	26.9 ± 2.89
nr[§]	*M*	*12 – 24*	*0.4 – 0.6*	*25.0 – 45.0*
	F	*11 – 28*	*0.4 – 0.7*	*21.7 – 46.7*

***** significantly different ($p < 0.05$) in males across the column; $^{\beta}$ significantly different among females ($p < 0.05$). $^{\alpha}$ significantly different ($p < 0.05$) from corresponding male values; CRF = commercial rat feed; GSL = golden syrup low; NHL = natural honey low; GSH = golden syrup high; NHH = natural honey high; nr = normal range of values; [§] adapted from Hilltop Lab Animals Inc, 2013.

2.3 – Discussion

The results presented constitute the first study on the long-term effects of orally administered natural honey compared with cane syrup on metabolism in growing male and female SD rats fed NH and GS supplemented diets starting from pre-weaning (7-day old) stage. The nutritional benefits and health importance of eating natural honey is extensively documented in the literature [Jones, 2001; Ramenghi *et al*, 2001; Busserolles *et al*, 2002a; Gharzouli *et al*, 2002; Mahgoub *et al*, 2002; Al-Quassemi and Robinson, 2003; Schramm *et al*, 2003; Al-Waili and Haq, 2004; English *et al*, 2004; Yahya, 2005; Al-Waili *et al*, 2006; Nasuti *et al*, 2006; Ajibola *et al*, 2007]. Some other workers reporting the beneficial values of honey consumption include Kilicoglu *et al,* 2008; Prakash *et al,* 2008; Yaghoobi *et al,* 2008; Bahrami *et al*, 2009; Chepulis *et al,* 2009; Korkmaz and Kolankaya, 2009; Munstedt *et al,* 2009; Alvarez-Suarez *et al*, 2010b; Cortés *et al*, 2011; Alagwu *et al*, 2011 and Erejuwa *et al*, 2012. The consumption of refined sugars provides energy for metabolic activities, but the prolonged intake is associated with various health problems [Johnson and Fray, 2001; Ludwig *et al*, 2001; Astrup *et al*, 2002; Elliott *et al*, 2002; Bray *et al,* 2004; Schulze *et al,* 2004; Gaby, 2005; Havel, 2005; Cao *et al*, 2007]. There are also other studies documenting the health hazards associated with excess intake of artificial and refined sugars, and these include Dubois *et al*, 2007; Johnson *et al,* 2007; Promdee *et al,* 2007; Rutledge and Adeli, 2007; Chepulis and Starkey, 2008; Couchepin *et al*, 2008; Miller and Adeli, 2008 ; Ruottinen *et al*, 2008; Teff *et al*, 2009; Tappy and Le, 2010. I hypothesized that the dietary supplementation with natural honey would provide energy for metabolism and improve growth without causing metabolic diseases or compromising health. Hence, the aim was to show that consumption of honey even from an early (pre-

weaning) stage in the long term is more nutritionally beneficial and healthier than eating processed and refined sugars such as HFCS and cane syrup.

The records on the health implications of eating fructose in form of HFCS, including its role in MetS pathogenesis abounds in scientific reports [Johnson and Fray, 2001; Ludwig *et al*, 2001; Bray *et al,* 2004; Schulze *et al,* 2004; Gaby, 2005; Havel, 2005; Johnson *et al,* 2007; Melanson *et al,* 2007; Rutledge and Adeli, 2007; Miller and Adeli, 2008; Teff *et al*, 2009; Tappy and Le, 2010]. However, there is scanty information on the effects of chronic consumption of cane syrup, a common dietary source of fructose in the developing countries [Tappy and Le, 2010; Samuel, 2011]. Thus, the study of the effects of prolonged eating of cane syrup becomes imperative due to this dearth of scientific information. The present study began with the establishment of animal models as representatives of human metabolic syndrome. This was done by feeding SD rats with two (low and high) doses of cane syrup from infancy to adult stage. The metabolic response of the rats to these dietary treatments at 91 days old was compared with the performance of similar groups nurtured simultaneously with two matched iso-caloric and iso-nitrogenous doses of NH supplemented diets. This facilitated the study of the effects of cane syrup compared with that of natural honey. Then, one could ascertain the protective roles of NH against MetS amidst the other several nutritional and health advantages of this functional liquid food (NH) over refined sugars. Thus, one could propose it (NH) as a source of dietary fructose for digestive and metabolic health, and also as substitute to the refined sugars such as cane syrup.

2.3.1 – Dietary treatments, Nutrients intake and Growth response

There was no disparity in the pups' dietary treatments as all the neonatal rats were fed sugar solutions with similar nutritional values, except for the higher amount of sucrose (26.03g/100g) in GS than NH (2.03g/100g). Consequently, GS pups were fed higher amount of dietary sucrose (Table 2.2). In addition, although not assayed in the diets used for this study, NH has been shown to contain minerals, and other micronutrients as shown in Tables 1.1 and 1.2 [White and Doner 1980]. These could account for the higher value of the undetermined (or other) nutrients of NH than that of GS. These were shown in Table 2.2 containing the proximate analyses of the undiluted honey and golden syrup used as dietary supplements in this study. All the diets fed to the rats at the adult stage were iso-caloric (except NHH with higher energy value), and dose-matched diets were iso-nitrogenous. Thus, the dietary treatments did not confer macro-nutritional advantage of one group over the other. However, the higher amount of sucrose consumed by the rats on GS-supplemented diets relative to the honey eaters (Tables 2.2 and 2.4) could expose these GS-fed rats to metabolic risks such as insulin resistance, diabetes mellitus, dyslipidaemia and obesity [Johnson and Fray, 2001; Ludwig *et al*, 2001; Betts *et al*, 2005; Cao *et al*, 2007]. In addition, eating excess sucrose hinders protein and calcium intake and utilization [Promdee *et al*, 2007; Ruottinen *et al*, 2008], with consequent poor bone mineralization [Tjaderhane and Larmas 1998], leading to growth retardation [Ruottinen *et al*, 2008]. The observations of reduced terminal BW of the GS-fed male rats (Figures 2.2) in the current study confirmed these earlier findings. Unlike, the GS-fed rats, the rats nurtured with the NH supplemented diets enjoyed normal growth and did not appear to suffer any metabolic abnormality.

The estimated feed intake showed preferential higher feed consumption by the low dose-diet groups than their high dose-diet counterparts. This differential feed intake by the experimental animals in these (low dose-diet) groups was possibly due to the preference for solid particulate substance in low-dose diets, against the pasty nature of high-dose diets. However, I did not test the dietary constituents' quality selectivity and feeding behaviour. Nonetheless, the preferential dietary intake by rats has been reported by other workers [Davis *et al*, 1976; Sieck *et al*, 1978]. The low viscosity of the high dose diets might have also influenced the pattern of feed intake, as one author opines that feed viscosity influences calorific intake [Davidson, 2005]. Another probable explanation is that these animals regulate their calorific intake based on fast-acting specific gustatory signals [Prabhakar and Rao, 1985].One author postulates that early life events influence life-long patterns of behavioural and physiological responsiveness [McEwen, 2007], thus the preferential consumption of low dose diets by the experimental rats could have resulted from the introduction of the dietary treatments to the rats in their neonatal period. In addition, it has been suggested that sweet receptors evolved in ancestral environments poor in sugars in most mammals including rats, and are thus not adapted to high concentrations of sweet tastants. [Lenoir *et al*, 2007]. Hence, these rats might have evoked some self-control mechanisms due to supra-normal stimulation of some receptors in order to deal with dose dependency and avoid over consumption of the high dose diets. This assumption is supported by the hypotheses of some workers in the field of neuroscience that early life experience has influence on physiological and behavioural patterns [Carr and Wolinsky, 1993; Berridge, 1996; Drewnowski, 1997; Sclafani, 2004].

Table sugar processed from sugarcane or sugar beet contains glucose and fructose in the ratio 1:1 (that is about 50 % of each monosaccharide), when refined [Anderson, 1995]. It has been established that honey contains about 53 – 60 % fructose [White and Doner, 1980; Bogdanov *et al*, 2008], which is higher than the 50 % fructose level in the processed refined sugars [Anderson, 1995; White and Doner, 1980; Bogdanov *et al*, 2008]. The calculation of the fructose quantity from the results of the proximate analyses of the artificial (GS) and natural (NH) sugars used as dietary supplements in the current study gave 49.2 % and 54.7 % respectively. These values were within the established ranges of the fructose levels expected to be obtained from these dietary sources [Anderson, 1995; White and Doner, 1980; Bogdanov *et al*, 2008]. As expected, after the GS and NH were added to the commercial rat feed to produce the treatment diets, the NH supplemented diets had significantly higher ($p < 0.05$) fructose contents than the GSL and control (CRF) diets (Table 2.4). Despite the high fructose content of the NH supplemented diets, and the known culpability of fructose in the epidemiology and pathogenesis of MetS [Johnson and Fray, 2001; Ludwig *et al*, 2001; Bray *et al,* 2004; Schulze *et al,* 2004; Gaby, 2005; Havel, 2005; Johnson *et al,* 2007; Melanson *et al,* 2007; Rutledge and Adeli, 2007; Miller and Adeli, 2008; Teff *et al*, 2009; Tappy and Le, 2010], it is interesting that the NH-fed rats appeared not to show any sign of metabolic dysfunction. This suggested that the amount of fructose eaten might not alone be enough to cause metabolic diseases, but the form in which the fructose is fed, as well as the other constituents in the diet may be also important factors.

Unlike the refined sugars containing only mainly the sugars: fructose, glucose and sucrose, honey has several other nutritional and healthy constituents [White and Doner, 1980; Al-Quassemi and Robinson, 2003; Bogdanov *et al*, 2008; Ajibola *et al*, 2012]. In addition, contrary to the fructose moiety of refined sugars which existed singly in isolation, the fructose in honey is often found as fructooligosaccharides (FOS), a prebiotic, which is not only devoid of any disease traits, but also helpful in promoting digestive functions of the GIT [Yun, 1996;]. In their extensive search for a natural cure for diabetes mellitus, Erejuwa and co-workers opine that 'Fructose might contribute to the hypoglycemic effect of honey' [Erejuwa *et al*, 2010, 2011a, b; 2012b; Erejuwa, 2012]. They also suggested in a review that Oligosaccharides might contribute to the antidiabetic effect of honey [Erejuwa *et al*, 2012a]. Other workers also align with the hypothesis that the fructose found in honey also enhances the protective properties of this natural liquid food [Al-Quassemi and Robinson, 2003; Bahrami *et al*, 2009; Alvarez-Suarez *et al*, 2010b; Cortés *et al*, 2011]. Honey also contains minerals, proteins, amino acids, phytochemicals and other micronutrients (Tables 1.3 – 1.5), that are not found in the cane syrup. These substances in synergy with the various sugars in honey could provide the required energy to drive metabolic activities, as well as facilitate the protective functions. Hence, these substances are collectively referred to as 'SMIF' meaning Synergistic Multiple Ingredients Factor. In addition, NH belongs to a class of foods known as glycaemic foods with the ability to control the blood glucose concentration [Truswell, 1992; Venn and Green, 2007].

The results of the proximate analyses of the dietary supplements used in the current study showed that NH and GS contained similar high concentrations of

glycaemic sugars (Table 2.2). In the same vein, the proximate analyses of the treatment diets yielded feeds with similar high concentrations of glycaemic sugars, except NHH diet containing significantly higher % ($p < 0.05$) of glycaemic sugars than all the treatment and the control diets. Although, there was no significant difference in the amount of the glycaemic sugars found in the NH and GS used in my study (Table 2.2), the presence of high level of these antidiabetic agents in this natural liquid food (NH) attest to the hypoglycaemic property of honey [White and Doner, 1980; Shambaugh *et al*, 1990; Al-Quassemi and Robinson, 2003; Al-Waili, 2004; Yaghoobi *et al*, 2008; Bahrami *et al*, 2009; Alvarez-Suarez *et al*, 2010b; Cortés *et al*, 2011; Erejuwa *et al*, 2010, 2011a, b; 2012b; Erejuwa, 2012].

Natural honey contains soluble fibre with prebiotic effects [White and Doner, 1980; Steinberg *et al*, 1996; Schley and Field, 2002; Parnell and Reimer, 2009], whilst the acid detergent fibre (ADF) and neutral detergent fiber (NDF) often measured during digestibility trials are insoluble/non fermentable fibres [Cheeke and Patton, 1980; Ajibola, 2000; Garci´a *et al*, 2000; Gidenne, 2003]. These fibres (ADF and NDF) constitute the plant materials (cereals) used in producing commercial animal feeds [Cheeke and Patton, 1980]. Generally, ADF is a laboratory estimate of the less digestible fibre consisting of cellulose, lignin, lignified nitrogen compounds (heat damaged protein), and insoluble ash; whilst NDF consists of ADF plus hemicellulose [Blas *et al*, 1994; Cameron-Smith *et al*, 1994; Gidenne, 2003]. The addition of NH to CRF reduced the ADF and NDF contents of NH supplemented diets; whilst there were no significant changes in the concentrations of these insoluble fibres (ADF and NDF) in the GS supplemented diets (Table 2.4). The soluble fibre present in NH-supplemented diets would be fermented in the caecum

[Munro *et al*, 1998; Garci´a *et al*, 2000], and could facilitate the production of short chain fatty acids (SCFA) from the less digestible fibres in the diets by the gut microbiota [Tehrani *et al*, 2012]. Thus, improving the caecal weight (Table 2.9) [Baltrop and Brueton, 1990; Pacha, 2000], and promoting intestinal villi growth (Table 2.10) [Satchithanadam *et al,* 1990; Podolsky, 1993; Dignass and Lynch-Devaney, 1995; Erlwanger and Cooper, 2008] in the rats nurtured with the NH supplemented diets in the current study.

The previous chemical analyses of NH show zero level of fat [White and Doner, 1980; Bogdanov *et al*, 2008]. The proximate analyses of NH and GS used for the current study gave a negligible fat level of 0.53 % and 0.62 % respectively (Table 2.2), and on addition of these supplements to the CRF to produce the adult diets, there was a reduction in the fat level of the control diet (CRF) from 3.71 % to 2.08 – 2.45 % in the high dose diets (GSH and NHH); whilst the low dose diets (NHL and GSL) had 3.51 % and 3.83 **%** fat respectively (Table 2.4). These values were low, and as such could not have any metabolic influence on the rats.

The consumption of honey by the NH groups resulted in growth similar to that of the control rats (Figures 2.2 – 2.4), and increased the terminal body weights of the male rats more than that of GS-fed males (Figure 2.2). This observation conformed to earlier findings that honey improves the growth of laboratory animals [Cho *et al*, 2001; Fritton *et al*, 2005; Ajibola *et al*, 2007; Ariefdjohan *et al*, 2008]. In a previous study, Cheplius and Starkey [2008] have a different opinion on the

growth response by honey-fed rodents. These authors report low body weight (BW) gain of the honey-fed and those fed sugar-free (control) diet relative to sugar-fed animals. These conflicting observations might be due to the difference in the growth phases of the rats used for the studies. In my earlier study reporting the growth influence of honey [Ajibola *et al*, 2007], young rats in their active growing phase were used, while Cheplius and Starkey started their study with two months old rats, and also administered the honey at a sub-pharmacological and lower (10 %) dose than the amount used in the current study (20 % and 50 %). In addition, it was not known whether the high BW of the sucrose-fed rats by these authors [Cheplius and Starkey, 2008] was due to muscle growth or high fat weight. It is noteworthy that, the other aspect of their findings suggests the latter, as these authors report a significantly higher body fat in the sucrose-fed rats than the honey-fed [Cheplius and Starkey 2008].

The use of body weight alone to assess growth has limitations in that body weight can change acutely in response to changes in some factors which include feed intake, environmental temperature, hydration status, GIT filling, attrition of fat or muscle, loss of hair/fur, genetic factors whereas bone growth is a long term process and the bones do not shrink acutely [Ja¨rvinen *et al*, 1998; Bennell *et al*, 2002; Yadav *et al*, 2003; Burrows, 2007; McVeigh *et al*, 2007; Thiruvenkadan *et al*, 2009; González *et al*, 2012]. Hence, the measurement of long bones is a better and more accurate method of growth assessment in growing animals and human participants [Eshet *et al*, 2004]. The tibial length has been used in several studies

[Hunziker and Schenk 1989; Tjaderhane and Lamas 1998; Eshet *et al*, 2004Fritton *et al*, 2005] as a more accurate indicator of linear growth instead of the BWG.

The results of the rats' linear growth in the present study (Table 2.6) further corroborated previous findings on the growth influence of honey in rodents. The femurs and tibias of the NH-fed rats were longer ($p < 0.05$) and of higher weights than of those animals fed with golden syrup. The marginal increase in the bone density of the honey-fed rats also showed that honey consumption influenced bone growth, probably due to the high calcium component of pure natural honey [White and Doner, 1980; Ariefdjohan *et al*, 2008]. According to one study [Tylavsky, 2004], the calcium constituent of a diet has an influence on bone growth. The findings of some authors also buttress this fact [Cho *et al*, 2001; Fritton *et al*, 2005]. The observations in the present study aligned with these previous reports. Thus, bone mineralization could manifest as increased bone density as shown by the bones radiology in Figures 2.5 and 2.6. Although, I did not analyse the calcium content of the honey used in this study, previous workers [White and Doner, 1980; Bogdanov *et al*, 2008] showed that NH contains several minerals [Table 1.1], which include between 3 to 31 mg calcium per 100 g honey [Table 1.2]. Hence, the increased bone weight of these experimental rats observed in the current study could be due to an increased deposition of calcium and some other organic components of the bone.

2.3.2 – Metabolic response to dietary treatments

The characteristics of MetS are abdominal obesity, dyslipidaemia, hyperglycaemia and hypertension. The main feature assuming epidemic dimension is obesity [Ford *et al*, 2004; Santos *et al,* 2004; Santos *et al,* 2005; Johnson *et al*, 2007; Isken *et al,* 2010; Tehrani *et al*, 2012]. When this abnormality (obesity) co-exists with any other two components of MetS in an individual, the patient is said to suffer the syndrome. This syndrome was established in the SD rat models nurtured with cane syrup from infancy (7-day old) for 12 weeks by recording abdominal obesity and visceral fat accumulation in the rats. The elevated visceral adiposity and excess fat deposit in the GS-fed livers could cause non-alcoholic fatty liver disease (NAFLD) in the GS-fed male rats. The NAFLD provoked the excessive release of FFAs into the bloodstream (due to increased lipolysis). This could partly explain the presence of high blood levels of FFAs seen in the GS-fed rats. The metabolism of high amount of sucrose consumed by the GS-fed rats into its constituent monosaccharides (glucose and fructose) could also lead to the increased blood concentrations of FFAs and TGs formed from their glycerol metabolite.

Furthermore, the fructose moiety in the GS supplemented diets could have contributed its carbon atoms to hepatic metabolism aggravating lipogenesis, and thus releasing low density lipoprotein (LDL), TGs and subsequently FFAs into circulation. These risk factors constitute dyslipidaemia and increased the susceptibility of the rats nurtured with cane syrup to MetS. In addition, the higher concentration of blood FFAs play a role in inflammation by activating pro-inflammatory pathways [Hirosumi *et al*, 2002; Makowski and Hotamisligil, 2004;

Tehrani *et al*, 2012]. Inflammation is also associated with metabolic diseases including MetS [Makowski and Hotamisligil, 2004; Hotamisligil, 2006; Hotamisligil, 2008; Wellen and Hotamisligil, 2005; Hotamisligil and Erbay, 2008; Tehrani *et al*, 2012]. The inflammatory response manifested as Kupffer cells accumulation around the central veins in the rat livers fed with cane syrup in the present study (Figures 2.15 and 2.16).

In addition to the fructose-induced hepatic lipogenesis, the fructose constituent of the GS diets could have also contributed to gluconeogenesis through its metabolite, glyceraldehyde increasing the fasting blood glucose concentration of the GS-fed rats (Table 2.7). The elevated fasting blood glucose levels may have altered the glucose homeostasis of the GS-fed rats, increasing insulin secretion into their blood stream and impairing cellular glucose uptake by the metabolic tissues, causing hyperglycaemia, another feature of MetS. The altered glucose homeostasis and the consequent insulin resistance (IR) were further shown by the OGTT results. The study demonstrated that 30 minutes after oral dosing, the blood glucose concentration (BGC) in the GS-fed male rats was higher ($p < 0.05$) than those of the other male groups (Figure 2.7). This was even more pronounced ($p < 0.001$) with the GSL group. Contrary to the other male groups, the BGC of the GS-fed rats did not return to basal value even two hours after the OGTT was done in the GS-fed male rats (Figure 2.7). The calculation of AUC is critical for evaluating the response of experimental animals to an OGTT. The results of the total AUC confirmed hyperglycaemic tendency of the GSL male rats (Figure 2.9). The total AUC results obtained from the female groups showed that all the female rats were normoglycaemic (Figure 2.10).

The above findings showed that the GSL-fed rats were representative models of human metabolic syndrome. The GS supplemented diets caused the elevation of the fasting blood levels of glucose and triglycerides, and induced high store of hepatic lipid. The high concentrations of these metabolic substrates in circulation and storage coupled with abdominal obesity and high visceral adiposity are components of MetS. In addition, the role of systemic inflammation is increasingly recognised to play a part in metabolic syndrome [Makowski and Hotamisligil, 2004; Hotamisligil, 2008; Sampey *et al*, 2011], and the study demonstrated GS-induced inflammation in the liver. This was manifested as infiltration and accumulation of the resident macrophages, Kupffer cells in the GS-fed livers (Figures 2.15 and 2.16).The activation of Kupffer cells is necessary to produce hepatic insulin resistance [Kremer *et al*, 2006; Lanthier *et al*, 2010]. The excessive store of lipids in the GS livers (Table 2.11), and the higher than normal concentrations of the liver marker enzymes in GS-fed rats (Table 2.12) were indications of hepato-pathology. These confirmations of diseased state of the GS livers were pointers to metabolic dysfunction of the rats nurtured with cane syrup supplements in the present study.

Contrary to the male rats nurtured with the GS supplemented diets, the NH-fed rats enjoyed healthy growth, with the manifestation of the protective properties of honey in all the parameters measured to assess the metabolic health of the animals. The honey-fed experimental animals showed tolerance to an oral glucose load. The blood glucose reached the peak after 30 minutes of oral glucose dosing and returned to basal values at 120 minutes (Figures 2.7 and 2.8) in these honey-fed rats. Unlike the results obtained from the rats fed with honey, the blood

glucose concentrations in GS-fed (GSL and GSH) male rats were significantly higher ($p < 0.05$), and even more prominent ($p < 0.001$) with GSL group than values obtained for other male groups. The high concentrations of circulating metabolic substrates, namely FBG, TGs, FFAs and cholesterol (Table 2.7) associated with cane syrup consumption by the experimental rats signifying the onset of MetS were not seen in NH-fed rats.

The consumption of honey as supplements mitigated hyperglycaemia, maintained glucose homeostasis, with consequent insulin responsiveness unlike GS intake, as observed by the HOMA-IR 21.8 ± 2.53 at low dose (NHL) and 23.2 ± 3.71 at high dose (NHH) compared to the GSL (46.1 ± 6.92) and GSH (72.7 ± 16.78) rats as shown in Table 2.7. These findings of lower FBG and HOMA-IR values in the honey-fed compared to the GS-fed rats confirmed previous studies which showed that eating natural honey reduces plasma glucose in hyperglycaemic subjects [Shambaugh *et al*, 1990; Al-Quassemi and Robinson, 2003; Al-Waili, 2004; Yaghoobi *et al*, 2008; Bahrami *et al*, 2009; Alvarez-Suarez *et al*, 2010b; Cortés *et al*, 2011; Erejuwa *et al*, 2010, 2011a, b; 2012b; Erejuwa, 2012].

However, the mechanistic properties of this natural liquid food (NH) have been puzzling for some time now. Some investigators subjected experimental rats to gastrointestinal assaults caused by alcohol, ammonia, aspirin and indomethacin [Ali, 1995a, b; Gharzouli *et al*, 2001, 2002], and proposed two mechanisms for the hepatoprotective and gastroprotective actions of honey. The first suggests that the

effect is due to the antioxidant properties of honey. NH was found to maintain or enhance the level of non-protein sulfhydryl substances (such as glutathione) in gastric tissue subjected to factors inducing ulceration [Ali, 1995a, b, 1997; Al Swayeh and Ali, 1998]. Similar observation was made when Anzer honey pre-treatment was used in one study to prevent N-ethylmaleimide (NEM) -induced liver damage in rats [Korkmaz and Kolankaya, 2009]. The findings imply that depletion of glutathione concentration plays an aetiological role in NEM-induced liver injury, and that the hepatoprotective effect of Anzer honey may be mediated through the sulfhydryl sensitive processes. The authors concluded that honey possess antioxidant properties. According to some authors, the second mechanism of action being proposed shows that honey intake stimulates the sensory nerves in the stomach, and this proprioceptive effect is in response to capsaicin [Ali, 1995a; Al Swayeh and Ali, 1998]. This mechanism involves the reduction of ulcer index, vascular permeability, and muscular activity of the stomach [Nasuti *et al*, 2006]. Other authors also explain this phenomenon by reporting the mitigating effect of dandelion honey intake against gastric juice acidity by more than 50 % [Baltuskevicius *et al*, 2001]. One study reported a slower passage rate of gastric content of saccharides after the intake of NH than that after ingestion of a mixture simulating honey that is glucose and fructose mixture [Pokorn and Vukmirovic, 1978], and thus, mitigating diarrhoea. The clinical uses of honey in infants and children revealed shorter duration of diarrhoea caused by bacteria. In the same vein, NH also reduced the pathogenesis and duration of viral diarrhoea unlike that associated with the use of conventional antibacterial therapy [Haffejee and Moosa, 1985]. In honey, there is little water available to promote the growth of bacteria and yeast. In addition, honey's natural acidity inhibits some pathogens, as it has

an amount of hydrogen peroxide and other substances contributing to its antibacterial effect [Molan, 2001b].

The presence of soluble fibre in honey is also a contributory factor to its protective properties [White and Doner, 1980; Alagwu *et al*, 2011]. Dietary fibre has been shown to aid digestion and confer gastroprotection amidst other metabolic and health benefits [Anderson *et al*, 2009]. Individuals with high intakes of dietary fibre appear to be at significantly lower risk for developing MetS risk factors such as dyslipidaemia, hypertension, diabetes and obesity [Satchithanandam *et al*, 1990; Kirby *et al*, 1981; Steinberg *et al*, 1996; Schley and Field, 2002; Parnell and Reimer, 2009; Reimer *et al*, 2011]. Increasing fibre intake also lowers serum cholesterol levels. In their extensive review on the health benefits of dietary fibre, Anderson and co-workers concluded that increased intake of soluble fibre improves glycaemic control and insulin sensitivity in non-diabetic and diabetic individuals [Anderson *et al*, 1995, 2009]. These observations confirm previous reports of reduced postprandial hyperglycaemia due to a meal containing dietary fibres [Jenkins *et al,* 1980; Lovejoy and DiGirolama, 1992]. This could adduce for an explanation of the tolerance to an oral glucose load shown by the NH-fed rats amidst other factors.

The hypocholesterolaemic effects of dietary fibre have been studied extensively and the protective mechanisms are best-characterized as the effects of fibre consumption on metabolic risks [Kirby *et al*, 1981; Wright *et al*, 1990; Anderson *et*

al, 2009]. In the opinion of some workers, soluble or viscous fibres appear to exert primary effects on serum cholesterol especially LDL by binding bile acids in the small intestine and increasing their excretion in the faeces [Kirby *et al*, 1981]. The above proposed mechanisms have been supported by some workers in a recent study that honey decreases the blood cholesterol levels through increase in bile cholesterol excretion [Alagwu *et al*, 2009, 2011]. Further investigation by other workers shows that fermentation of fibres in the caecum yields short-chain fatty acids, and this may contribute to hypocholesterolaemia by attenuating cholesterol synthesis [Wright *et al*, 1990].

In addition, NH contains antioxidants and between 0.10 to 0.20 mg/g niacin (Table 1.4), and both possess hypocholesterolaemic properties [Alagwu *et al*, 2011]. The vitamin, niacin has been used as a pharmacologic agent to control the abnormalities of plasma lipid metabolism, and its mechanism of action includes the ability to reduce TGs and LDL by decreasing FFAs mobilization from adipose tissue, and inhibiting hepatic lipid store [Ganji *et al*, 2003; Kamanna and Kashyap, 2008]. The observations in the NH fed rats (Tables 2.7, 2.11 and 2.12) confirmed these mechanistic properties of honey. The current study suggested that the synergy of all the substances in NH could be the mechanism of action responsible for the various protective capabilities of honey. The pointer to this fact is the synergistic actions of prebiotics (FOS, propolis and oligosaccharides) with the probiotics of honey (bacterial flora) in the GIT, which have been shown in several experimental studies (both in vivo and in vitro) to produce resultant beneficial health effects [Cameron-Smith *et al*, 1994; Steinberg *et al*, 1996; Yun, 1996;

Schley and Field, 2002; Sanz *et al*, 2005 [Bianchi, 1977; Yun, 1996; Ramenghi *et al*, 2001; Busserolles *et al*, 2002a; Shin and Ustunol 2005 Newburg, 1997; Busserolles *et al*, 2002a; Shin and Ustunol 2005]].

Pure natural honey is said to contain prebiotics which include FOS, propolis and oligosaccharides [Cameron-Smith *et al*, 1994; Steinberg *et al*, 1996; Yun, 1996; Schley and Field, 2002; Sanz *et al*, 2005]. These competitively activate beneficial intestinal bacteria flora (probiotics) in consumers [Newburg, 1997; Busserolles *et al*, 2002a; Shin and Ustunol 2005], inhibit infectious agents [Kunz and Rudloff, 1993], and enhance digestive functions [Bianchi, 1977; Yun, 1996; Ramenghi *et al*, 2001; Busserolles *et al*, 2002a; Shin and Ustunol 2005]. Oligosaccharides cause a reduction of the flora pathogens, increase bifidobacteria (beneficial bacteria), and an increase of availability of minerals [Rivero-Urgell and Santamaria-Orleans, 2001; Sanz *et al*, 2005]. This facilitates metabolic activities and brings about healthy growth in the honey eaters.

In addition to the intestinal villi growth caused by high fibre diets in rodents, increasing the soluble fermentable fibre content of laboratory animals reduces the susceptibility to gastric infections, and was also found to ameliorate gastroenteritis. High dietary fibre is protective against insulin resistance and obesity [Isken *et al*, 2010] which are components of MetS. This protective effect was shown earlier by Cheeke [1982] who observed a decrease in diarrhoea in rabbits fed with high dietary fibre. In one recent study, it was found that the ingestion of dandelion honey reduced gastric juice acidity by more than 50% [Baltuskevicius *et al*, 2001].

In another previous study, the gastric emptying of saccharides after the ingestion of honey was slower than that after ingestion of a mixture of glucose and fructose [Pokorn and Vukmirovic, 1978], and thus, mitigating diarrhoea. This beneficial property of honey might have informed the decision to treat infants and children suffering from bacterial and viral diarrhoea with honey [Haffejee and Moosa, 1985]. This suggested a potential nutritional and health benefits of substituting honey for refined sugars such as cane syrup in the animal feed and by extension human diet.

2.3.3 – Morphological changes of the abdominal viscera

The study showed that there were differences in the absolute weights and lengths of the small and large intestines amongst all the groups of experimental animals (Table 2.8). The use of absolute organ weight may be misleading as it does not account for the differences in body weight of the animals [Bailey *et al*, 2004]. The differences in body weight often occur between and within groups of experimental animals due to the changes in some parameters. These include alterations in growth caused by modification of hormone secretion; hormonal status being modified by maturational patterns; feed consumption due to changes in neurotransmitters or reduced palatability of diets; and other non-specific systemic factors [Bailey *et al*, 2004]. Hence, the ratio of the organ weight to body weight (expressed as % BW), and referred to as relative organ weight is commonly used for the analysis of organ weight. When these organs' weights were evaluated relative to the rats' body weights, the differences were found to be insignificant in both sexes (Table 2.8). Further evaluation of the weight : length ratio (g/cm) of the intestines also showed no significant difference amongst the male rats as well as

the female groups. This plausibly suggested that the variations observed in the absolute weights and lengths were of no biological significance. Thus, no experimental diet had any undue advantage over the other diets to influence the gross anatomical configuration and functional disposition of the GIT.

The differential impact of the dietary treatments was observed at the microscopic level (Table 2.10). The sections of the small intestine showed that NH had trophic effects on the intestinal cells of the male rats, whilst the SI villus height : crypt depth ratio showed that there was no difference ($p > 0.05$) amongst the female rats (Table 2.10). The low honey group (NHL) of the male rats showed enhanced development of the intestinal morphology than the control and other treatment groups. The villus height, villus width and crypt depth of NHL rats were significantly higher ($p < 0.05$) than those of the other male groups. Satchithanandam *et al* [1990] reported an influence of high dietary fibre in their experimental rats where they found an increase in the number of intestinal cells.

Honey has been shown to contain the biologically active soluble fibre, propolis [White and Doner, 1980; Steinberg *et al*, 1996; Schley and Field, 2002; Parnell and Reimer, 2009], which could have produced the trophic effects in the intestinal villi (Table 2.10). The influence of high dietary fibre on intestinal villi growth has also been documented in different domestic and laboratory animals [Cheeke, 1982; Yamauchi and Isshiki, 1991; Yu and Chiou, 1996; Ajibola, 2000]. The NH-enhanced villi and crypts dimensions could have provided more surface area for

digestive functions and enzymes activities in the GIT. In the words of other workers, the broader villi provide a greater surface area and, therefore, more brush border for nutrients absorption [Yamauchi and Isshiki, 1991]. This could be attributable for improved growth of the honey-fed rodents. These findings were emphasized by Yamauchi and Isshiki [1991], when they fed their broiler chickens with high fibre diet, and made similar observations of larger intestinal villi resulting in faster growth than the laying birds used in their study.

The comparison of the high dose-diets groups showed that all the GIT microscopic values did not differ significantly, except that the NHH villi had the same width as control, but wider ($p < 0.05$) than that of GSH rats. However, all the other intestinal morphological parameters obtained from NHH-fed rats were higher, albeit insignificantly different than the corresponding values from high dose cane syrup eaters (GSH). The evaluation of villus height to crypt depth ratio further showed in Table 2.10 that honey eaters could have enhanced digestive functions and better utilisation of their food. This agreed with the opinion of McAnuff *et al* [2003] in one dietary supplementation study. The workers demonstrate that a decrease in the villus height to crypt depth ratio suggests reduced overall capacity for digestion and absorption of nutrients, and vice versa [McAnuff *et al*, 2003].

The determination of organ weights is a long established practice to ascertain their functional integrity and establish the health status of experimental animals [Baltrop and Brueton, 1990]. Honey increased the absolute and relative weights of some

visceral organs which include caecum, stomach, pancreas and kidneys in the male rats (Table 2.9), and these contributed positively to the animals' TBW.

The predominant influence of natural honey ($p < 0.05$) on organs' macroscopic growth was shown by the pancreas and caecum in the male rats as increased relative weights compared with the GS-fed (Table 2.9). The pancreas is one of the largest glands associated with the GIT, whose growth is influenced by intestinal luminal nutrients and hormonal factors [Williamson, 1978; Bloom and Polak, 1982; Dowling *et al*, 1985; Miazza *et al*, 1985]. The response of the pancreas to dietary supplements could be due to hypertrophic and hyperplastic changes within the organ. Some authors demonstrate pancreatic hypertrophy in rats fed with diets from chickpea seeds [Tavano *et al*, 2005]. Other workers show that short (4 weeks) and long (95 weeks) terms dietary exposure of rats to dietary supplements with soy and potato produce hypertrophy and hyperplasia respectively in the pancreas [Gumbmann *et al*, 1989]. These pancreatic changes are in response to gastrointestinal hormonal factors consistent with pancreatic adaptation to dietary treatments that is interactive with the nutritional status of the rats [Bloom and Polak, 1982; Dowling *et al*, 1985; Lee *et al*, 1986; Calam *et al*, 1987]. The exposure of animals to long-term dietary treatments produces functional adaptation of the pancreas to different diets [Buddington and Lepine, 1999]. According to a report from some workers, dietary treatments of rats, mice, chicks and other species greatly stimulates the exocrine pancreas, leading to increased enzyme production [Gumbmann *et al*, 1989]. Feeding the rats with the

supplements from infancy to adult stage could have led to pancreatic adaptability with consequent enhanced functions.

Previous studies with rats fed erythritol or alcohol extracts of African potato resulted in significantly heavier caecal weight [Munro *et al*, 1998; Erlwanger and Cooper, 2008]. The mechanism underlying the increase in caecal weight is yet to be fully elucidated, but it was attributed to the trophic effect on the caecum by osmotically active or fermentable substances which include soluble fibres that are not absorbed from the small intestine [Munro *et al*, 1998]. The fermentation of these soluble fibres present in the NH-supplemented diets in the caecum could facilitate the production of short chain fatty acids (SCFA) from the less digestible fibres in the diets by the gut microbiota [Munro *et al*, 1998; Garci´a *et al*, 2000; Tavano *et al*, 2005; Tehrani *et al*, 2012]. Thus, improving the caecal weight (Table 2.9) [Baltrop and Brueton, 1990; Pacha, 2000]. The increase in caecal weight could also be due to the presence of some phytochemicals and various sugars in the honey, promoting favourable conditions for the growth and development of the intestinal flora [Reddy, 1971]. The proximate analyses of honey's chemical constituents showing probiotics and prebiotics [White and Doner, 1980; Bogdanov *et al*, 2008], give credence to these facts. Probiotics are live microbial feed supplements which when administered in adequate amounts beneficially affect the host animal by improving its intestinal microbial balance, while prebiotics are non-digestible, fermentable carbohydrates and fibres that selectively stimulate the growth and/or activities of a limited number of bacteria (i.e., probiotics) to exhibit health promoting properties to the host [Fuller, 1989; FAO/WHO, 2001; Parracho *et al*, 2007; Roberfroid *et al*, 2010; Yan and Polk, 2011]. Some of the bacteria

constituting the probiotics of honey are Lactobaccilli, Bifidobacteria while the prebiotics found in honey include propolis and fructooligosaccharides (FOS). According to some workers, the improved caecal weight could also be due to the bulking effect of prebiotics in the caecum [Parnell and Reimer, 2009; Reimer *et al*, 2011]. This bulking effect on the caecum could contribute to TGs excretion [Parnell and Reimer, 2009], hence the hypotriglyceridaemia observed in the NH-fed male rats (Table 2.6). The increased relative weights of the pancreas and caecum of the honey-fed male rats in the current study could enhance enzymatic production by the pancreas and digestive functions by intestinal microflora of the caecum. These findings demonstrated that eating NH could improve the digestive and metabolic health.

The absolute kidneys' weights of the male rats at high dose of both diets (GSH and NHH) were reduced ($p < 0.05$) compared to control (Table 2.9), however the relative measurements (% BW) showed that there were no significant differences ($p > 0.05$) amongst all the groups (Table 2.9). These figures were also within the normal ranges of reference values found in the literature [Melby and Altman, 1976; Davies and Morris, 1993; Gur and Waner, 1993]. Hence, the observed high organ weight of the control (CRF) rats and the low dose-diets (GSL and NHL) groups, could not be of any biological significance. In addition, the values of urea/creatinine ratio obtained from all the experimental rats were not significantly different ($p < 0.5$) and were within normal ranges (Table 2.14). This confirmed that all the experimental rats neither suffered any obvious renal pathological trait, nor showed any benefit accruable to the renal system due to the dietary treatments.

Nonetheless, chronic consumption of natural honey improves renal function [Alagwu *et al*, 2009]. These Nigerian Scientists fed unprocessed honey to male Albino rats (200 – 210 g BW) for 22 weeks, and observed decrease in the rate of bile flow, increase in bile cholesterol excretion, and consequent reduction in plasma cholesterol concentration in the test rats. The prolonged feeding of the unprocessed Nigerian honey to the Albino rats by these workers did not produce any significant difference in both the test and control groups when the bile electrolytes and bilirubin concentrations were measured [Alagwu *et al*, 2009]. In their opinion, increase plasma HDL may account for the increase bile cholesterol excretion with decrease plasma cholesterol concentration observed in their test group. Increased plasma HDL is reported to increase cholesterol transport from the plasma and peripheral tissues to the liver for breakdown and excretion (Ganong, 2012). In addition, the slow rate of bile flow observed in this study [Alagwu *et al*, 2009] could facilitate improved bile secretion and cholesterol excretion, thus the hypocholesterolaemia observed, similar to the honey-fed rats in the current study (Table 2.7). Although, most of these parameters were not specifically within the purview of the current study, the reduced plasma cholesterol and indifferent total bilirubin concentrations relative to the control rats, aligned with the findings of Alagwu and co-workers [2009].

The excess consumption of artificial sugar causes accumulation of body fat [Cheplius and Starkey, 2008]; hepatomegaly, fatty liver and other liver pathologies [Neyrinck *et al*, 2009; Lanthier *et al*, 2010; Papackova *et al*, 2012]. In the present study, the higher visceral fat weight of the male rats fed with cane syrup (3.64 –

3.75 %) relative to NH-fed groups (2.39 – 2.48 %) also confirmed that eating refined sugars induced high visceral adiposity (Table 2.9). The accumulation of excess body fats is inimical to health in children and adults [Ludwig *et al*, 2001; Cook *et al*, 2003; Bray *et al*, 2004; Cruz and Goran, 2004; Johnson *et al*, 2007; Melanson *et al*, 2007]. Although the BWG by the GS-fed male rats was lower than that of the honey-fed males, the visceral fat weight of these GS animals was significantly higher ($p < 0.0001$) than that of the NH-fed rats. This contributed substantially to their (GS-fed rats) body weight**.** However, the increase in visceral fat weight was significantly ($p < 0.05$) greater in the male rats than the females (Table 2.9). The low dose-diet male rats had the highest visceral adiposity than the other groups. This slight predominance of high visceral fat weight in the GSL male rats over high dose-diet group (GSH) was a reflection of the high level of exposure to dietary fructose of the former (GSL). This could be attributable to a high feed intake, due to rodent's preference for dry feed to pasty or liquid material [Davis *et al*, 1976; Sieck *et al*, 1978], as mentioned earlier. The observation of excess visceral adiposity in this study is linked to abdominal obesity [Santos *et al,* 2004; Santos *et al,* 2005; Johnson *et al*, 2007; Tehrani *et al*, 2012] which is a central component of metabolic syndrome [Ford *et al*, 2004; Grundy *et al*, 2004a, 2004b; Santos *et al,* 2004; Santos *et al,* 2005; Ogden *et al*, 2007; Petrucelli, 2008; Flegal *et al*, 2010]. This aligned with previous studies that excessive consumption of refined sugars is culpable in the pathogenesis of MetS [Cook *et al*, 2003; Bray *et al*, 2004; Cruz and Goran, 2004; Johnson *et al*, 2007; Melanson *et al*, 2007; Rutledge and Adeli, 2007; Tappy and Le, 2010].

The pathological trait associated with chronic cane syrup intake was further shown by the development of hepatomegaly in the GS-fed animals (Table 2.9). Contrary to GS induced hepatic abnormality, NH-fed rats had significantly attenuated absolute (g) and relative (% BW) liver weights compared to GS groups as shown in Table 2.9. The hepatic degenerative changes observed under the light microscope (Figures 2.9 and 2.10) were also recorded in Table 2.10. The rats fed CRF and those nurtured with either of the two NH diets showed liver sections with normal cytology and intact architecture. There were various degrees of fatty degenerations in hepatic sections of golden syrup fed animals. These include early stage fatty degenerations, fatty degeneration around the portal triad, diffuse fatty degeneration, hepatic cords disruption and hepatic damage. The GS groups had severe hepatic degenerations with scores of 3.3 to 3.9 compared to a range of 0.7 to 1.3 recorded for the control and NH animals. In addition, the chronic exposure of the rats to cane syrup diets caused hepatic assault which consequently provoked inflammatory response in the liver. The inflammation was shown by the accumulation of resident macrophages, the Kupffer cells around the central veins in the livers of GS-fed rats. Previous studies report that hepatomegaly, fatty liver and the inflammatory milieu are indices of metabolic diseases [Rai *et al*, 1997; Clementi *et al*, 2009; Neyrinck *et al*, 2009; Lanthier *et al*, 2010]. Some of these authors describe the enhanced presence and activation of Kupffer cells as a participatory response in the dietary-induced hepatic insulin resistance [Neyrinck *et al*, 2009; Lanthier *et al*, 2010], which is a component of MetS. The degenerative changes coupled with enhanced visceral adiposity could potentiate hepatic steatosis (onset of NAFLD) in the cane syrup-fed rodents. Despite the high fructose content of honey, it was absolved of culpability in all the pathological

changes associated with excess fructose intake recorded with GS-fed rats. Thus, the honey eaters enjoyed digestive and metabolic health, unlike the GS-nurtured animals.

2.3.4 – Gender differences in response to dietary supplementation

The growth patterns of all the experimental animals from the beginning of the study at the age of 7 days to termination at 91 days showed that all the treatment diets induced significant body weight gain in both sexes. The comparative gender influence on BWG of the experimental animals fed NH and GS for the 12-week study period was recorded at termination in Figure 2.4. As is normal for SD rats, there was a highly significant gender difference in the growth response with the males gaining 45 % in BW more than females at termination, despite their similar weight at birth, as well as being subjected to the same dietary treatments and experimental conditions. SD male rats are noted for faster growth and higher body weight gain than females [Klinger *et al*, 1996].

When the linear growth of the animals was assessed, the gender difference to the dietary treatments of the animals came to fore again as the males were observed to have higher linear growth than the female rodents. The enhanced bone weight and length of the male rats might be due to the effects of testosterone on bone formation. Human and animal studies show that testosterone secretion by males promote linear growth through the coordination of bone matrix synthesis and

mineralization [Peralta *et al*, 1994; Prakasam *et al*, 1999; Chiang *et al*, 2009; Sinnesael *et al*, 2011].

There was similar response to the dietary treatments by the female rats in the growth of the small and large intestines (Table 2.8), but grossly the weights and lengths of these intestinal organs were lower in the females than male rats. This gender difference was a reflection of the higher growth by the male rodents than the females as shown by higher BWG of the males at termination (Figure 2.4). The microscopic morphometric measurements of the small intestine of the female animals showed higher values ($p < 0.05$) of intestinal villus height and crypt depth when the dietary supplements were fed at low doses (GSL and NHL) than the high dose (GSH and NHH) and control rats. This was a reflection of the estimated higher feed consumption by the low dose groups, due to preferential dietary intake [Davis *et al*, 1976; Sieck *et al*, 1978]. As recorded in Table 2.10, the villus height and width of the honey-fed females were higher than the values of those animals nurtured with cane syrup (GS). Similar to my observation in male rats, all the microscopic measurements of high honey dose (NHH) female groups were higher than those obtained from their cane syrup (GSH) counterparts (Table 2.10). However, these values did not attain any statistical significance. The gender difference in the microstructure of the small intestine of the rats were reduced villus height of the females relative to their male counterparts on the same dietary treatments; whilst there were no differences in the other parameters (villus width, crypt depth and villus height/crypt depth ratio), except reduced villus width of the NHL females compared to NHL male rats and higher crypt depth of GSL females than GSL males (Table 2.10). The factors responsible for these intestinal

microstructural gender differences could not be ascertained, but according to some authors dietary treatments and other metabolic manipulations have influence on the rat's intestinal morphology [McAnuff *et al*, 2003; Pacha *et al*, 2003]. These include maturational changes and the development of intestinal transport mechanisms [Pacha, 2000].

There were differences in the absolute weights of the other abdominal visceral organs, mainly the induction of higher liver and visceral fat weights by all the dietary treatments compared with the control (Table 2.9). However, the organs' increased weights did not attain any statistical significance to influence BWG in the females. This was evident in the similar body weights in the female animals at the termination of the study (Figure 2.3). The observed lower absolute weights of all the female abdominal viscera compared to males (Table 2.9) was also a reflection of the higher TBW of the male rats (Figure 2.2) relative to the females TBW shown in Figure 2.3.

When the weights of the viscera were measured relative to the BW, there was no difference ($p > 0.05$) in the visceral fat weight of all the female rats (Table 2.9). This suggested that exposure of female rodents to cane syrup dietary supplementation could not cause high visceral adiposity, contrary to previous findings in human subjects and laboratory animals [Anderson *et al*, 1989; Kasim-Karakas *et al*, 1996; Astrup *et al*, 2002; Elliott *et al*, 2002]. All the female animals were also insulin responsive and tolerant to an oral glucose load, as the female

rats had lower HOMA-IR values (Table 2.7), and the BGC returned to base line after two hours of oral glucose dosing (Figure 2.8). This was a confirmation of previous studies which demonstrate less susceptibility of females to dietary induced IR and MetS [Hollenbeck, 1993; Horton *et al*, 1997; Bantle *et al*, 2000; Busserolles *et al*, 2002b ; Galipeau *et al*, 2002]. This opinion aligns with the other findings of some authors that oestrogen is protective against the effect of pro-oxidants [Nathan and Chaudhuri, 1997; Garcia-Segura *et al*, 2001; Bhathena and Velasquez, 2002; Galipeau *et al*, 2002; Bureau *et al*, 2003]. Although, the female rats fed cane syrup in the present study did not suffer multiple risk factors of MetS, the high FBG levels and hypercholesterolemia induced at low dose (GSL), as well as the dyslipidaemia shown as high TGs concentrations at high dose (GSH) appeared contradictory to the notion of the females' having a lower susceptibility to developing MetS. This could be due to the fact that the dietary interventions initiated in the neonatal rats before the onset of puberty exposed the pups to higher sucrose intake (26.3 g /100 g) in the GS supplement than the sucrose level of 2.6 g /100 g NH (Table 2.2). In addition, the rats fed with the GS supplemented diets would have been nurtured with exceedingly high free fructose as the sucrose would have also increased the fructose concentration in their diets through metabolism to the monosaccharide moieties (fructose and glucose). Hence, the GS pups were unable to benefit from the post pubertal elevation of oestrogen levels [Ramirez and Sawyer, 1965; Busserolles *et al*, 2002b], suggested to be responsible for the protection against MetS risks [Ramirez and Sawyer, 1965; Galipeau *et al*, 2002] at that stage. The interpretation of these findings is that consumption of refined sugars such as cane syrup by female rats may not be totally devoid of metabolic risks.

In conclusion, the reports from experimental studies [Horton *et al*, 1997; Galipeau *et al*, 2002] and human clinical trials [Hollenbeck, 1993; Bantle *et al*, 2000] show that females are safe from the metabolic risks inherent in excess consumption of diets containing high amount of refined sugars. In one study supporting the concept that fructose fed to females provides protection against MetS, some workers show that 6-day fructose overfeeding produces hypertriglyceridaemia (71%) and hepatic insulin resistance in men, but these effects are markedly blunted and mild (16%) in healthy females [Couchepin *et al*, 2008]. In the same vein, the return of blood glucose levels to basal values two hours after the OGTT in the female rats used for the current study suggested a normal postprandial glucose homeostasis. The total AUC (Figure 2.10) calculated from the female OGTT results shown in Figure 2.8 further confirmed the reduced susceptibility of female rodents to developing MetS than the males. The study showed that extrapolation of results from studies involving male rats to females should be done with caution due to potential gender differences in response to dietary manipulations. These conflicting findings suggested that there is need for further gender based studies to clarify the protective status of female subjects exposed to metabolic risks from dietary intake of excess refined sugars, and probably resolve the controversy.

2.4 – Conclusion

I established an animal model of metabolic syndrome in the SD rats nurtured with cane syrup from pre-weaning stage (7-day old) for 12 weeks by recording visceral fat accumulation and dyslipidaemia in the rats amidst other metabolic risk factors. Contrary to GS effects, the consumption of honey was protective against MetS due to the pure natural honey (PNH)'s dietary fibre constituent, and probably its high level of antioxidants. Further studies to identify the protective agent(s) in honey should be intensified. In an attempt to identify these agent(s), a renowned researcher on the Biochemical and Nutritional components of honey, Peter Molan resorted to the use of an acronym UMF known as Unique Manuka Factor as a nomenclature of the 'mystery factor' of protective capabilities in the jellybush (manuka) honey of Australia [Knox, 2004; Yahya, 2005]. Although, manuka honey seems to have higher potency and antioxidant potentials than non-manuka honeys, other honey types also have protective capabilities [Molan, 1992; Cooper, Molan and Harding, 2002; Brady, Molan and Bang, 2004; Lin, Molan and Cursons, 2008]. The present study used a monofloral Sunflower honey from South Africa, and still recorded digestive and metabolic health effects. The findings of the present study attested to the nutritional and health benefits of eating natural honey. Several studies with different types of honey from various geographical origins point to the complimentary roles of the carbohydrates, phytochemicals and other honey constituents as producing its intestinal, digestive and metabolic health as well as other protective effects. Some of the studies have been extensively discussed in chapter one of this thesis. This shows that protective factor is not restricted to Manuka honey, but resides in all honey types at varying degrees. The main factor behind the gastroprotection, hepatoprotection and other protective

effects of this natural functional liquid food, honey is the complimentary role of the phytonutrients and the other chemical constituents. These can be collectively referred to as 'SMIF' meaning synergistic multiple ingredients factor, as the various honey constituents combine to promote and enhance one another's functions, and ultimately collectively provide the nutritional and metabolic health effects. The several honey constituents contributing to SMIF and their roles in gastroenterology, animal and human health have been extensively discussed in chapter one. Although, the synergy of these ingredients was not specifically tested, a pointer to the existence of this beneficial relationship among the various constituents of honey is the symbiotic relationship of probiotics and prebiotics formed in the GIT of honey eaters [Fuller, 1989; FAO/WHO, 2001; Parracho *et al*, 2007; Roberfroid *et al*, 2010; Yan and Polk, 2011]. Nonetheless, there is need for further studies to establish the physiological mechanisms behind this metabolic protection and the health benefits of honey. One could suggest animal experimentation and clinical trials in humans involving feeding of honey for a prolonged period of time in future studies. Future studies could also involve inducing metabolic syndrome in animal models with the initial oral administration of GS, and followed by feeding honey to observe any reversal or neutralizing effects of NH on the adversity of GS. In addition, feeding cane syrup simultaneously with NH could be further used to evaluate the protective strength of honey against metabolic syndrome.

The incidence and prevalence of MetS has also been reported in children due to their high intake of refined sugars [Birch *et al*, 1989; Bellisle *et al*, 2001; Ludwig *et*

al, 2001; Cook *et al*, 2003; Cruz and Goran, 2004; Schulze *et al*, 2004; Betts *et al*, 2005; Dubois *et al*, 2007; Ruottinen *et al*, 2008], with susceptibility rate as high as 30% globally [Cook *et al*, 2003; Cruz and Goran, 2004; WHO, 2005]. The search for a healthy substitute becomes imperative to also curb the MetS menace in children. Honey appears to be a good source of natural sugars as substitute for the refined sugars, due to its protection against the hypertriglyceridemic and pro-oxidative effects of fructose [Busserolles *et al*, 2002a]. Although honey is mainly consumed by adults, some traditional rites include the feeding of honey to infants at birth [Al-Bukhari, 1976; Haffejee and Moosa, 1985; MacMillan, 1999; Jones, 2001; Santos and Antonini, 2008]. However, there is a dearth of scientific information on the effect of feeding honey to the neonates [Bianchi, 1977; Ramenghi *et al*, 2001]. The inclusion of sugars in infant dietary formulations has also been documented [Rivero-Urgell and Santamaria-Orleans, 2001]. Thus, the need to evaluate the safety of honey consumption vis-à-vis refined sugars in children becomes imperative. In addition, the GIT which is the first point of contact for all orally administered substances is relatively immature and undergoes structural and functional changes in the infants as they grow to adult stage. The neonatal stage also imposes the challenges of adaptation on the young ones during the transition from a milk diet to weaning diet [Pacha, 2000], which is even introduced to babies prematurely now especially by working mothers [Rivero-Urgell and Santamaria-Orleans, 2001; WHO, 2005]. The transition from suckling to other diets induces changes in the infants' body systems including the GIT [Baltrop and Brueton, 1990; Pacha, 2000]. Feed intake and nutritional composition of the diets are paramount in the proper development and adaptation of the GIT [Pacha,

2000]. This further strengthens the need to investigate and compare the metabolic effects of orogastric administration of honey vis-à-vis cane syrup.

CHAPTER THREE

EFFECTS OF SHORT TERM FEEDING

CHAPTER THREE

EFFECTS OF SHORT TERM FEEDING

3.0 – Introduction

After the long term animal model of metabolic syndrome was established by the 12-week cane syrup dietary treatments vis-à-vis the protection conferred on the other littermates fed with NH supplemented diets, the study on rat pups was done. This was with a view to investigate and document metabolic and any other acute changes in the suckling pups fed GS or NH supplemented diets. The investigation of the acute effects of the dietary treatments on the gastrointestinal tract and other abdominal visceral organs became imperative. This was due to changes induced by dietary manipulation at early age, sometimes with irreversible consequences at adulthood [Pacha, 2000]. The study could also show the onset of any deleterious short term effects. This was even more paramount due to the increasing global use and consumption of refined sugars by children [Birch *et al*, 1989; Ludwig *et al*, 2001; Dubois *et al*, 2007; Ruottinen *et al*, 2008], in spite of the reports of adverse health consequences [Bellisle and Rolland-Cachera, 2001; Cook *et al*, 2003; Cruz and Goran, 2004; Betts *et al*, 2005]. There was the need for a healthy substitute to nourish the human infants, provide energy and drive their metabolic activities without any health hazards. The use of honey as a palliative medicinal and functional food in experimental studies [Ali *et al,* 1990; Ali, 1995a, b, 1997; Busserolles *et al*, 2002a; Ajibola *et al*, 2007; Alagwu *et al*, 2009, 2011], and clinical trials [Bianchi, 1977; Haffejee and Moosa, 1985; MacMillan, 1999; Jones, 2001; Ramenghi *et al*, 2001] appears promising. This informed the decision to investigate and establish the effects of short term feeding of natural (honey) and artificial (cane syrup) sweeteners in children.

At birth, children are dependent entirely on their mothers, and breast feeding constitutes the main source of these babies' nourishment [Heinig and Dewey, 1996], until weaning when they (babies) begin to eat solid foods. The rat pups are born with their eyes closed at birth until 14 days old [Kohn and Clifford, 2002], and survive this neonatal period through nutritional dependency on their dams by suckling. Afterwards, the pups then begin to nibble their lips on small solid particles in their environment. Both man and rats are mammalian species, whose digestive system responds to dietary changes by structural and functional adaptability [Pacha, 2000; Sorensen, 2009]. This structural adaptability influences the GIT maturational changes during the transition from neonates to adulthood [Pacha, 2000]. Thus, dietary manipulation in early life plays a prominent role in the development of normal GIT function [Strasburger, 2004]. The similarities in the developmental stages, which include nutritional dependency on their mothers at birth and the GIT development of both mammalian species (man and rat) in response to dietary manipulation [Pacha, 2000] makes the rat pups good models for the investigation of the effects of dietary supplements on human infants. Therefore, the pups' short term response to NH or GS dietary supplements constituted phase two of this study.

3.0.1 – Phase Two: Effects of honey and cane syrup on neonatal rats

This comparative study of the effects of natural honey and cane syrup on neonatal rats was done using eight nursing Sprague Dawley (SD) dams with their litters of a total of 69 pups (8 to 10 pups each). The sixty-nine pups consisting of 36 males and 33 females with their respective dams were provided by the Central Animal Services of the university in batches based on the availability of animals during the

research period. The 69 suckling (7-day old male and female) SD rats were randomly divided into five groups by completely randomized design, based on dietary treatments. The dietary treatments with either natural honey (NH) or golden syrup (GS) were replicated on the basis of sex as in phase one. Each dam had pups belonging to all the groups for fair representation among the littermates. The pups had colour codes marked on their tails with a non-toxic, non-invasive, superficial permanent ink marker for identification purposes. This phase of the study lasted 10 days (age 10 – 20 days), with their date of birth being noted as day one.

3.1 – Materials and Methods

3.1.1 – Experimental Animals

The 69 SD male and female rats were weighed at the commencement of the study. The mean body weight was 23.36 g (SEM 0.33 g), (range 18 – 26 g). The mean BW of the male pups was 23.97 g (SEM 0.91 g), (range 20 – 26 g), and was not significantly different ($p > 0.05$) from that of the females (22.70 g (SEM 1.14 g), (range 18 – 26 g)).

3.1.2 – Housing of animals

The housing was as described for the pre-weaning period of the long term study (phase one – Adult phase (see section 2.1.2).

3.1.3 – Dietary treatments

The treatments were as described in chapter 2, section 2.1.3.1, except that the pups were gavaged with their respective supplements twice daily at 12 hours interval, every morning (07h00 – 08h00) and evening (19h00 – 20h00) for 10 days. The rats in the low dose-diet groups were given 10 ml/kg BW of 50 % solution (volume/ volume, v/v), twice a day; whilst the high dose-diet groups were given 20 ml/kg BW of 50 % solution (v/v) twice daily. The pups in the control group were similarly gavaged with distilled water (10 ml/kg BW). All the rats were kept with their respective dams and allowed to suckle *ad libitum* between gavages and weighed daily to monitor growth performance. The groups were thus Group 1, Control (CON); Group 2, Golden syrup Low (GSL); Group 3, Natural honey Low (NHL); Group 4, Golden syrup High (GSH); and Group 5, Natural honey High (NHH). There were 7 rats per group except CON males, and GSH and NHH female groups that had 6 rats each; whilst n = 8 in GSH and NHH male groups. The details of groupings, dietary treatments and interventions were as shown in Figure 3.1 overleaf.

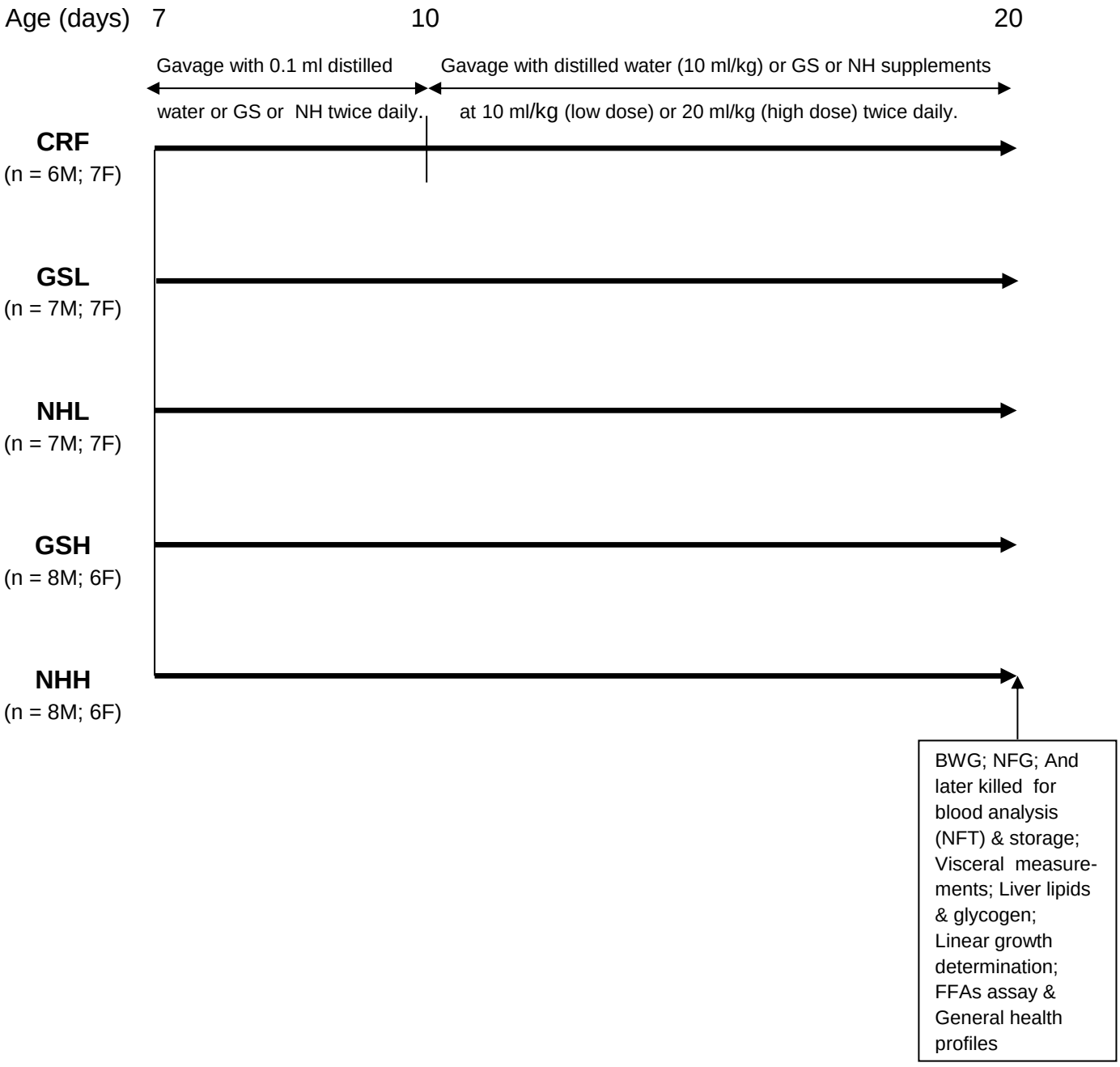


Figure 3.1: Flow chart showing the groupings, treatments and interventions.

KEY

BWG: Body weight gain
NFG: Non-fasting blood glucose
NFT: Non-fasting blood triglycerides
FFAs: Free fatty acids
CON: Control (10 ml/kg distilled water twice daily)
GSL: Golden syrup Low (10 ml/kg 50 % golden syrup solution twice daily)
NHL: Natural honey Low (10 ml/kg 50 % natural honey solution twice daily)
GSH: Golden syrup High (20 ml/kg 50 % golden syrup solution twice daily)
NHH: Natural honey High (20 ml/kg 50 % natural honey solution twice daily)

3.1.4 – Growth of animals

The growth (body weight gain and linear growth) of the experimental animals was monitored as described earlier (chapter 2, section 2.1.4).

3.1.5 – Non-fasting blood glucose

The non-fasting blood glucose (NFG) was determined in the 20-day old rats using a calibrated glucometer according to the technique described for the measurement of fasting blood sugar in phase one (section 2.1.5), except that the rat pups were not fasted as done in phase one. The NFG was done to assess the sugar metabolism and post prandial sugar retention in infants.

3.1.6 – Tissue collection

a. Blood: The rats were euthanized with sodium pentobarbitone (150 mg/kg BW) (Euthanaze®, Centaur Laboratories, Johannesburg, South Africa) injected intraperitoneally. The thorax of each animal was cut open and 2 ml of blood was collected by cardiac puncture using 2 ml syringes and 21G needles. The blood sample was transferred into heparin coated tubes (Greiner Bio-one GnebH, Austria), and the tubes were gently inverted so that the blood mixed with heparin to prevent the blood from clotting. The blood samples were subsequently centrifuged with a Sorvall® RT 6000B (Du Pont, USA) at 4 ^{o}C and 5000 x G for 15 minutes to obtain plasma. The plasma samples collected were frozen at – 20 ^{o}C for later clinical biochemistry measurements.

b. Viscera: The visceral organs of the 20 days old rats were carefully removed , measured and stored as done in the adult rats during phase one.

3.1.7 – Experimental procedures

Most of the experimental procedures done in the adult rats (Phase one) were repeated in the unweaned rats. These included body weight gain, linear growth, metabolic substrates, gross viscera measurements, histology, liver function analysis and clinical biochemistry.

The few exempted tests and measurements that were not performed in the neonatal rats due to the following reasons:

i. Visceral fat determination because there were negligible amounts deposited in the pups.

OGTT because suckling rats could not be subjected to fasting, as this would require separating the pups from their dams. Early-life maternal separation of rat pups is a confounding factor in analyses causing alteration in clinical chemistry [Clarke *et al*, 2009]. In addition, suckling neonates including rat pups are susceptible to hypoglycaemia when subjected to fasting [Pegorier *et al*, 1982; Ogata, 1986; Ogata *et al*, 1981; Ogata *et al*, 1985].

ii. Hormones (insulin and leptin) and AST because of inadequate blood volume due to the small size of the rats.

The experimental procedures carried out on the samples from these rat pups after appropriate modifications of the techniques used for analyses in the adult phase were as follow:

i. Non-fasting blood glucose (NFG) and non-fasting blood triglycerides (NFT) done at the end of the phase (20 days old) instead of FBG and TGs, as suckling rats could not be subjected to fasting. As mentioned above, the fasting of suckling rats would be tantamount to separation from their dams. Consequently, imposing early-life stress of maternal separation on the rodents with profound alteration of their biochemical parameters [Clarke *et al*, 2009].
ii. Smaller liver sample (1.0 – 1.5 g) was used for liver lipids extraction instead of the 5.0 – 7.0 g used in the older rats, because of small organ size.

3.2 – Results

The results of the short term effects of dietary supplements with natural honey and cane syrup administered orally (over a 10-day period) to 10-day old suckling male and female SD rats were as presented below.

3.2.1 – Overall body growth

The body weight gain (BWG) was obtained from the difference in the initial weight and terminal weight, and were used to assess the growth pattern of the experimental rats on gender basis (male and female). The terminal body weight

showed that the treatment diets induced significant BWG ($p < 0.001$) of 94.8 % in males and 96.2 % in the female rats relative to initial weight (Figures 3.2 and 3.3). Figure 3.4 showed that there was no difference in BWG amongst all groups and there were also no gender differences.

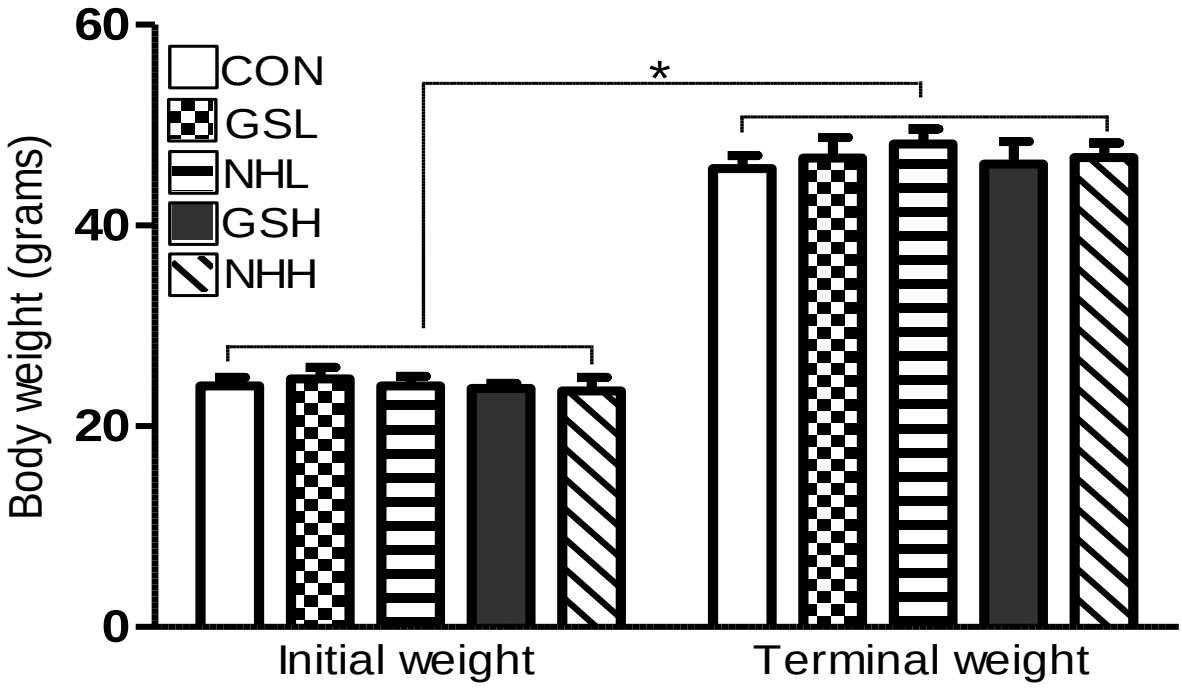


Figure 3.2: Effects of treatment diets on the growth pattern of suckling male rats at 20 days of age after feeding with NH and GS supplements for 10 days.

CON, control, 10 ml/kg BW distilled water (n = 6); GSL, golden syrup low dose, 10 ml/kg BW 50 % golden syrup [GS] solution (n = 7); NHL, natural honey low dose, 10 ml/kg BW of 50 % natural honey [NH] solution (n = 7); GSH, golden syrup high dose, 20 ml/kg BW 50 % GS solution (n = 8); NHH, natural honey high dose, 20 ml/kg BW 50 % NH solution; (n = 8); all treatments were given twice daily. * Significant increase ($p < 0.001$) of body weight gain in all groups after 10-day treatment *vs* initial weight.

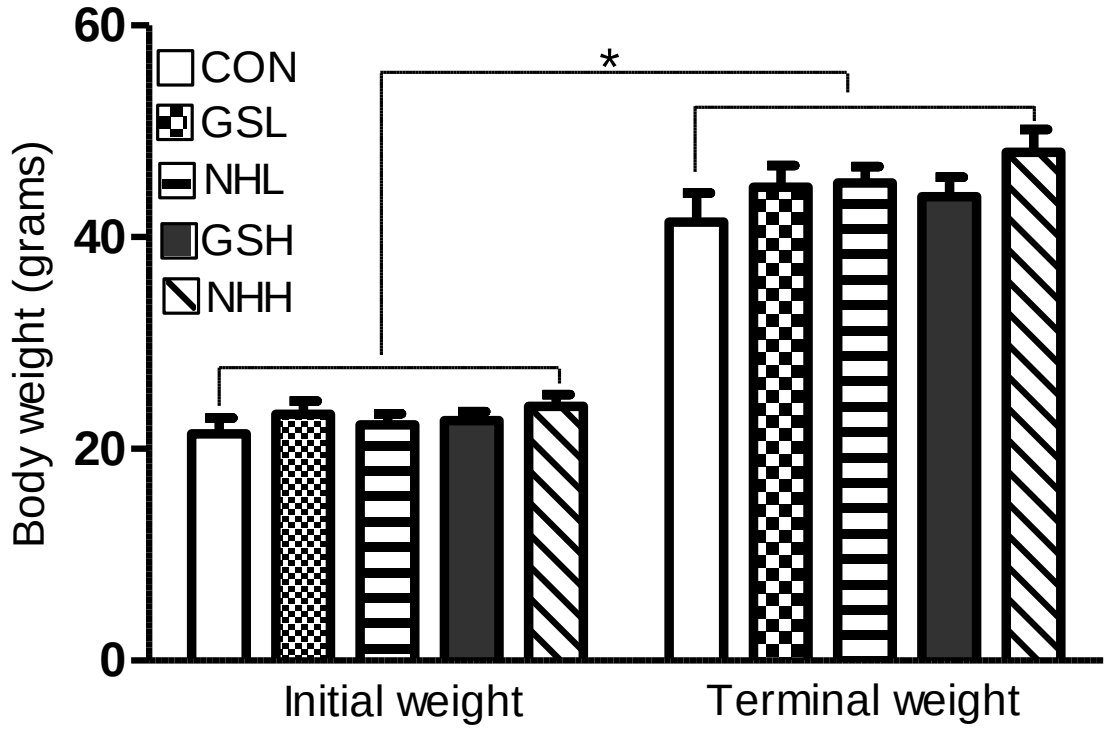


Figure 3.3: Effects of treatment diets on body weight gain of suckling female rats at 20 days of age after feeding with NH and GS supplements for 10 days.

CON, control, 10 ml/kg BW distilled water (n = 7); GSL, golden syrup low dose, GSL, 10 ml/kg BW 50 % golden syrup [GS] solution (n = 7); NHL, natural honey low dose, 10 ml/kg BW of 50 % natural honey [NH] solution (n = 7); GSH, golden syrup high dose, 20 ml/kg BW 50 % GS solution (n = 6); NHH, natural honey high dose, 20 ml/kg BW 50 % NH solution (n = 6); all treatments were given twice daily. * Significant increase ($p < 0.001$) of body weight gain in all groups after 10-day treatment *vs* initial weight.

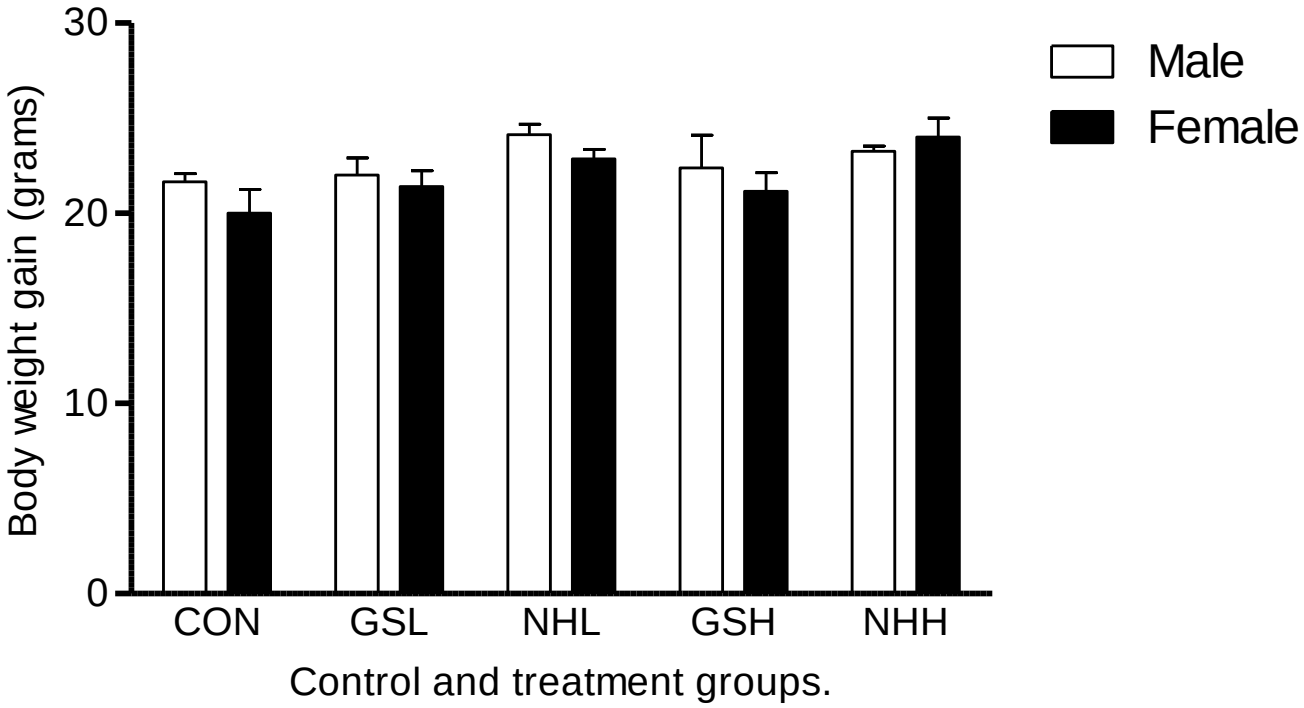


Figure 3.4: Effects of treatment diets on body weight gain of suckling rats at 20 days of age after feeding with NH and GS supplements for 10 days.

CON, control, 10 ml/kg BW distilled water; GSL, 10 ml/kg BW 50 % golden syrup [GS] solution; NHL, 10 ml/kg BW of 50 % natural honey [NH] solution; GSH, 20 ml/kg BW 50 % GS solution; NHH, 20 ml/kg BW 50 % NH solution; all treatments were given twice daily. No significant difference ($p > 0.05$) in body weight gain amongst all groups and no gender difference after 10-day treatment.

3.2.2 – Linear growth in Neonates

The results of the linear growth of the experimental rats evaluated through the measurements of weight and length of the long bones (femur and tibia) are shown in Table 3.1. The values obtained showed that the bones were not significantly different ($p > 0.05$) in weight, length and density amongst all the groups. There was no significant difference between males and females in all parameters measured.

Table 3.1: Weight, length, and bone density (mg/mm) of femur and tibia in suckling rats at 20 days of age after feeding with NH and GS supplements for 10 days.

Diet	**Sex**	**Femur**			**Tibia**		
		Weight (mg)	**Length** (mm)	**Density** (mg/mm)	**Weight** (mg)	**Length** (mm)	**Density** (mg/mm)
CON	M	65.3 ± 2.3	15.0 ± 0.3	4.35 ± 0.1	51.3 ± 1.7	17.7 ± 0.2	2.9 ± 0.1
	F	67.3 ± 1.7	14.9 ± 0.3	4.54 ± 0.1	51.6 ± 2.2	17.6 ± 0.2	2.9 ± 0.1
GSL	M	66.4 ± 3.6	15.0 ± 0.2	4.42 ± 0.2	49.4 ± 3.7	17.7 ± 0.3	2.8 ± 0.2
	F	62.1 ± 4.0	14.3 ± 0.3	4.34 ± 0.3	50.6 ± 3.4	17.3 ± 0.2	2.9 ± 0.2
NHL	M	68.9 ± 1.5	15.1 ± 0.3	4.55 ± 0.1	53.1 ± 1.4	17.6 ± 0.2	3.0 ± 0.1
	F	63.7 ± 2.0	14.9 ± 0.5	4.30 ± 0.1	50.7 ± 2.1	17.6 ± 0.2	2.9 ± 0.1
GSH	M	62.8 ± 3.5	14.6 ± 0.2	4.29 ± 0.2	47.8 ± 2.6	17.0 ± 0.3	2.8 ± 0.1
	F	59.5 ± 3.2	14.5 ± 0.3	4.10 ± 0.2	46.3 ± 2.4	17.2 ± 0.3	2.7 ± 0.1

Table 3.1 Continued

Diet	Sex	Femur			Tibia		
		Weight (mg)	**Length** (mm)	**Density** (mg/mm)	**Weight** (mg)	**Length** (mm)	**Density** (mg/mm)
NHH	M	64.8 ± 2.6	14.9 ± 0.3	4.33 ± 0.1	49.9 ± 3.4	17.1 ± 0.3	2.9 ± 0.2
	F	65.2 ± 3.3	14.8 ± 0.4	4.38 ± 0.1	49.5 ± 2.3	17.5 ± 0.3	2.8 ± 0.1

No significant difference ($p > 0.05$) in weight, length, and density of the long bones amongst the control and treatment groups after 10-day treatment; CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50 % GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50 % honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50 % GS solution twice daily); NHH = Natural honey High (20 ml/kg 50 % honey solution twice daily).

3.2.3 – Metabolic substrates

Cane syrup supplementation induced highly significant increase (* $p < 0.0001$) in the blood concentration of FFAs in the male and female pups at both low and high doses compared to NH and control rats (Table 3.2). The differences in the blood concentrations of NFG and NFT, as well as the plasma concentration of cholesterol measured were not significant amongst the control and treatment groups (Table 3.2). There were no gender differences in the concentrations of all these circulating metabolic substrates.

The hepatic store of metabolic substrates (lipids and glycogen) was recorded in Table 3.3. The GS induced significantly higher ($p < 0.001$) fat deposit in the male and female livers at both doses than in the control and NH livers. However, there were no gender differences amongst all the groups in the hepatic lipid contents (Table 3.3). There was no significant difference ($p > 0.05$) in the hepatic store of glycogen amongst the male and female rats and similarly no gender differences existed (Table 3.3).

Table 3.2: Circulating metabolic substrates in suckling rats at 20 days of age after feeding with NH and GS supplements for 10 days.

Diet	**Sex**	**NFG** (mg/dL)	**NFT** (mmol/L)	**FFAs** (mmol/L)	**Cholesterol** (mmol/L)
CON	M	5.37 ± 0.11	1.90 ± 0.26	0.17 ± 0.01	4.26 ± 0.39
	F	5.26 ± 0.58	1.66 ± 0.20	0.15 ± 0.01	4.24 ± 0.41
GSL	M	5.91 ± 0.12	1.80 ± 0.25	0.25 ± 0.01*	3.95 ± 0.35
	F	5.81 ± 0.10	2.00 ± 0.26	0.23 ± 0.01*	4.29 ± 0.15
NHL	M	5.73 ± 0.16	1.49 ± 0.12	0.16 ± 0.02	3.74 ± 0.25
	F	5.27 ± 0.41	1.66 ± 0.27	0.16 ± 0.01	3.66 ± 0.26
GSH	M	6.28 ± 0.17	1.52 ± 0.26	0.22 ± 0.01*	3.89 ± 0.18
	F	6.02 ± 0.21	1.68 ± 0.25	0.23 ± 0.01*	3.80 ± 0.30

Table 3.2 Continued

Diet	**Sex**	**NFG** (mg/dL)	**NFT** (mmol/L)	**FFAs** (mmol/L)	**Cholesterol** (mmol/L)
NHH	M	5.59 ± 0.18	1.45 ± 0.21	0.17 ± 0.01	3.96 ± 0.20
	F	5.07 ± 0.40	1.26 ± 0.09	0.16 ± 0.01	3.74 ± 0.34

* value highly significant ($p < 0.0001$) down the column; No significant difference ($p > 0.05$) in the other circulating metabolic substrates amongst the control and treatment groups after 10-day treatment; CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50 % GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50 % honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50 % GS solution twice daily); NHH = Natural honey High (20 ml/kg 50 % honey solution twice daily); NFG = non-fasting blood glucose; NFT = non-fasting blood triglycerides; FFAs = free fatty acids.

Table 3.3: Liver lipid and glycogen content (as glucose equivalents) in suckling rats at 20 days of age after feeding with NH and GS supplements for 10 days.

Diet	**Sex**	**Liver lipid** (% Liver weight)	**Liver glycogen** (as glucose equivalents) (mmol/L)
CON	M	4.41 ± 0.46	2.97 ± 0.40
	F	5.65 ± 0.33	2.76 ± 0.39
GSL	M	6.96 ± 0.61*	3.04 ± 0.47
	F	7.87 ± 0.16*	2.03 ± 0.43
NHL	M	4.79 ± 0.30	2.91 ± 0.48
	F	5.70 ± 0.47	2.79 ± 0.67
GSH	M	6.98 ± 0.30*	3.01 ± 0.46
	F	7.40 ± 0.61*	2.21 ± 0.63
NHH	M	4.32 ± 0.13	2.58 ± 0.35
	F	5.34 ± 0.13	2.79 ± 0.36

* value significantly different down the column ($p < 0.001$); CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50 % GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50 % honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50 % GS solution twice daily); NHH = Natural honey High (20 ml/kg 50 % honey solution twice daily).

3.2.4 – Viscera and morphometric measurements

The rats fed treatment diets (NH and GS) had greater ($p < 0.05$) absolute (g) and relative (% BW) weights, as well as weight/length ratio (g/cm) of the small intestine (SI) than controls in both sexes (Table 3.4). NH also had increased absolute large intestine (LI) weight at high dose (NHH) compared to the other diets in both sexes, however when expressed relative to body weight there was no significant difference noted. There were no significant differences ($p > 0.05$) in the values of SI length, LI length, LI relative weight and LI weight/length ratio amongst the groups in both sexes (Table 3.4). For the caecum, I recorded significantly higher ($p < 0.05$) absolute weight of the treatment groups compared to control in both sexes, whilst there were no differences in the absolute and relative weights of the stomach in both sexes (Table 3.4)

The weights of the non-gastrointestinal tract abdominal visceral organs were recorded in Table 3.5. There were no significant differences ($p > 0.05$) in the absolute (g) and relative (% BW) weights of the visceral organs amongst all the rat pups (Table 3.5).

The photomicrographs of the histological sections presented normal cytology of the control, NHL and NHH livers; whilst there were fat deposits in the livers of the cane syrup fed rats (GSL and GSH) (Figures 3.5 and 3.6).

Table 3.4**:** Gross measurements of the gastrointestinal tract (small intestine (SI), large intestine (LI), caecum and stomach) in suckling rats at 20 days of age after feeding with NH and GS supplements for 10 days.

Organ	**Unit**	**Sex**	**CON**	**GSL**	**NHL**	**GSH**	**NHH**
SI	g	M	1.79 ± 0.08*	2.26 ± 0.15	2.20 ± 0.13	2.31 ± 0.10	2.29 ± 0.10
		F	1.62 ± 0.10*	2.30 ± 0.13	2.24 ± 0.14	2.29 ± 0.16	2.43 ± 0.20
	cm	M	63.17 ± 0.99	68.14 ± 2.80	68.36 ± 1.15	70.06 ± 1.03	70.26 ± 2.56
		F	64.57 ± 1.41	70.43 ± 1.79	67.07 ± 0.92	69.92 ± 2.95	70.75 ± 2.17
	g/cm	M	0.03 ± 0.00*	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
		F	0.03 ± 0.00*	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
	% BW	M	0.04 ± 0.00*	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
		F	0.04 ± 0.00*	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00

Table 3.4 Continued

Organ	Unit	Sex	CON	GSL	NHL	GSH	NHH
LI	g	M	0.26 ± 0.02	0.31 ± 0.02	0.29 ± 0.02	0.32 ± 0.02	0.35 ± 0.02*
		F	0.27 ± 0.01	0.34 ± 0.02	0.31 ± 0.02	0.32 ± 0.03	0.39 ± 0.04*
	cm	M	8.55 ± 0.27	9.19 ± 0.29	9.36 ± 0.30	9.09 ± 0.55	8.75 ± 0.28
		F	8.43 ± 0.32	9.43 ± 0.34	8.93 ± 0.37	8.50 ± 0.29	8.75 ± 0.82
	g/cm	M	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00
		F	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
	% BW	M	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
		F	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Table 3.4 Continued

Organ	Unit	Sex	CON	GSL	NHL	GSH	NHH
Caecum	g	M	0.21 ± 0.02*	0.33 ± 0.03	0.31 ± 0.02	0.35 ± 0.02	0.35 ± 0.02
		F	0.24 ± 0.02*	0.27 ± 0.02	0.28 ± 0.01	0.33 ± 0.03	0.35 ± 0.02
	% BW	M	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
		F	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Stomach	g	M	0.42 ± 0.03	0.49 ± 0.03	0.49 ± 0.03	0.47 ± 0.01	0.50 ± 0.03
		F	0.44 ± 0.03	0.48 ± 0.02	0.49 ± 0.02	0.48 ± 0.03	0.54 ± 0.05
	% BW	M	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
		F	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Absolute (g) and relative (% BW) weights of GIT organs; length (cm), weight : length ratio calculated as g/cm of SI and LI in male and female suckling animals. Data are expressed as mean ± SEM. *Data in the same row with superscripts are significantly different ($p < 0.05$); CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50% GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50% honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50% GS solution twice daily); NHH = Natural honey High (20 ml/kg 50% honey solution twice daily).

Table 3.5: Absolute (g) and relative (% BW) weights of the non gastrointestinal tract abdominal visceral organs in male and female suckling rats at 20 days of age.

Organ	Unit	Sex	CON	GSL	NHL	GSH	NHH
Liver	g	M	1.71 ± 0.05	1.78 ± 0.11	1.79 ± 0.10	1.81 ± 0.10	1.77 ± 0.08
		F	1.48 ± 0.09	1.69 ± 0.11	1.67 ± 0.07	1.74 ± 0.08	1.90 ± 0.09
	% BW	M	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
		F	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
Pancreas	g	M	0.18 ± 0.02	0.15 ± 0.01	0.17 ± 0.03	0.19 ± 0.03	0.18 ± 0.02
		F	0.15 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.14 ± 0.02	0.18 ± 0.02
	% BW	M	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
		F	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Spleen	g	M	0.25 ± 0.02	0.27 ± 0.02	0.26 ± 0.02	0.31 ± 0.05	0.25 ± 0.02
		F	0.23 ± 0.02	0.28 ± 0.03	0.25 ± 0.02	0.27 ± 0.02	0.29 ± 0.02
	% BW	M	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
		F	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Table 3.5 Continued

Organ	Unit	Sex	CON	GSL	NHL	GSH	NHH
Kidneys	g	M	0.54 ± 0.03	0.51 ± 0.04	0.56 ± 0.02	0.56 ± 0.03	0.54 ± 0.02
		F	0.52 ± 0.02	0.53 ± 0.03	0.55 ± 0.02	0.53 ± 0.03	0.56 ± 0.04
	% BW	M	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
		F	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50 % GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50 % honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50 % GS solution twice daily); NHH = Natural honey High (20 ml/kg 50 % honey solution twice daily).

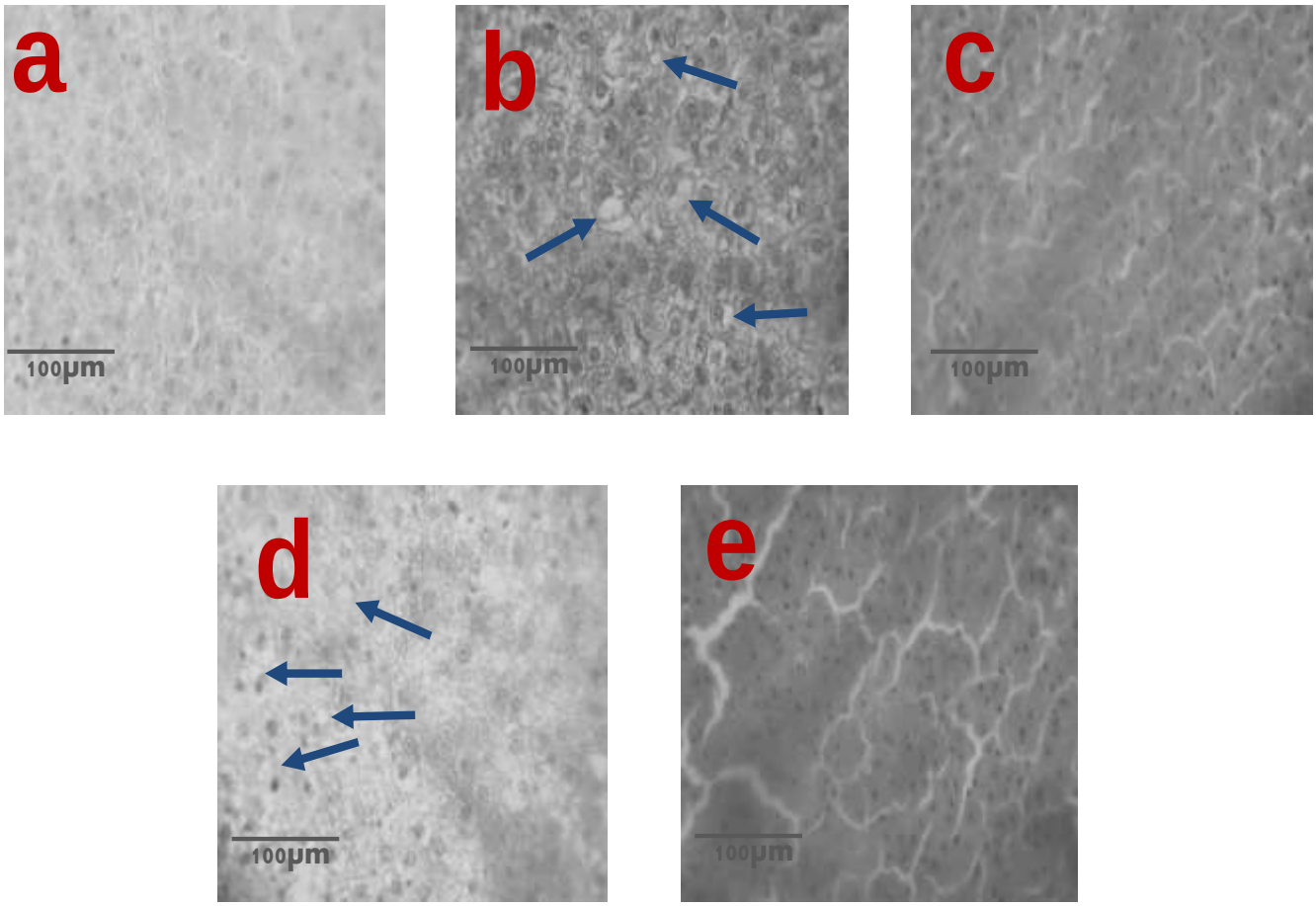


Figure 3.5 Photomicrographs of the livers (Haematoxylin & Eosin stain) from suckling male rats at 20 days of age after feeding with NH and GS supplements for 10 days [magnification is 400 x, Scale bar is 100 µm]: (a) CON, control, 10 ml/kg distilled water; (b) GSL, 10 ml/kg BW 50% golden syrup [GS] solution; (c) NHL, 10 ml/kg BW of 50 % natural honey [NH] solution (d) GSH, 20 ml/kg BW 50 % GS solution (e) NHH, 20 ml/kg BW 50 % NH solution; all treatments given twice daily; CON, NHL and NHH livers showed normal cytology; Arrows showed fat deposits in GSL and GSH livers.

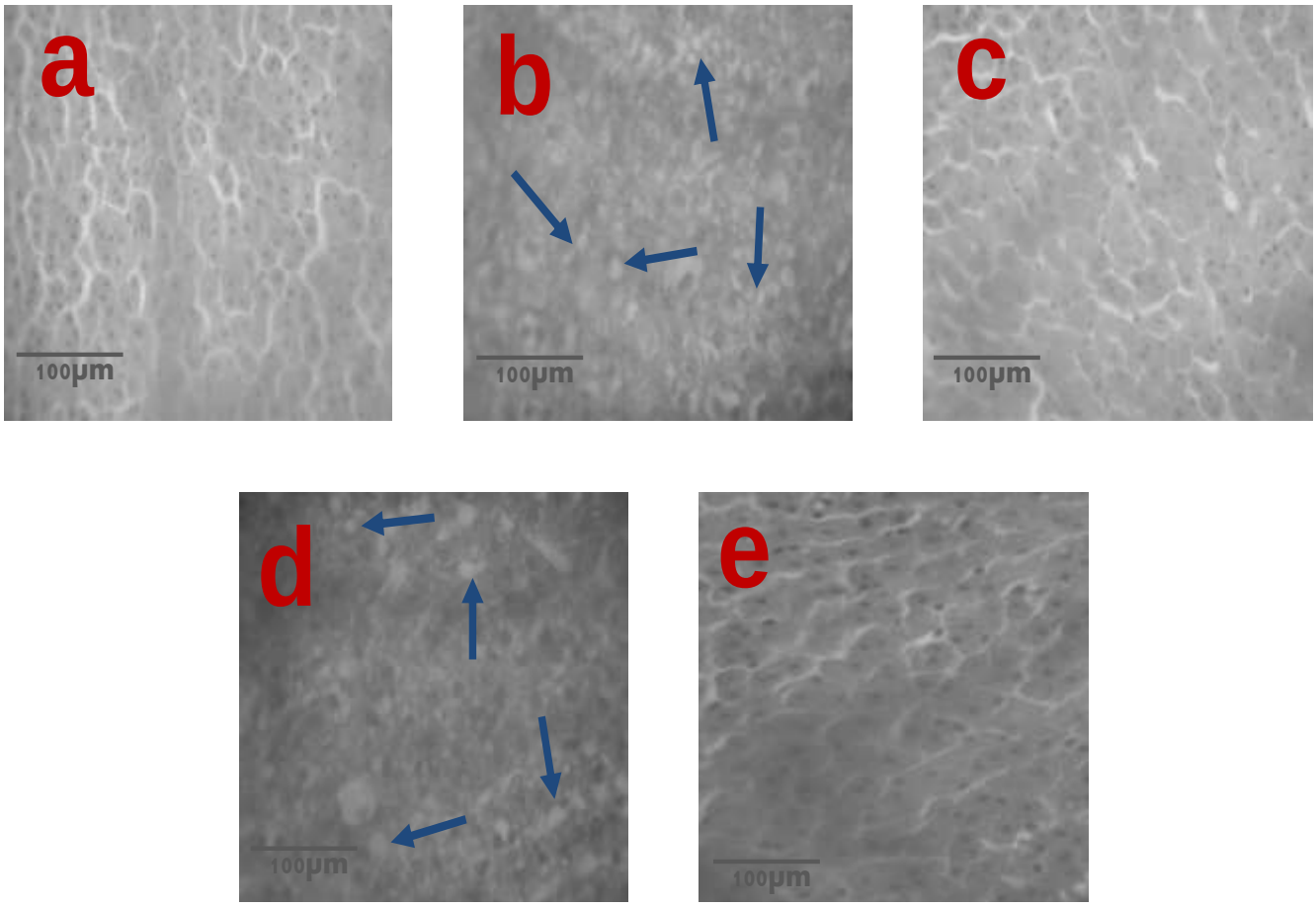


Figure 3.6 Photomicrographs of the livers (Haematoxylin & Eosin stain) from suckling female rats at 20 days of age after feeding with NH and GS supplements for 10 days [magnification is 400 x, Scale bar is 100 µm]: (a) CON, control group, 10 ml/kg distilled water; (b) GSL, 10 ml/kg BW 50 % golden syrup [GS] solution; (c) NHL, 10 ml/kg BW of 50 % natural honey [NH] solution (d) GSH, 20 ml/kg BW 50 % GS solution (e) NHH, 20 ml/kg BW 50 % NH solution; all treatments given twice daily; CON, NHL and NHH livers showed normal cytology; Arrows showed fat deposits in GSL and GSH livers.

3.2.5 – Health profiles

The plasma concentrations of surrogate markers of the liver function were summarised in Table 3.6. The plasma concentrations of alanine transaminase (ALT) in GS-fed animals were higher ($p < 0.05$) than control and normal, whilst the values of the other liver function markers (ALP and T bil) were within the normal ranges (Table 3.6). The other clinical biochemistry parameters showing the surrogate markers of general health assayed in the plasma of the neonatal animals were documented in Table 3.7. This table showed that there were no significant differences ($p > 0.05$) in all the general health markers amongst the control and treatment groups (Table 3.7). The parameters of the kidney functionality (plasma concentration of urea and creatinine and urea/creatinine ratio) listed in Table 3.8 showed that there were no differences ($p > 0.05$) amongst all the rat pups.

There were no gender differences in all the clinical biochemistry and general health parameters except the alkaline phosphatase. As shown in Table 3.6, the ALP values obtained from all the female rats were significantly ($p < 0.05$) lower than that of their corresponding male groups. The differences between the male and female experimental animals in the renal and other health indices did not attain any statistical significance (Tables 3.6, 3.7 and 3.8).

Table 3.6: Surrogate markers of liver function in male and female suckling rats at 20 days of age after feeding with NH and GS supplements for 10 days.

Diet	**Sex**	**ALT** (U/L)	**ALP** (U/L)	**T bil** (µmol/L)
CON	M	26.0 ± 3.4	399.2 ± 40.9	6.0 ± 1.4
	F	26.8 ± 5.0	320.5 ± 30.7$^{\beta}$	7.7 ± 3.1
GSL	M	36.7 ± 7.6*	355.2 ± 13.2	6.4 ± 0.3
	F	35.9 ± 5.6*	311.3 ± 20.3$^{\beta}$	7.0 ± 1.0
NHL	M	27.2 ± 5.4	381.7 ± 7.7	5.0 ± 0.7
	F	28.0 ± 3.5	341.4 ± 18.9$^{\beta}$	5.6 ± 1.0
GSH	M	31.3 ± 2.8	380.8 ± 16.4	5.1 ± 0.7
	F	33.0 ± 6.0*	317.0 ± 27.1$^{\beta}$	6.8 ± 3.9

Table 3.6 Continued

Diet	**Sex**	**ALT** (U/L)	**ALP** (U/L)	**T bil** (µmol/L)
NHH	M	27.1 ± 2.6	434.7 ± 31.1	6.2 ± 1.0
	F	30.3 ± 2.0	363.8 ± 23.0$^{\beta}$	5.1 ± 0.5
nr§		*25 – 32*	*245 – 445*	*5 – 8*

* significantly different down the column ($p < 0.05$); $^{\beta}$ significantly different from corresponding male values ($p < 0.05$); CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50 % GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50 % honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50 % GS solution twice daily); NHH = Natural honey High (20 ml/kg 50 % honey solution twice daily); ALT = alanine transaminase; ALP = alkaline phosphatase; T bil = total bilirubin; nr = normal range of values; § adapted from Kurata *et al*, 2002.

Table 3.7: Surrogate markers of general health assayed in the plasma of suckling male and female rats at 20 days of age after feeding with NH and GS supplements for 10 days.

Diet	**Sex**	**Ca** (mmol/L)	**P** (mmol/L)	**TP** (g/L)	**Alb** (g/L)	**Glob** (g/L)	**Amyl** (U/L)
CON	M	2.8 ± 0.1	3.2 ± 0.2	45.8 ± 2.0	24.5 ± 2.1	21.3 ± 1.3	1389.3 ± 69.0
	F	2.7 ± 0.1	3.1 ± 0.0	43.7 ± 2.2	23.2 ± 1.1	20.5 ± 1.3	1188.3 ± 17.6
GSL	M	2.7 ±0.1	2.9 ± 0.1	44.0 ± 2.2	22.4 ± 0.9	22.0 ± 1.4	1320.4 ± 95.3
	F	2.7 ± 0.1	3.2 ± 0.1	45.1 ± 0.9	22.7 ± 0.9	22.3 ± 0.9	1140.3 ± 46.3
NHL	M	2.6 ± 0.1	3.0 ± 0.1	42.3 ± 1.4	23.9 ± 0.6	18.6 ± 1.7	1318.0 ± 89.5
	F	2.8 ± 0.1	3.4 ± 0.3	44.4 ± 1.3	22.4 ± 0.1	22.0 ± 0.1	1247.6 ± 116.9
GSH	M	2.7 ± 0.1	3.0 ± 0.1	42.9 ± 0.8	21.1 ± 0.9	21.7 ± 0.9	1237.1 ± 67.5
	F	2.8 ± 0.1	3.1 ± 0.3	48.6 ± 2.4	23.4 ± 1.6	25.0 ± 1.0	1322.0 ± 82.0

Table 3.7 Continued

Diet	**Sex**	**Ca** (mmol/L)	**P** (mmol/L)	**TP** (g/L)	**Alb** (g/L)	**Glob** (g/L)	**Amyl** (U/L)
NHH	M	2.8 ± 0.0	3.0 ± 0.1	41.9 ± 1.1	20.1 ± 0.8	21.8 ± 0.7	1205.9 ± 60.9
	F	2.7 ± 0.1	3.0 ± 0.1	43.3 ± 1.3	22.3 ± 0.8	21.2 ± 1.4	1187.5 ± 23.0
nr[§]		*2.4 – 2.9*	*2.5 – 3.5*	*40 – 50*	*20 – 25*	*20 – 25*	*1050 – 1400*

10-day dietary treatments had no effect on all the health values ($p > 0.05$) across the column; CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50 % GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50 % honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50 % GS solution twice daily); NHH = Natural honey High (20 ml/kg 50 % honey solution twice daily); Ca = calcium; P = phosphorus; TP = total protein; Alb = albumin; Glob = globulin; Amyl = amylase; nr = normal range of values; [§] adapted from Kurata *et al*, 2002.

Table 3.8: Plasma concentration of urea, creatinine and urea/creatinine ratio in male and female suckling rats at 20 days of age after feeding with NH and GS supplement for 10 days.

Diet	**Sex**	**Urea** (mg/dL)	**Creatinine** (mg/dL)	**Urea/creatinine ratio**
CON	M	17.20 ± 0.83	0.46 ± 0.03	37.4 ± 6.2
	F	17.87 ± 1.39	0.45 ± 0.02	39.7 ± 5.0
GSL	M	19.72 ± 1.39	0.40 ± 0.02	49.3 ± 5.5
	F	20.47 ± 1.11	0.43 ± 0.02	47.6 ± 7.1
NHL	M	17.51 ± 1.11	0.42 ± 0.03	41.7 ± 7.8
	F	17.94 ± 1.39	0.46 ± 0.02	39.0 ± 5.5
GSH	M	19.16 ± 1.39	0.40 ± 0.02	48.1 ± 6.6
	F	19.68 ± 1.95	0.41 ± 0.04	48.0 ± 11.6

Table 3.8 Continued

Diet	**Sex**	**Urea** (mg/dL)	**Creatinine** (mg/dL)	**Urea/creatinine ratio**
NHH	M	19.61 ± 1.11	0.43 ± 0.02	45.6 ± 6.3
	F	18.69 ± 1.11	0.42 ± 0.05	44.5 ± 11.1
nr[§]	*M*	*11 – 20*	*0.4 – 0.6*	*21.7 – 50.0*
	F	*9 – 34*	*0.4 – 0.6*	*22.0 – 85.0*

10-day dietary treatments had no effect on all the renal function values ($p > 0.05$) across the column; CON = 10 ml/kg distilled water twice daily; GSL = Golden syrup Low (10 ml/kg 50 % GS solution twice daily); NHL = Natural honey Low (10 ml/kg 50 % honey solution twice daily); GSH = Golden syrup High (20 ml/kg 50 % GS solution twice daily); NHH = Natural honey High (20 ml/kg 50 % honey solution twice daily); nr = normal range of values; [§] adapted from Hilltop Lab Animals Inc, 2013.

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3.3 – Discussion

The investigations constitute the first study on the acute effects of natural honey compared with cane syrup administered orally (over a 10-day period) on metabolic activities in 10-day old suckling male and female Sprague-Dawley (SD) rats. The cane syrup supplement was fed in form of golden syrup to the rat pups. Although, there are anecdotal reports encouraging the feeding of honey to new born babies by some customs and traditions [Menshikov and Feidman, 1949; Khotkina, 1955; Slobodianiuk and Slobodianiuk, 1969; Al-Bukhari, 1976], few studies give scientific credence to this practice [Haffejee and Moosa, 1985; MacMillan, 1999; Santos and Antonini, 2008]. The inclusion of sugars in infant dietary formulations has also been documented [Rivero-Urgell and Santamaria-Orleans, 2001].

Rats are altricial species, whose pups are born after a short gestation period [Henning, 1981]. As such the pups closely depend on their dams for nutrition, and do not achieve independence before weaning (21 days). The intervention for sampling was done at the age of 20 days in these pups in order to minimise confounding factors such as feeding on other substances in the environment apart from suckling and the experimental dietary supplements. As it is known that the pups eyes are closed at birth, and normally open at about 14 days of age [Kohn and Clifford, 2002], and during the third week of life become gradually conscious of their environment. Thus, the pups could start to explore the ingestion of more particulate materials in their environment as soon as their eyes open. Furthermore, the pre-weaning period is a developmental window characterized by the transition from immature GIT of the pups to a mature intestinal system at adulthood [Teichberg *et al*, 1992; Pacha, 2000]. At birth, the intestinal epithelium of the

immature GIT has a relatively high permeability for macromolecules (in the milk and other supplements) through the transcellular pathway. During the transitional period from birth to weaning, the macromolecules transport and nutrients absorption decreases, and terminates at about 21 days after birth [Teichberg *et al*, 1992]. These macromolecules from colostrum and milk include immunoglobulins, growth factors and food antigens [Teichberg *et al*, 1992; Pacha, 2000]. Thus, at 20 days old, the pups could still benefit from the nutritional values of suckling as well as the NH and GS supplements.

The undiluted forms of the NH and GS 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 macronutrient values. However, NH contains minerals, vitamins and other micronutrients according to previous report [White and Doner, 1980], and thus has a higher value of ash than GS (Table 2.2). These micronutrients could have influence on the milk intake of the NH-fed pups from their dams, and consequently affects nutrients utilization of the rat pups. Nonetheless, the differential dietary composition of the supplements appeared to translate into neither nutritional benefit, feed efficiency, nor body weight gain (BWG) of any of the groups. This reflected in similar body weight gain of all the experimental animals over the 10-day dietary treatment period. This observation of identical BWG in the current study was contrary to the findings of previous workers. According to Heidi *et al* (1984), the rate of growth and development of rat pups can be greatly altered by varying supplements administered to them during the suckling period. This was also corroborated by results from other animal infants [Miller and Miller, 1960; Ott and Asquith, 1989], and human babies

[Bianchi, 1977; Rivero-Urgell and Santamaria-Orleans, 2001]. However, BWG alone is not a reliable measure of growth because according to some reports it (BWG) may be influenced by various factors like food intake, obesity and hydration status [Hunziker and Schenk, 1989; Deng *et al*, 2001]. As discussed in phase 1 (chapter 2), the tibial length has been used in several studies [Hunziker and Schenk, 1989; Tjaderhane and Larmas, 1998; Fritton *et al*, 2005] as a more accurate indicator of linear growth instead of the BWG. In addition, I hypothesised in chapter 1 (see section 1.6, sub section i), that consumption of natural honey would promote body weight gain and linear growth (compared to the normal diet and the sucrose diet).

In order to test the second part of the above hypothesis of my study, and further explore the growth effects of these supplements, the linear growth of the pups was evaluated. This assessment was done through the measurements of weight and length of the long bones (femur and tibia) as shown in Table 3.1. The results obtained showed that the bones were not significantly different in length amongst the groups. There were also marginal insignificant differences in the bones' weight and the bone density calculated as mg/mm (Table 3.1). There were no differences in weight and density of both bones between male NHH and control rats, as well as amongst all the female groups. There were also no gender differences in the linear growth. Oestrogen and testosterone are sex hormones with influence on bone formation, growth and maintenance [Chiang *et al*, 2009; Cunha *et al*, 2010; Peralta *et al*, 1994; Prakasam *et al*, 1999]. These hormones are secreted in large amounts at puberty [Peralta *et al*, 1994; Prakasam *et al*, 1999; Sinnesael *et al*,

2011]. As the intervention in these animals in the acute phase (phase one) of the present study was done pre-weaning, the rats could not benefit from the post-pubertal influence of these hormones on linear growth. This may explain the absence of significant difference in bone growth, as well as the absence of gender differences in linear growth amongst the animals.

In suckling animals, the intestinal degradation of substances such as the dietary supplements (NH and GS) is low unlike in weanling animals [Rao *et al*, 1993, 1998; Phillips *et al*, 1995; Shen and Xu, 1998; Pacha, 2000]. This is due to the protection of the colostrum present in the milk of suckling animals and infants on gastrointestinal hormones such as gastrin, secretin and cholecystokinin (CCK) from luminal hydrolysis in the small intestine [Xu *et al*, 1996; Rivero-Urgell and Santamaria-Orleans, 2001; Pacha *et al*, 2003; Strader and Woods, 2005]. These hormones are more stable, and thus exert their effects for a longer period on the digestive tracts of suckling animals relative to weanlings [Xu *et al*, 1996]. This causes reduction in the passage rate of luminal contents of the intestines [Britton and Koldovsky, 1988, 1989; Pacha, 2000]. This could facilitate the absorption of intact biologically active substances and nutrients from the intestine. These substances could influence the proliferation and differentiation of intestinal cells [Burrin *et al*, 1995, 1996; Philipps *et al*, 1995, 1997; Houle *et al*, 1997; Pacha, 2000]. Consequently, this will promote intestinal growth as seen in the present study whereby the absolute and relative values (g, % BW and g/cm) of the treatment animals' small intestines were significantly higher ($p < 0.05$) than those recorded in the control rats. In addition, incomplete absorption or loss of any nutrients from the small intestine of suckling animals could be salvaged by

bacterial fermentation in the caecum [Mackie *et al*, 1999], with consequent increased weight of the caecum [Pacha, 2000; Muegge *et al*, 2011]. The higher absolute weight of the treatment caeca relative to the caecal weight of the control rats in the present study lends some support to these theories. These findings tallied with the improvement in the growth of the caecum resulting from dietary manipulation earlier reported by some workers [Munro *et al*, 1998; Erlwanger and Cooper, 2008]. These workers observed a significantly heavier caecal weight in the rats fed erythritol or alcohol extracts of African potato relative to that of control rats.

The eating of NH by animal and human infants could not be associated with any metabolic risks. In 2009, Chepulis and co-workers concluded from their New Zealand study on rats that early introduction of honey diet has nutritional and health benefits compared with artificial sugars. In a review on the importance of honey relative to sucrose in children's nutrition, honey-fed infants were found to have improved haematological profiles and calcium uptake, no digestion problem, lighter and thinner faeces, better skin colour, less susceptibility to diseases, and steady weight gain [Bianchi, 1977]. These positive effects of honey in infant nutrition are attributed to its effects on the digestion process, and one possible cause is the effect of honey constituent, oligosaccharides on intestinal flora of these children [Rivero-Urgell and Santamaria-Orleans, 2001].

The NFG and NFT concentrations in suckling male and female rats at 20 days of age shown in Table 3.2 indicated no significant difference ($p > 0.05$) amongst the treatment and control groups. The results of these circulating metabolic substrates suggested that the experimental animals in this study were exposed to sub-adverse levels of refined sugar, as several previous studies have associated high sugar intake with hyperglycaemia and hypertriglyceridemia [Elwood *et al*, 1970; Birch *et al*, 1989; Bellisle and Rolland-Cachera, 2001; Ludwig *et al*, 2001; Elliot *et al*, 2002; Cook *et al*, 2003; Ford and Giles, 2003; Bray *et al*, 2004; Cruz and Goran, 2003; Deen, 2004; Schulze *et al*, 2004; Betts *et al*, 2005; Gaby, 2005; Cao *et al*, 2007; Dubois *et al*, 2007; Promdee *et al*, 2007; Ruotinnen *et al*, 2008; Tappy and Le, 2010]. This may be the evidence that the dietary supplements did not alter the endocrine pancreatic function, which could have an indirect influence on the blood glucose concentration.

Lipid is the principal source of energy for metabolism in the suckling rats, and the immature intestine is also able to absorb fatty acids and cholesterol [Meddings and Theisen, 1989; Perin *et al*, 1997]. The lipolytic products are easily resynthesized into triglycerides, phospholipids, and cholesterol because the activity of reesterification enzymes in the immature intestine is as high as it is in adulthood [Shiau *et al*, 1979]. The proximate analyses of the dietary supplements shown in Table 2.2 indicated that the GS-fed pups would have consumed a higher amount of sucrose (26.03 g/100g) than the NH pups (2.03 g/100g). The breakdown of the excess sucrose eaten by the GS rats into its constituent monosaccharides (glucose and fructose) placed additional metabolic burden on the GS livers, leading to increased blood concentrations of FFAs and TGs formed from their

glycerol metabolite. Consequently, the FFAs produced from the hepatic lipolysis entered the blood system, and there is excess circulating FFAs in the GS rats, which is significantly higher ($p < 0.0001$) than that recorded for the suckling rats fed with NH supplements (Table 3.2). In addition, the presence of fat droplets in the photomicrographs of the GS pups' livers further strengthened the clinical chemistry results. This portends the development of hepatic steatosis in the GS-fed pups, that could later manifest as NAFLD with prolonged feeding as observed with the adult GS rats in phase one.

There was no gender differences in all the parameters measured in this phase of the study, except the lower values of alkaline phosphatase of the female groups relative to their male littermates. However, these values were within the normal range as shown in Table 3.6. The absence of gender differences in the neonatal rats fed with cane syrup in this study plausibly confirmed that the resistance to MetS by the female rats could be associated with age. The pointer to this theory is that the hormone, oestrogen responsible for the antioxidative protection of the females manifest in large amount during the adult age of the rodent's life [Ramirez and Sawyer, 1965; Busserolles *et al*, 2002b; Galipeau *et al*, 2002]. These findings suggested that male and female pups, and by extension human babies (irrespective of sex) are equally susceptible to the diet induced metabolic dysfunction.

3.4 – Conclusions

The findings showed that both artificial (GS) and natural (NH) sugars were well tolerated when consumed by neonatal animals. However, the marginal insignificant differences of most of the metabolic substrates amongst the groups suggested that adverse metabolic changes could be influenced by the cumulative effects of chronic intake of refined sugars, exposure level and age effects. These findings can be extended to human babies, and as such it is advisable that children should eat refined sugars with caution by avoiding excessive consumption.

The study showed that despite the rapid growth phase of the neonatal pups, the adverse effects associated with the consumption of refined sugars, did not manifest prominently within the short term. However, the presence of fatty livers in the GS-fed pups attested to the fact that metabolic diseases could develop with chronic exposure as elaboratorily discussed during the adult phase (chapter 2).

The lack of gender differences in this acute phase of the study informed the conclusion that the factors facilitating metabolic protection are adult hormones. Thus, the little amount of oestrogen secreted during the neonatal life did not attain protective level in the unweaned rats.

CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATIONS

CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATIONS

4.0 – Introduction

This study investigated the long and short terms effects of natural honey as a dietary substitute to artificial sugars, established animal models of metabolic syndrome in cane syrup fed rats, and described the protective values of natural honey against metabolic diseases. This chapter summarizes the findings of the study. Thereafter, it focuses on the strengths and limitations of the study, the recommendations for future experimental research and applications of the findings to the food and health industries.

4. 1 – Conclusions on the study objectives in adult phase

I hypothesized that consumption of natural honey could not promote body weight gain and linear growth; the intake of natural honey would increase the concentrations of metabolic substrates in circulation and storage; there could be adverse influence on the morphology and morphometry of the GIT and metabolic tissues due to the consumption of natural honey; and feeding of natural honey as dietary supplement to rats could not have any gender effects in animals. My findings were as follow:

i. Honey-fed rats had normal body weight gain and improved linear growth showing the healthy growth effects of eating natural honey, unlike the GS-fed rats observed to have suffered reduced growth at termination.

ii. Natural honey (NH) is a healthy dietary source of sugars. Thus, natural honey did not result in pathological changes such as dyslipidaemia, abnormal high circulating and stored metabolic substrates, hepatic dysfunction, and fatty livers observed with cane syrup consumption.

iii. Natural honey induced enhanced structural development of the intestinal morphology predominantly improved intestinal villi, pancreas and caecal growth in the male rats relative to the GS-fed rats. These could translate to enhanced functional capabilities of the pancreas and the gastrointestinal tract. These findings demonstrated that eating NH could improve the digestive and metabolic health of honey consumers.

iv. The gender effects of eating NH or cane syrup were higher body weight gain and linear growth of the males compared to females

v. There was reduced susceptibility of female rats to metabolic syndrome (MetS) shown by the presence of few MetS traits in the female GS-fed rats contrary to the full blown metabolic syndrome observed in the male GS rats.

4. 2 – Conclusions on the study objectives in neonatal phase

My findings from the neonatal phase of the study were as described below. The 10-day dietary supplements appeared not to confer any nutritional benefit on the rat pups, and as such all pups had similar body weight gain and linear growth. The signs of dyslipidaemia and presence of fatty livers in the GS-fed pups attested to the fact that cane syrup could cause susceptibility to metabolic syndrome in

neonates. The marginal insignificant higher levels of the metabolic substrates induced by GS feeding showed that adverse metabolic changes could become full blown metabolic syndrome with increased age and increased exposure to cane syrup feeding, and as such it is advisable that children should eat refined sugars with caution, and avoid its excessive consumption.

The feeding of NH or GS dietary supplement had a positive influence on the immature intestinal morphometry shown mainly as induction of caecal growth in the suckling rats.

The presence of MetS traits in the female GS-fed pups similar to their male littermates suggested equal susceptibility of both sexes to MetS.

4.3 – Study limitations

This investigation was essentially a descriptive study, and issues such as physiological mechanisms were not investigated. However, speculation supported by appropriate literature has been included to explain the findings. Nonetheless, further studies are recommended to support these speculations, establish physiological mechanisms, and strengthen the findings of the present study. The limitations of this study that necessitated further investigation include the following:

a. The age effects of the influence of honey on metabolic health could have been progressively monitored at several stages over a long period of time. This would have provided information on whether or

not ageing or senility, which is associated with cellular and degenerative changes play any role in the reduction of the honey's protective effects.

b. Lack of molecular investigations such as measurement of glucose transporters (Gluts) genes and proteins expressions using RT-PCR (Reverse transcription Polymerase Chain reaction) and Western blot techniques respectively. These could be used to corroborate the findings on the blood concentrations of metabolic substrates (glucose and lipids). The determination of adenosine monophosphate-activated protein kinase (AMPK) is also another molecular assessment that could be used to monitor the regulation of metabolic activities and energy homeostasis of metabolic tissues mainly the intestines, liver, kidneys and adipose tissues.

c. The non-inclusion of dual energy X-ray absorptiometry (DEXA) scans assessment for subcutaneous fat distribution. Thus, the scope of fat measurements in these experimental rats was restricted to visceral fat alone.

d. The inflammatory response to cane syrup intake observed as accumulation of Kupffer cells in the GS-fed rats could also have been further established with the measurement of markers of inflammation such as cytokines, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and plasma viscosity.

These limitations coupled with the rising profiles of natural honey in the food and health industries make it imperative for further experimental and clinical trials. Thus, these investigations are being envisaged for future studies.

4. 4 – Recommendations for further studies

The normal BWG and enhanced linear growth of the NH-fed rats should be explored further, through the assay of the key growth regulating factors such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1), and microscopic assessment of the growth plates. In addition, the dual energy X-ray absorptiometry (DEXA) scanners could be used to measure the bone mineral content (BMC) and bone mineral density (BMD). The evaluation of the degree of bone mineralization and density would not only strengthen the linear growth determination, but also complement the results of the plasma concentrations of calcium and phosphorus.

Future experimental studies could feed control diet made of sugar solutions with vitamin and mineral supplements to animals and/or to human participants in clinical trials. This control solution is to simulate the nutritional composition of natural honey. When comparative investigations involving the dietary use of NH alongside the control diet, any differential effects observed can be attributed to the phytochemicals in honey. In addition, other studies could also be directed at the identification, isolation, characterisation and extraction of the different phytochemical constituents of NH individually for further investigation of their properties. This is with a view to ascertain which of the components has high nutritional and health benefits. If identified such components could probably be

simulated and produced in commercial quantities for use as food supplements and other relevant use in the health industry.

There is also the need for further work to establish the physiological mechanisms behind this metabolic protection and the health benefits of honey. Further studies could also entail inducing metabolic syndrome in animal models with the initial oral administration of artificial sugars. The induction of fully developed characteristics of metabolic syndrome could then be followed by feeding honey for short and long periods to observe any probable reversal or neutralizing effects of NH on the metabolic adversity of GS. In addition, feeding cane syrup simultaneously with NH could be used to evaluate the protective strength of honey against MetS. This is even more important as the current study and other animal studies as well as human clinical trials show that adult obesity could be primed from an early age.

There is need for further studies to assess the microfloral population of the GIT vis-à-vis the role of dietary supplements such as natural honey and cane syrup. In this search, the effect of honey and its phytochemical constituents on the gut microbial composition should be investigated.

The conflicting findings on female metabolic response to artificial and natural sugars dietary treatments suggested that there is need for further gender based studies to clarify the protective status of females exposed to metabolic risks from dietary intake of excess refined sugars, and probably resolve the controversy.

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APPENDIX

ETHICS CLEARANCE CERTIFICATE

AESC 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

CLEARANCE CERTIFICATE NO. 2010/29/2B

APPLICANT: **Dr A Ajibola**

SCHOOL: Physiology
DEPARTMENT:
LOCATION:

PROJECT TITLE: **Effects of dietary supplementation with pure natural honey on metabolism in growing Sprague Dawley rats**

Number and Species

20 female Sprague Dawley rats and 200 Sprague Dawley pups

Approval was given for the use of animals for the project described above at an AESC meeting held on 25.05.2010. This approval remains valid until 25.05.2012

The use of these animals is subject to AESC guidelines for the use and care of animals, is limited to the procedures described in the application form and to the following additional conditions:

None

Signed: _____ Date: 02/06/2010
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 02/06/2010
(Registered Veterinarian)

cc: Supervisor:
Director: CAS

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