

Biomarkers of Metabolic Syndrome in Honey Fed Wistar Rats

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ABSTRACT

Recent findings indicate that a high fructose diet induces features of metabolic syndrome in both humans and laboratory rats. Honey contains a high amount of fructose and its effects on the risk of metabolic syndrome have not been fully documented. Therefore, the aim of this study was to assess the effects of the changes in risk biomarkers of honey feeding on metabolic syndrome. Forty-nine (49) Wistar rats (n=7/group) were fed for 4 weeks with semi-purified diet containing 65g/100g carbohydrate (control) and 20% (group B), 30% (group C) and 40% (group D) honey or fructose/glucose quantities equivalent to the amount in 20% (group E), 30% (group F) and 40% (group G) honey. After biochemical assay, it was observed that honey and fructose caused significant increase ($P < 0.05$) in serum cholesterol, triacylglycerol (TAG), LDL-cholesterol, uric acid, glucose and body weight of the experimental animals, but reduced ($P < 0.05$) HDL-cholesterol. Thus honey or fructose feeding has a measure of risk regarding metabolic syndrome. Like fructose, the consumption of honey may confer similar metabolic disorder. A considerable amount of research is required to completely appreciate the implications of the increased biomarkers of metabolic syndrome.

Key words: Metabolic syndrome, honey fructose, cholesterol, triacylglycerol, glucose.

INTRODUCTION

The new millennium has witnessed the emergence of a modern epidemic, the metabolic syndrome, with frightful consequences to the health of humans world wide. The westernization of diets, with an increase in availability of high calorie foods contributes to the epidemic (Basciano, *et al.*, 2005). Metabolic syndrome is a combination of medical disorders characterized mainly by insulin resistance, cardiovascular diseases and oxidative stress which may result from risk factors like diet, obesity, genetic disposition, diabetes mellitus, stress and sedentary lifestyle. Increased consumption of high fructose diet has been observed to induce features characteristic of metabolic syndrome in both laboratory animals and humans (Basciano, *et al.*, 2005). Honey, a complex biological product of bees is presently being promoted as sugar substitute (sweetener), flavour enhancer, and in the treatment of certain ailments leading to increased demands and consumption in

our society. Honey contains high amounts of fructose (Al-Waili, 2004), and whether its consumption is also associated with the risk of metabolic syndrome is not well documented. Attempt is therefore made to report the influence of honey consumption on biomarkers used to characterize the medical disorders (insulin resistance, cardiovascular dysfunction and oxidative stress) of the syndrome.

MATERIAL AND METHODS

Animal Handling and Experimental Design

Forty-nine (49) Wistar albino rats of both sexes were obtained from Animal Unit, Faculty of Pharmacy, University of Benin, Nigeria. They were adult rats weighing approximately 83.9g. They were maintained in Animal Laboratory Unit, Faculty of Basic Medical Sciences, Delta State University Abraka, Nigeria and housed in wired bottomed cages (seven rats per cage) at room temperature

(25°-28° C) with a twelve hour light/twelve hour dark cycle.

All rats were first adapted to a starch based semi-purified diet (growers' mash) obtained from Flour Mill Company, Sapele, Delta State. They were then randomly assigned to seven groups (A-G) and fed for 28 days with diets composed from the growers' mash and honey or fructose as shown (Table 1). They were allowed free access to water and feed.

The honey sample was obtained from a local farmer in Kafachan, Kaduna State, Nigeria. The food grade fructose and glucose were obtained from BDH Chemicals Limited, Poole, England.

Animal care and handling conform to the guidelines of the National Institute of Health (NRC, 1985) and the recommendations of the INRA Ethics Committee in accordance with degree no. 87-848.

Sample Collection

At the end of the 28 days of dietary treatment, the rats were starved overnight for 12 hours then anaesthetized with chloroform soaked cotton wool and blood samples were collected by heart puncture into plain, sterile tubes (Bioblock Scientific, Ilikrich, France) using disposable hypodermic needle and syringe. Blood samples were thereafter centrifuged at 2000 x g for 15 min to remove serum which was stored frozen in bijoux bottle until required for analysis.

Assays

Triacylglycerol was determined in serum by enzymatic procedures (Fossati and Principle, 1982) and serum HDL-cholesterol level by enzymatic- colorimetric method (Burstin and Mortin, 1969). Glucose concentration in serum was estimated by the glucose oxidase method (Barham and Trinder, 1972). The uric acid contents in serum were quantified by enzymatic-colorimetric method (Caraway, 1963). Total cholesterol level in serum was estimated by enzymatic method (Holvey, 1972). Reagents used were supplied in commercial kits by Randox Laboratories Ltd., Ardmore, United Kingdom.

RESULTS

Changes in biomarkers of the features (cardiovascular dysfunction, insulin resistance and oxidative stress) characteristic of metabolic syndrome induced by honey feeding are shown in Table 2.

The results (Table 2) indicate that honey or fructose feeding significantly increased ($P<0.05$) serum TAG concentration in a dose dependent manner when compared with the control rats. Honey and fructose fed rats show increased serum glucose concentrations when compared with the control group and the increases were dose dependent. Differences among the experimental groups (honey and fructose) were not significant ($P>0.05$), but these experimental values were higher ($P<0.05$) than the control value.

Table 1: Diet composition for the various groups of rats

Diet composition	Group						
	A	B	C	D	E	F	G
Growers mash (g)	100	80	70	60	87.2	80.8	74.4
Honey (g)	-	20	30	40	-	-	-
Fructose (g)	-	-	-	-	7.8	11.7	15.6
Glucose (g)	-	-	-	-	5.0	7.5	10.0
Total (g)	100	100	100	100	100	100	100

100g growers mash contain: protein (13%), fat (11%), fibre (7.5%), starch (50%), glucose (15%), calcium (1.0%), average phosphorus (0.5%), sucrose (0.5%), fructooligosaccharide, FOS (1.2%), minerals, vitamins, polyphenols (0.03%) (Stefan, *et al.*, 2008).

Table 2: Changes in risk biomarkers of metabolic syndrome induced by honey in Wistar rats

Features of metabolic syndrome	Groups						
	A	B	C	D	E	F	G
Cardiovascular dysfunction markers.							
Cholesterol (mmol/L)	2.97±0.32	2.82±0.26	2.74±0.23	2.68±0.35	3.44±0.52	3.23±0.31	3.02±0.29
‘ HDL-Cholesterol (mmol/L)	0.94±0.08	0.76±0.04	0.52±0.06	0.46±0.03	0.68±0.12	0.48±0.07	0.44±0.06
‘ TAG(mmol/L)	0.53±0.14	0.79±0.07	0.88±0.15	0.94±0.10	0.77±0.09	0.79±0.06	0.82±0.09
‘ LDL-Cholesterol (mmol/L)	0.50±0.21	1.90±0.37	2.04±0.22	2.41±0.18	2.61±0.36	2.60±0.24	2.42±0.30
Insulin resistance markers							
‘ Glucose (mmol/L)	2.86±0.17	3.19±0.10	3.22±0.09	3.25±0.06	3.28±0.16	3.33±0.17	3.44±0.31
‘ %Weight gain	13.34	48.86	49.49	42.96	48.81	39.03	37.85
‘ %Weight gain vs glucose ratio	4.65	15.30	15.34	13.20	14.87	11.71	10.99
Oxidative Stress markers							
‘ Uric Acid (mmol/L)	0.18±0.03	0.20±0.04	0.20±0.04	0.21±0.01	0.21±0.02	0.21±0.03	0.21±0.03
‘ Glucose (mmol/L)	2.86±0.17	3.19±0.10	3.22±0.09	3.25±0.06	3.28±0.16	3.33±0.17	3.44±0.31

Values are written as Mean ± Standard Deviation.

n=7rats/group

Group A-control group (100% growers' mash)

Group B-20% honey (20% honey +80% growers' mash)

Group C-30% honey (30% honey + 70% growers' mash)

Group D-40% honey (40% honey + 60% growers' mash)

Group E-Fructose (7.8%) and glucose (5%) amounts equivalent in 20% honey

Group F-Fructose (11.7%) and glucose (7.5%) amounts equivalent in 30% honey

Group G-Fructose (15.6%) and glucose (10%) amounts equivalent in 40% honey.

HDL-cholesterol concentration shows a gradual decrease in experimental rats based on the dosage of either honey or fructose administered. The serum total cholesterol concentration of honey fed rats decreased gradually as doses increase but that of fructose fed rat increased.

The LDL-cholesterol concentration of honey and fructose fed rats increased based on the dose administered and these levels were significantly ($P < 0.05$) different from the control value. Data (Table 2) show that honey and fructose diets increased serum uric acid and glucose levels, indicating increased measure of oxidative stress.

Weight changes were highest among the animals fed with 30% honey (group C).

DISCUSSION

Hypercholesterolemia is as a result of higher concentration of LDL-cholesterol and lower concentration of HDL-cholesterol and is strongly associated with cardiovascular diseases, a risk factor of metabolic syndrome. LDL-cholesterol contributes to cardiovascular diseases by promoting atheroma development in arteries. High HDL level is associated with reduced cardiovascular diseases and conversely, low HDL-cholesterol level is associated with increased cardiovascular risk. Honey or fructose fed rats show increasing LDL-cholesterol and decreasing HDL-cholesterol, thus, linking honey or fructose consumption to metabolic syndrome.

Honey and fructose at the highest dose (40%) significantly ($P < 0.05$) increased glucose level and weight gain of the experimental rats indicating a measure of glucose intolerance and probably insulin resistance. Increased in weight have been observed to induce glucose intolerance and exacerbate insulin resistance (Elliot, *et al.*, 2002).

Observations reveal high risk of glucose intolerance, evidenced by the increased amount of glucose in serum, greater susceptibility to cardiovascular dysfunction (Mercola, 1997), and enhanced vulnerability to oxidative stress shown by the levels of serum uric acid and glucose among

the honey and fructose fed rats compared with the control. These biochemical features characterize insulin resistance as have been reported in isolated studies (Festa, 2000; Busserolles, *et al.*, 2002; O'Doherty, *et al.*, 1997). It follows that honey diets like fructose could also induce insulin resistance, though the risk is higher with fructose diets as study reveals.

Insulin is also known to regulate lipid synthesis and secretion (Benneth, *et al.*, 1995). Feeding rats fructose stimulated fatty acid synthesis and created a 56% increase in TAG secretion rate and an 86% increase in plasma TAG (Kazumi, *et al.*, 1997). Hallfrisch, *et al.* (1983) have also demonstrated that in humans, TAG concentration increases as the amount of fructose taken also increased. Maybe because of its high fructose content, honey also has the potential of increasing serum TAG levels, but in addition, it reduces the HDL-cholesterol content – an antiatherogenic lipoprotein fraction. Abnormally high levels of TAG may be a risk factor for atherosclerosis. It is however, difficult to conclusively prove that elevated TAG by itself can cause atherosclerosis. It is however, associated with risk factor of atherosclerosis such as obesity, low HDL-cholesterol, high LDL-cholesterol and insulin resistance.

Again, fructose-induced changes in TAG modify hepatic VLDL secretion, and this modification has been demonstrated to cause less protection to oxidative stress (Noguchi and Tanaka, 2003), and certain intermediates (glyceraldehydes) in the metabolism of fructose are known to generate superoxide anions, a reactive oxygen species (ROS) (Hallfrisch, 1990). ROS interact with NO and oxidize it to NO_3 (Bailey, *et al.*, 1999). The generated NO_3 stimulates the activity of xanthine oxidase (XO) – an ROS generating enzyme (Nishino, 1994). Although uric acid, the catalytic product of XO is an antioxidant, it appears that the rate of ROS generation overwhelms its antioxidant capacity because increased levels of uric acid arising from a stimulated XO activity exacerbate insulin resistance and promote oxidative stress (Nishino, 1994). The metabolism of honey also appears to enhance the generation of ROS, as indicated by the increased level of uric acid.

Evidence shows that honey or fructose feeding to rats might contribute to the development of metabolic syndrome. This is so, because such feeding increased the risk biomarkers of metabolic syndrome, though proportionately, fructose caused a higher risk compared with honey. The consumption of honey should be cautioned until further researches define its metabolic safety, since experimental data from present study appears to implicate honey as risk factor of metabolic syndrome in experimental animals.

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