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Beneficial Effects of Low-Calorie-Carbohydrate/High-Agar Diet on Cardiometabolic Disorders Associated with Non-Alcoholic Fatty Liver Disease in Obese Rats

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ABSTRACT: Energy restriction and low carbohydrate diets are recommended as nutrition therapies to prevent becoming overweight or obese. However, their beneficial effects in non-alcoholic fatty liver disease (NAFLD) are less well investigated. In addition, the effects of the type of polysaccharides incorporated into these diets and their contents have been scarcely studied. Therefore, this study aimed to elucidate whether low-calorie-carbohydrate high-agar diets could improve liver metabolic dysfunction, membrane fluidity, oxidative damage, and endothelial dysfunction in obese rats. Obesity was induced by feeding rats a high-fat diet (HFD) for 10 weeks. The obese rats were then divided into two homogenous groups: the first group was fed low-calorie-carbohydrate/high-agar diet (LCC/HA) and the second continued to consume the HFD for 4 weeks [obese control (Ob-C)]. Normo-ponderal rats were fed a normal diet during the entire study, and were used as the control (N-C). Compared with the Ob-C group, body weight, hepatic lipids, low density lipoproteins cholesterol (C), the non esterified cholesterol/phospholipids ratio, serum transaminases activities, and lipid peroxidation markers (thiobarbituric acid reactive substances and lipid hydroperoxides) were reduced in LCC/HA group ($P<0.05$). However, the serum concentration of high density lipoproteins-C was enhanced ($P<0.05$). In addition, we observed improved antioxidant defence and endothelial dysfunction associated with antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase ($P<0.05$), and nitric oxide level ($P<0.05$). These findings suggest that hypocaloric diets low in energy and carbohydrates and rich in agar may be beneficial against HFD-induced hepatic steatosis damage, and may be a promising therapeutic strategy to counteract NAFLD development associated with obesity.

Keywords: liver steatosis, low carbohydrate diet, agar, oxidative stress, nitric oxide

INTRODUCTION

Non-communicable diseases are the most prevalent cause of morbidity and mortality in the world (Mozaffarian, 2017), even in developing countries (Mensah et al., 2017). Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease worldwide, with prevalence reaching 25% in the global population (Araújo et al., 2018). Obesity, hyperlipidemia, diabetes mellitus, metabolic syndrome, and insulin resistance (IR) are well-known pathological features of NAFLD (Lonardo et al., 2015; Younossi, 2019). Hepatic abnormalities were characterized principally by triacylglycerol (TG) accumlation that induces liver fat (hepatic steatosis) (Nassir et al., 2015). Liver lipids storage results from an imbalance between lipid acquisition [from adipose tissue-derived fatty acids (FA) and *de novo* lipogenesis] and lipid clearance (TG-rich lipoprotein secretion) (Cohen et al., 2011; Gong et al., 2017). An increasing body of evidence shows that NAFLD is not only a potentially progressive liver disease, but also has systemic consequences. Among mechanisms linking cardiovascular disease (CVD) risk with hepatic steatosis, the most prominent factors appeared to be IR, low-grade chronic inflammation and hyperlipidemia (Bhatia et al., 2012; Nseir et al., 2011). Oxidative stress plays a key role in the initiation and progression of both NAFLD and CVD. An excessive production of reactive oxygen species (ROS) is responsible for low density lipoprotein (LDL) oxidation (Mangge et al., 2014), which may promote transformation of macrophages into foam cells; this represents the first step in the formation of atherosclerotic lesions. In many clinical studies, elevated

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systemic markers of oxidative stress, specifically lipid peroxidation, have been found in patients with NAFLD (Morita et al., 2012; Gaens et al., 2012).

The relationship between type of diet and NAFLD development is complex and extends beyond total energy intake. Low-calorie (LC) diets have been commonly recommended as nutrition therapy to prevent patients becoming overweight or obese, and protect against associated complications. Studies have shown potentially beneficial effects of LC diets (low in carbohydrate and rich in fiber) on obesity by suppressing the lipogenesis pathway in the liver and attenuating hepatic steatosis, inflammatory processes, and oxidative stress (Linden et al., 2016; Kim et al., 2016). Independently of high energy intake, the macronutrient composition of the diet, particularly consumption of carbohydrates (CHO), sugars, fats, and proteins, and low fiber intake may be associated with risk of NAFLD (Eslamparast et al., 2017). The low-CHO energy-restricted diets (<50 g/d and ≤45% of daily calorie intake) had become a strategy for management of weight and metabolic disorders. In this context, several studies have indicated that low-CHO diets may be effective in rapidly improving intrahepatic lipid levels and biochemical markers of liver function (Rodríguez-Hernández et al., 2011; Browning et al., 2011; Volynets et al., 2013). The existing literature do not describe the specific or simultaneous action of energy and nutrient intake, but just highlight foods and/or functional compounds with anti-obesity properties (Eslamparast et al., 2017).

Previous studies have suggested that high soluble fiber intake reduces body weight and is inversely correlated with LDL-cholesterol (LDL-C) level, hepatic lipid accumulation, plasma transaminases concentration, IR, and NAFLD fibrosis score (Chen et al., 2017; Krawczyk et al., 2018; Cantero et al., 2017). Agar-agar is the main component of the cell wall of marine red algae, and is widely used as functional food for reducing body weight (Hong et al., 2017). The health benefits of agar-agar are attributed to the antioxidant, anti-inflammatory, anti-diabetic, anti-obesity, and anti-atherogenic properties of soluble fiber (Maeda et al., 2005; Kim et al., 2008; Seo et al., 2012; Kang et al., 2016), which represents 80% of agar-agar. A recent study showed that agar extracted from *Gelidium amansii* is beneficial for reducing body weight, reducing amounts of adipose tissue, plasma total cholesterol (TC) and TG, reducing TG content in adipose tissue, and reducing hepatic lipid accumulation, by decreasing cholesterol absorption and increasing bile acid and fecal fat excretion in an animal model (Yang et al., 2017; Yang et al., 2019). However, studies examining the effects of diets low in energy and carbohydrate and high in agar on preventing NAFLD progression and obesity-associated CVD risk are missing. Therefore, the aim of this study was to investigate the effects of low-calorie-carbohydrate/high-agar diet (LCC/HA) on overall liver function, including intrahepatic lipid and aminotransferases activities, membrane fluidity, oxidative stress, and nitric oxide (NO) bioavailability in the serum and tissues of obese rats.

MATERIALS AND METHODS

Animals and diets

Albinus male Wistar rats (Pasteur Institute, Algiers, Algeria) (n=18) aged four weeks old and weighing 63±5 g were used during this study. The animals were housed in standard environmental conditions (temperature 24°C, humidity 60%, and with a 12-h light/dark cycle) and had free access to food and water. The experimental protocol used was approved by the Institutional Animal Care and Use Committee at University Oran 1, Oran, Algeria (Approval no. PNR 047, LNCMW0911000). The ethical aspects were carefully followed according to the Council of European Communities (1986) general guidelines for the care and use of laboratory animals. In order to induce obesity, an experimental group (n=12) was received a high-fat diet (HFD) for ten weeks. After this period and at a body weight (BW) of 405±14 g, the obese rats were divided into two groups (n=6). The first group was fed a LCC/HA diet (277 kcal/100 g with 34% CHO and 31% agar) and the second continued to consume the HFD (456 kcal/100 g) for 28 days [obese control (Ob-C)]. The normo-ponderal rats (N-C group) (n=6) were fed with the normal diet (381 kcal/100 g) throughout the experiment.

Body weight and food consumption were recorded weekly and daily, respectively. Detailed compositions of all diets are shown in Table 1.

Sample collection

On the 28th day of the experiment, after 12 h of fasting, six rats from each group were anesthetized with sodium pentobarbital (60 mg/kg BW) (French Pharmaceutical Cooperation, Melun, France). Blood was extracted from the abdominal aorta into dry test tubes and serum was prepared by low-speed centrifugation (1,000 *g* for 20 min at 4°C) (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Organs (liver, heart, and aorta) were removed, rinsed with cold saline solution (0.9% NaCl), and weighed. A serum aliquot was preserved in tubes containing 0.1% Na_2ethylenediaminetetraacetic acid and 0.02% sodium azide for lipoprotein assays. Tissue samples were stored at −70°C until analysis.

Biochemical study

Lipid and lipoprotein content and transaminase activity analysis: The amount of total lipids (TL) in the liver was determined by a gravimetric method after lipid extraction

Table 1. Composition of experimental and control diets

Ingredients	LCC/HA		HFD		N-C	
	g/100 g	Energy (%)	g/100 g	Energy (%)	g/100 g	Energy (%)
Casein	20	29	20	17	20	21
Corn starch	34	49	45	39	60	63
Sucrose	4	6	4	4	4	4
Sunflower oil	5	16	20	40	5	12
Agar agar	31	–	5	–	5	–
Mineral mix	4	–	4	–	4	–
Vitaminic mix	2	–	2	–	2	–
Total (kcal/100 g)		277		456		381

LCC/HA, low-calorie-carbohydrate/high-agar diet; HFD, high-fat diet; N-C, normal control diet.

(Folch et al., 1957). TG contents were estimated using an enzymatic colorimetric methods (kit CHOD-PAP; Biocon Diagnostik, Vöhl/Manenhagen, Germany). Serum and liver non-esterified cholesterol (NEC) concentrations were estimated using an enzymatic method (kit CHOD-PAP; Biolabo S.A.S., Maizy, France). Phospholipid (PL) levels were assessed by enzymatic determination of PLs (kit CHO-POD; Cypress Diagnostics, Langdorp, Belgium) in serum and liver. Non-esterified fatty acid (NEFA) contents were determined using enzymatic colorimetric assay kit (Sigma-Aldrich Co., St. Louis, MO, USA). The NEC/PL ratio was calculated to estimate membrane fluidity. Lipoprotein fractions were separated using the precipitation method. Serum LDLs were isolated by precipitation (Burstein et al., 1970) using $MgCl_2$ and phosphotungstate (Sigma-Aldrich Chimie S.a.r.l., Lyon, France). High density lipoprotein (HDL_2 and HDL_3) extractions were separated by precipitation (Burstein et al., 1989) using $MgCl_2$ and dextran sulfate MW 500,000 Da (Sigma-Aldrich Chimie S.a.r.l.). The cholesterol in each fraction was determined by enzymatic colorimetric methods described previously. Serum aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities were estimated using a colorimetric method (Chronolab Systems, S. L., Barcelona, Spain).

Lipid peroxidation analysis: Thiobarbituric acid reactive substances (TBARS) levels were measured using malondialdehyde (MDA) as a reference (Quintanilha et al., 1982; Ohkawa et al., 1979). In serum, lipid hydroperoxides (LOOH) concentrations were determined by ferric ions oxidation (Nourooz-Zadeh et al., 1996). In tissues, LOOH contents were determined according to the method of Eymard and Genot (2003). Serum IsoP (15-isoprostane F2t) levels were determined by the competitive enzyme immunoassay (EIA) method (Oxford Biomedical Research, Oxford, MI, USA).

Antioxidant enzymes activities measurement: The enzymatic activities of superoxide dismutase (SOD) (EC 1.15.1.1), glutathione peroxidase (GSH-Px) (EC 1.11.1.9), and glutathione reductase (GSSH-Red) (EC 1.6.4.2) were estimated using kits methods (Cayman Chemical, Ann Arbor, MI, USA). Catalase (CAT) (EC 1.11.1.6) activity was determined as described previously (Aebi, 1974).

NO bioavailability: Serum and tissue nitrite and nitrate levels were determined using the Griess reaction (Cortas and Wakid, 1990). NO has a half-life of only a few seconds because it is readily oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-), which serve as index parameters for NO production.

Statistical analysis

Statistical analysis was carried out using STATISTICA (version 10, StatSoft Inc., Tulsa, OK, USA). Values were expressed as the mean±standard error of mean (SEM) for six rats per group. Data were analysis by analysis of variance (one-way ANOVA), and differences in the means were assessed using Duncan's new multiple range tests (Duncan, 1955). The means with different letters were considered significantly different ($P<0.05$).

RESULTS AND DISCUSSION

Effects of LCC/HA on body and liver weight and energy intake

After four weeks of the nutritional experiment, the BW and liver weight of rats in the LCC/HA and Ob-C groups were reduced by a similar amount (−14%). However, the BW remained higher than the N-C group (+15%) (Table 2). The energy intake (kcal/rat/d) was reduced in the LCC/HA group compared with the Ob-C and N-C groups (−36% and −26%, respectively) (Table 2).

Changing eating habits (associated with physical activity) is the recommended cornerstone for weight loss. Low energy diets are the most efficient therapeutic target for NAFLD (Marchesini et al., 2016). In rodents, nutrients such as CHO and casein found in low energy diet are effective regulators that counteract obesogenic effects of high-fat diets (Marsset-Baglieri et al., 2004; Madsen et al., 2008). Variations in energy density and satiating quality of foods, and differing macronutrient composition may influence the potential of low-CHO diets for aiding

Table 2. Body weight, liver weight, and food and energy intake

	LCC/HA	Ob-C	N-C
Body weight (g)	376±22[b]	436±08[a]	326±13[c]
Weight gain (g)	−1.07±0.25[b]	1.24±0.31[a]	1.58±0.92[a]
Food intake (g/rat/d)	24.10±0.46	23.32±0.80	24.01±0.56
Total calorie intake (kcal/rat/d)	66.52±1.80[c]	106.36±3.29[a]	91.22±2.35[b]
Liver weight (g)	7.98±0.66[b]	11.12±0.49[a]	7.95±0.60[b]

Data are shown as mean±SEM for six values per group.
After analysis of variance, classification of the means was performed using the Duncan's multiple range test.
The means with different letters (a-c) are considered significantly different (P<0.05).
LCC/HA, low-calorie-carbohydrate/high-agar diet; Ob-C, obese control; N-C, normal control diet.

weight loss (Juanola-Falgarona et al., 2014). Most low energy diets with low glycemic indexes have a high fiber content, which prolongs distension of the gastrointestinal tract, resulting in increased and prolonged secretion of cholecystokinin, ghrelin, glucagon, glucagon-like peptide 1, and glucose dependent insulinotropic peptide, all of which are considered satiety modulating factors (Radulian et al., 2009; Rebello et al., 2013; Juanola-Falgarona et al., 2014). In this study, the LCC/HA was characterized by a high fiber content (31%) and low in CHO (34%), compared with the control diets (high fat and normal diets) (Table 1). This difference in nutrient composition may be indicative of the effectiveness of the diets for weight loss. Evidence from animal and human studies suggest that the composition and function of the gut microbiota play critical roles in energy homeostasis and development of obesity (Khan et al., 2016), which may also explain the observed benefits of high-fiber carbohydrates, independent of total calorie intake. Several studies reported that dietary carbohydrates, especially those that are not digested in the upper part of the gut, can enhance growth and the functions of the gut microbiota community (Huaman et al., 2018). Increasing dietary fiber intake through specific prebiotics may stimulate satiety hormones and enhance appetite control, which can help aid body weight control (Parnell et al., 2012).

Liver weight was decreased for rats in the treated group compared with the Ob-C group. This reduction in weight may be due to a decrease in fat deposition (especially at the visceral level), probably by inhibition of lipogenesis, which may explain the weight loss in rats receiving with LCC/HA. Several studies have demonstrated that low-calorie-carbohydrate diets reduce liver weight through elevating catabolic processes with a concomitant decrease of insulin and/or glucose levels in hepatocytes (Moura et al., 2012; Margolis et al., 2016). In rats, lipogenesis in the liver is as important as in adipose tissue (Axen et al., 2013). The lower liver weight in rats fed the LCC/HA compared with the Ob-C was probably the result of a reduction in liver lipids, thus reducing hepatic steatosis induced by the HFD. It has been demonstrated that dietary fiber intake may reduce intestinal absorption of nutrients and increase the quantity of fat excreted in faeces (Harrat et al., 2019); this confirms the probable ability of agar to bind dietary fat, as recently hypothesized (Yang et al., 2019). These results suggest that diets low in energy and carbohydrates but rich in agar effectively increase the liver's ability to excrete excess fat.

Effects of a LCC/HA on liver lipids profile

The LCC/HA reduced contents of TG, NEC, and NEFA

Table 3. Liver total lipids, lipid profile, lipoproteins-cholesterol contents, transaminases activities, and membrane fluidity

Parameters	LCC/HA	Ob-C	N-C
Liver lipids amounts			
TL (mg/g)	173.16±0.64[b]	198.32±1.55[a]	166.81±2.16[b]
TG (μmol/g)	27.90±0.58[c]	57.07±1.38[a]	42.40±1.89[b]
NEC (μmol/g)	45.38±0.98[b]	54.49±0.67[a]	44.63±0.56[b]
PL (μmol/g)	62.48±0.43[a]	43.01±0.50[b]	41.79±0.43[c]
NEFA (μmol/g)	37.56±0.41[b]	65.64±0.56[a]	38.30±0.56[b]
Lipoprotein-cholesterol contents			
LDL-C (mmol/L)	0.50±0.01[b]	1.57±0.02[a]	0.54±0.02[b]
HDL-C (mmol/L)	0.47±0.03[a]	0.30±0.02[b]	0.43±0.03[a]
Transaminases activities			
ALAT	15.46±0.42[b]	39.07±0.70[a]	14.91±0.70[b]
ASAT	17.74±0.32[c]	37.63±0.83[a]	20.81±0.43[b]
Membrane fluidity			
NEC/PL	0.32±0.02[b]	1.62±0.10[a]	0.39±0.02[b]

Data are shown as the mean±SEM for six values per group.
After analysis of variance, the classification of the means was performed using the Duncan's multiple range test.
The means with different letters (a-c) were considered significantly different (P<0.05).
LCC/HA, low-calorie-carbohydrate/high-agar diet; Ob-C, obese control; N-C, normal control diet; TL, total lipids; TG, triacylglycerols; NEC, non-esterified cholesterol; PL, phospholipids; NEFA, non esterified fatty acids; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; ALAT, serum alanine aminotransferase; ASAT, aspartate aminotransferase.

hepatic lipid. The liver TL content was lowered by −13% in LCC/HA group compared with the Ob-C group. Similarly, lower amounts of TG (−51%), NEFA (−43%), and NEC (−17%) levels was recorded for the LCC/HA group compared with the Ob-C group (Table 3). In contrast, the liver PL concentration was higher in the LCC/HA group (+45%) (Table 3). The liver contents of TG and NEFA were 34% and 21% lower, respectively, for rats in the LCC/HA group compared with the N-C group, whereas the hepatic PL concentration was increased by 50% (Table 3).

Exposure of the liver to high intakes of lipids may result in rapid stimulation of lipogenesis and TG accumulation, which may contribute to the reduction of insulin sensitivity and hepatic glucose intolerance (Bays et al., 2013). Several mechanisms may explain these effects, including reduction or inhibition of the activity of hydroxy methyl glutaryl CoA reductase (key enzyme in cholesterol biosynthesis) and stimulation of cholesterol 7 alpha hydroxylase activity (enzyme responsible for the conversion of cholesterol to bile acids). The LCC/HA contains 10-fold more fiber in the agar-agar form compared with the N-C group. Agar is a substance that consists of agarose (active compound of the agar-agar responsible for the gelling action) and agaro-pectin.

Agar has numerous functional properties. In addition to being a gelling product, agar saturates fat sensors. Currently, it is considered to be one of the best bioactive products for prevention and treatment of obesity (Mohamed et al., 2014). The low-calorie-carbohydrate diet used in the present study contained 31% agar fiber. Thus, one of the reasons why the LCC/HA can improve liver lipid metabolism may be related to its high levels of agar soluble fiber (80%).

Supplementation of agar extract (3%) in the diets of HFD-induced obese hamsters for 9 weeks induced reductions in body and adipose tissue weights, decreases in plasma TC/TG levels, hepatic TC/TG accumulation and adipose TG contents, and increases in fecal TC/TG contents and adipose tissue lipolysis. The authors suggested that agar extracts down-regulate hepatic lipid metabolism through phosphorylating adenosine monophosphate-activated protein kinases, up-regulating peroxisome proliferator activated receptor-α, and upregulating uncoupling protein-2 in HFD-induced obese hamsters; this subsequently increases fatty acid β-oxidation in the liver (Yang et al., 2019).

Further, LC diets improve lipid profiles *via* mechanisms such as increasing β-oxidation of FA and/or decreasing *de novo* lipogenesis in the liver (Baumeier et al., 2015). In addition, consumption of a low-CHO diet (<20 g/d) for 2 weeks may effectively alleviate metabolic abnormalities, by decreasing the accumulation of hepatic TGs and reducing development of hepatic steatosis (Browning et al., 2011). These findings suggest that diets low in energy and carbohydrates and rich in agar are beneficial for reducing lipid accumulation in the liver of obese rats, and for improving lipid metabolism. It is possible that the high dietary fiber content in the form of agar plays an important role in determining the effectiveness of low-energy-carbohydrates for preventing progression of NAFLD in obese rats.

Effects of LCC/HA on serum transaminases activities

Serum ALAT and ASAT activities were decreased by 60% and 53%, respectively, in LCC/HA group compared with the Ob-C group. Further, ALAT activity was similar between rats in the N-C and LCC/HA groups, whereas the ASAT level was lowered by −15% (Table 3).

Findings from the present study indicate that consumption of a LCC/HA reduces serum concentrations of liver transaminases. Rodríguez-Hernández et al. (2011) showed that weight reduction improves serum levels of aminotransferase irrespective of dietary macronutrient composition (low-CHO diet or low-fat diet). Other investigators have shown that beside weight reduction, low-CHO diets had a greater number of beneficial effects for treatment of patients with NAFLD compared with high carbohydrate diets (de Luis et al., 2010; Browning et al., 2011). Another determinant for the change in liver enzymes may be related to hepatic fat content since some investigators have reported that lower ALAT and ASAT levels in patients with NAFLD improve liver histology (Jang et al., 2018). Other possible reason for the variation in transaminase responses may be related to the increased amounts of dietary agar fiber in the LCC/HA. Increased soluble dietary fiber can positively affect NAFLD, and decrease serum ALAT and ASAT levels (Parnell et al., 2012). Previous study have shown that agar protects against liver damage in rats by efficiently inhibiting malondialdehyde formation and decreasing ASAT and ALAT levels (Chen et al., 2006). This study confirms that LCC/HA possesses hepatoprotective potential against HFD-induced hepatic steatosis damage.

Effects of LCC/HA on HDL-C, LDL-C, and membrane fluidity

LDL-C was reduced 68% and HDL-C was increased 57% in LCC/HA group compared with the Ob-C group (Table 3). These values were comparable with those reported for rats in the N-C group.

For rats in the LCC/HA group, the NEC/PL ratio was decreased 5-fold compared with rats in the Ob-C group, however the ratio did not significantly differ from that reported for the N-C group (Table 3).

The increase in HDL-C and decrease in LDL-C in rats receiving the LCC/HA are consistent with favoring a cardio-protective effect. The improvement in HDL-C ob-

served in the treated rats is probably a consequence of an increase in expression of adiponectin owing to its anti-atherogenic effects (Ruan and Dong, 2016). Our results are agreement with a previous study (Chen et al., 2015) which showed that hypocaloric-low-CHO diets (containing 34% carbohydrates) regulate serum lipoprotein concentrations (LDL-C and HDL-C), and are associated with production of plasma apolipoprotein A-1. The low LDL-C levels observed following consumption of the LCC/HA may also result from a reduction of cholesterol at the level of the mitochondrial membrane (Harmancey et al., 2010), a potent modulator of membrane fluidity. Previous studies have reported that membrane fluidity of rats fed a HFD is altered by changes in the content of hepatic cholesterol and phospholipids (Tanaka et al., 1989; Ciapaite et al., 2011). Our findings are agreement with these studies. Consumption of a LCC/HA has been previously shown to decrease NEFA concentrations (indicative of oxidation), probably due to changes in the fat composition of liver mitochondria and impaired oxidative phosphorylation (Vial et al., 2011). Therefore, the reduction of liver lipid accumulation observed for the LCC/HA group may be explained by an increase in adiponectin owing to liver lipid infiltration having favourable action on mitochondria membranes.

Effects of LCC/HA on prooxidant markers and antioxidant enzymes activities

Serum TBARS levels were decreased (1.8-fold) in rats fed the LCC/HA compared with the Ob-C. Similarly, serum LOOH was similar between the LCC/HA and N-C groups, but was reduced 1.8-fold in the LCC/HA group compared with the Ob-C group (Table 4). Serum IsoP concentrations were 2.7- and 1.9-fold lower in the LCC/HA group compared with the Ob-C and N-C groups, respectively (Table 4). Reductions in TBARS concentrations were recorded in the livers (−30%), hearts (−54%) and aortas (−56%) of rats fed the LCC/HA compared with the Ob-C (Table 4). However, TBARS were increased 11% in the LCC/HA group compared with the N-C group in liver, and was decreased 11% in heart and 38% in the aorta (Table 4). Similarly, decreases in LOOH levels were noted in the liver (2.1-fold), heart (2-fold) and aorta (3.3-fold) of the LCC/HA group compared with the Ob-C group (Table 4); however, compared with the N-C group, LOOH levels was higher in liver (1.1-fold higher) but lower in heart (1.7-fold) and aorta (2.2-fold).

In serum, rats fed the LCC/HA showed increases in SOD (1.8-fold), CAT (5.4-fold), GSH-Px (1.1-fold), and GSSH-Red (1.3-fold) activities compared with rats in the Ob-C group (Table 5). Further, SOD, serum GSSH-Red, and CAT activities were 1.4-, 1.2-, and 1.3-fold higher, respectively, in the LCC/HA group compared with the N-C group (Table 5).

For all organ tissues analyzed, SOD activity was increased for rats in the LCC/HA group compared with the Ob-C group ($P<0.05$). In addition, compared with the N-C group, SOD activity in the LCC/HA group was higher in the liver, heart, and aorta (+52%, +9%, and +16%), respectively (Table 5). Further, increases in CAT activity was noted in the liver (1.8-fold), heart (1.5-fold), and aorta (1.7-fold) in the LCC/HA group compared with the Ob-C group (Table 5).

LCC/HA treatment significantly enhanced the activity of GSH-Px in the liver and heart (both 1.4-fold) (Table 5). In addition, increases in GSSH-Red activity was re-

Table 4. Prooxydants biomarkers in serum and tissues

Groups	LCC/HA	Ob-C	N-C
Serum			
TBARS (nmol Eq MDA/L)	30.01±2.65^{b}	55.95±1.59^{a}	29.45±0.73^{b}
LOOH (nmol Eq CUOOH/L)	56.84±7.06^{b}	105.29±2.43^{a}	51.86±1.47^{b}
IsoP (ng/L)	253.58±15.16^{c}	680.41±71.67^{a}	477.51±28.55^{b}
Tissues			
Liver			
TBARS (nmol Eq MDA/g)	460.23±4.07^{b}	653.66±18.63^{a}	416.10±15.58^{c}
LOOH (nmol Eq CUOOH/g)	350.85±8.45^{b}	751.78±13.31^{a}	319.86±12.52^{c}
Heart			
TBARS (nmol Eq MDA/g)	217.98±39.47^{b}	475.73±14.18^{a}	246.15±15.45^{b}
LOOH (nmol Eq CUOOH/g)	262.11±58.05^{c}	522.68±48.87^{a}	449.91±26.62^{b}
Aorta			
TBARS(nmol Eq MDA/g)	249.44±25.86^{c}	569.62±52.42^{a}	402.96±31.08^{b}
LOOH (nmol Eq CUOOH/g)	117.98±17.83^{c}	390.75±25.37^{a}	263.05±14.18^{b}

Data are shown as the mean±SEM for six values per group.
After analysis of variance, the classification of the means was performed using the Duncan's multiple range test.
The means with different letters (a-c) were considered significantly different ($P<0.05$).
LCC/HA, low-calorie-carbohydrate/high-agar diet; Ob-C, obese control; N-C, normal control diet; TBARS, thiobarbituric acid reactive substances; LOOH, hydroperoxides; IsoP, isoprostanes.

Table 5. Antioxidant enzyme activities in serum and tissues

Groups	LCC/HA	Ob-C	N-C
Serum			
SOD (U/mL)	248.34±7.62[a]	139.11±6.93[c]	177.80±3.46[b]
GSH-Px (nmol/min/mL)	90.14±0.00[a]	82.77±1.81[b]	90.42±1.79[a]
GSSH-Red (nmol/min/mL)	132.44±7.20[a]	99.33±3.60[b]	113.34±1.80[b]
CAT (U/min/mL)	62.47±0.72[a]	11.57±0.68[c]	48.07±1.03[bs]
Tissues			
Liver			
SOD (U/g)	25.64±1.93[a]	8.56±1.14[d]	16.82±0.54[c]
CAT (U/min/g)	15.11±0.42[a]	8.35±0.02[d]	13.39±0.37[b]
GSH-Px (nmol/min/g)	177.01±9,00[a]	123.53±9.00[c]	169.37±1.80[ab]
GSSH-Red (nmol/min/g)	160.46±3.60[a]	115.88±1.80[c]	138.81±1.80[b]
Heart			
SOD (U/g)	26.02±0.30[a]	19.76±0.60[d]	23.77±0.48[b]
CAT (U/min/g)	13.02±0.13[a]	8.47±0.36[c]	13.53±1.19[a]
GSH-Px (nmol/min/g)	202.48±5.40[a]	145.17±3.60[d]	192.29±1.80[a]
GSSH-Red (nmol/min/g)	276.34±1.80[a]	225.40±5.40[c]	268.70±1.80[a]
Aorta			
SOD (U/g)	23.43±0.48[a]	15.16±0.48[d]	20.19±0.12[b]
CAT (U/min/g)	12.79±1.01[a]	7.59±0.23[c]	12.48±0.76[a]
GSH-Px (nmol/min/g)	163.00±3.60[a]	148.99±1.80[b]	162.73±1.80[a]
GSSH-Red (nmol/min/g)	216.49±3.60[a]	90.42±1.80[c]	182.10±5.40[b]

Data are shown as the mean±SEM for six values per group.
After analysis of variance, the classification of the means was performed using the Duncan's multiple range test.
The means with different letters (a-c) were considered significantly different (P<0.05).
LCC/HA, low-calorie-carbohydrate/high-agar diet; Ob-C, obese control; N-C, normal control diet; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; GSSH-Red, glutathione reductase.

corded in most tissues (1.4-fold in liver, 1.2-fold in heart, and 2.4-fold in aorta) in the LCC/HA group compared with the Ob-C group; however, compared with the N-C group, GSSH-Red activity was only higher in the liver and aorta (both 1.2-fold) (Table 5).

These results show that the LCC/HA may protect serum and tissues against the cytotoxic action and oxidative stress of HFDs. Our results clearly show a decrease in lipid peroxidation markers (TBARS, LOOHs, and IsoP levels) for rats receiving the LCC/HA; efficient antioxidant enzymatic defense may be linked to the decrease in lipids levels. It is well known that generation of free radicals is positively correlated with liver lipid concentrations (Volek et al., 2009). The reduction in lipid peroxidation may be the result of significant modifications to the cellular redox status in favor of antioxidants, associated with the reduction of hepatic lipids accumulation. In addition, assessment of antioxidant defense at the tissue level shows a high activity of tissue antioxidant enzymes (SOD, CAT, GSH-Px, and GSSH-Red) in obese rats receiving the LCC/HA, which may contribute to better anti-radical protection. However, increased GSH-Px activity at the hepatic and cardiac level could indicate an elevation of glutathione production since the latter is the specific substrate of this enzyme. The LCC/HA maintained the pro/antioxidant balance of cells and tissues by reducing their exposure to oxidative damage caused by the HFD. This work shows that a low calorie, LCC/HA decreases the occurrence of lesions related to oxidative aggression by reducing production of free radicals and lipid peroxides in the liver, and by increasing antioxidant enzymatic defense. This could probably be the result of reduced accumulation of lipids in the liver. Agar has been shown to enhance the activities of antioxidant enzymes, including SOD and GSH-Px (Chen et al., 2006). Agar had been indicated to improve hepatoprotective effects (Alam et al., 2017). Antioxidant enzymes such as SOD and GSH-Px are considered a primary defense system against oxidative damage; agar exerts antioxidant activity through both its own radical scavenging activity and by boosting the host's antioxidant enzyme system (Chen et al., 2006). Therefore, our findings suggest that high content of agar in diets (in the context of energy restriction) can exert a hepatoprotective effect through scavenging ROS to prevent oxidative damage.

Effects of LCC/HA on NO bioavailability

NO level was elevated 1.9-fold in the LCC/HA group compared with the Ob-C group, but was comparable to that of the N-C group (Table 6). In tissues, NO concentrations were higher in liver (1.6-fold), heart (3.4-fold), and aorta (3.6-fold) in rats fed the LCC/HA compared with the Ob-C. In contrast, compared with the N-C group, NO levels were decreased in liver (1.6-fold) and

Table 6. Nitric oxide (NO) bioavailability in serum and tissues

Groups	LCC/HA	Ob-C	N-C
NO-serum (μmol/L)	2.42±0.09[a]	1.27±0.08[b]	2.46±0.03[a]
NO-tissues (μmol/g)			
Liver	3.03±0.14[b]	1.86±0.06[c]	4.68±0.06[a]
Heart	5.53±0.26[a]	1.61±0.56[c]	3.51±0.78[b]
Aorta	4.33±0.72[b]	1.18±0.10[c]	5.73±0.71[a]

Data are shown as the mean±SEM for six values per group. After analysis of variance, the classification of the means was performed using the Duncan's multiple range test.
The means with different superscripts were considered significantly different (P<0.05).
LCC/HA, low-calorie-carbohydrate/high-agar diet; Ob-C, obese control; N-C, normal control diet.

aorta (1.3-fold), and enhanced in heart (1.6-fold) of rats in the LCC/HA group (Table 6).

In the present study, the LC diet improved bioavailability of NO in rats with hepatic steatosis. The LC diet could have a protective effect against endothelial dysfunction (Chou et al., 2010; Donato et al., 2013) through underlying mechanisms such as by increasing bioavailability of NO (through enhancing expression and/or eNOS activity (Blanquicett et al., 2007), suppressing vascular oxidative stress associated with superoxide anion ($O_2^{\bullet -}$) production, and attenuating lipid peroxidation (Chou et al., 2010; Donato et al., 2013). Animal experiments and clinical studies have shown that abnormal production of NO and elevated concentrations of endotheline-1 (ET-1) may be important contributors to endothelial dysfunction development, an early step in development of atherosclerosis in obesity (Weil et al., 2011; Sánchez et al., 2014). Soluble fiber can reduce CVD risk by its ability to regulate lipid metabolism, via reducing blood pressure, decreasing proinflammatory mediators, improving oxidative stress, and reducing endothelial dysfunction (Behall et al., 2006; Qi et al., 2006; Brock et al., 2006). However, the mechanism responsible for the protective effect of fiber on endothelial dysfunction is not well described. Dietary soluble fiber may stimulate NO generation and reduce ET-1 level (Xiao et al., 2009). In this study, it is possible that the protective effects of agar fiber on the vascular endothelium may be related to an improvement in NO production and/or a reduction in ET-1 concentration. Further research is needed to address these points.

This study shows that the LCC/HA exerts several mechanisms of action to improve HFD-induced hepatic steatosis damage, probably due to their specific or interactive actions. The LCC/HA attenuate cardiometabolic disorders associated with NAFLD in obese rats by reducing the BW and the weight of the liver, hepatic lipid accumulation, transaminases content, and by improving membrane fluidity, the pro/antioxidant balance and endothelial dysfunction.

The present study provides substantial evidence for the beneficial effect of this type of hypocaloric diet against HFD-induced hepatic steatosis damage and suggests it might be a promising therapeutic strategy to counteract NAFLD development associated with obesity.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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