Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 153–162

Journal of Nutritional Biochemistry

Lipid redistribution by α-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats

Hemant Poudyal[a,d], Sunil K. Panchal[d], Jennifer Waanders[b], Leigh Ward[c], Lindsay Brown[a,d,*]

[a]School of Biomedical Sciences, The University of Queensland, Brisbane, 4072, Australia
[b]School of Land, Crop and Food Science, The University of Queensland, Brisbane, 4072, Australia
[c]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, 4072, Australia
[d]Department of Biological and Physical Sciences, University of Southern Queensland, Toowoomba 4350, Australia

Received 5 September 2010; received in revised form 30 October 2010; accepted 9 November 2010

Abstract

Chia seeds contain the essential fatty acid, α-linolenic acid (ALA). This study has assessed whether chia seeds attenuated the metabolic, cardiovascular and hepatic signs of a high-carbohydrate, high-fat (H) diet [carbohydrates, 52% (wt/wt); fat, 24% (wt/wt) with 25% (wt/vol) fructose in drinking water] in rats. Diets of the treatment groups were supplemented with 5% chia seeds after 8 weeks on H diet for a further 8 weeks. Compared with the H rats, chia seed-supplemented rats had improved insulin sensitivity and glucose tolerance, reduced visceral adiposity, decreased hepatic steatosis and reduced cardiac and hepatic inflammation and fibrosis without changes in plasma lipids or blood pressure. Chia seeds induced lipid redistribution with lipid trafficking away from the visceral fat and liver with an increased accumulation in the heart. The stearoyl-CoA desaturase-1 products were depleted in the heart, liver and the adipose tissue of chia seed-supplemented rats together with an increase in the substrate concentrations. The C18:1*trans*-7 was preferentially stored in the adipose tissue; the relatively inert C18:1n-9 was stored in sensitive organs such as liver and heart and C18:2n-6, the parent fatty acid of the n-6 pathway, was preferentially metabolized. Thus, chia seeds as a source of ALA induce lipid redistribution associated with cardioprotection and hepatoprotection.

Keywords: Chia seed; α-Linolenic acid; Metabolic syndrome; High-carbohydrate, high-fat diet

1. Introduction

All three nutritionally important n-3 fatty acids, α-linolenic acid (ALA; C18:3n-3), eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), reduce one or more risk factors of cardiovascular disease as shown by epidemiological, human, animal and cell culture studies [1–5]. In human diets, ALA, the essential n-3 fatty acid, is usually derived from plant sources such as flax seed, while EPA and DHA are ingested from fish, fish oil supplements and other sea foods [1]. The richest botanical source of ALA is the oil from chia seeds containing about 60% (wt/vol) [6–10]. Chia seed (*Salvia hispanica* L.) was a major dietary component in the Mayan and Aztec populations [6]. In sucrose-fed rats, dietary chia seed prevented the onset of dyslipidemia and insulin resistance and reduced visceral adiposity without affecting glucose homeostasis [10]. Rats fed a 16% chia seed diet showed increased plasma ALA, EPA and DHA concentrations, decreased serum triglyceride content and increased serum high-density lipoprotein content [9]. In humans receiving conventional therapy for diabetes, chia seed reduced systolic blood pressure and C-reactive protein (CRP) concentrations and increased serum ALA and EPA concentrations without affecting body weight [11]. In another human intervention study, dietary chia seed increased plasma ALA concentrations, but EPA and DHA concentrations, body composition, biomarkers of inflammation and oxidative stress were unchanged [12].

Despite ALA being the primary fatty acid of the pathway, it has generated less scientific interest than EPA or DHA among the n-3 fatty acids possibly due to the inefficient conversion of ALA to EPA and DHA by humans [13]. Epidemiological and clinical evidence suggests

Abbreviations: ALA, α-linolenic acid; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; C, corn starch diet; CC, corn starch+chia seed diet; CK, creatine kinase; CPT-1, carnitine palmitoyltransferase I; CRP, C-reactive protein; DHA, docosahexaenoic acid; DXA, dual-energy X-ray absorptiometry; EPA, eicosapentaenoic acid; H, high–carbohydrate, high-fat diet; HC, high-carbohydrate, high-fat diet+chia seed; LDH, lactate dehydrogenase; LV, left ventricle; LVIDd, left ventricle internal diameter in diastole; LVIDs, left ventricle internal diameter in systole; LVPWd, left ventricle posterior wall thickness in diastole; NEFA, nonesterified fatty acids; SCD, stearoyl-CoA 9-desaturase; SREBP-1, sterol regulatory element binding protein-1.

* Corresponding author at: Department of Biological and Physical Sciences, University of Southern Queensland, Toowoomba 4350, Australia. Tel.: +61 7 4631 1319; fax: +61 7 4631 1530.
E-mail address: Lindsay.Brown@usq.edu.au (L. Brown).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2010.11.011

that higher consumption of ALA is associated with reduced risk of cardiovascular diseases [14–20]. A meta-analysis of five prospective cohort studies showed that increased ALA consumption reduced heart disease mortality [14]. The Lyon Heart Study concluded that diets rich in ALA were more efficient than presently used diets in the secondary prevention of cardiovascular diseases [15]. The cardiovascular health study [16]; The Nurses' Health study [17,18]; the National Heart, Lung, and Blood Institute Family Heart Study [19] and the Health Professionals Study [20] concluded that higher ALA intake was associated with a reduced risk of coronary heart disease and related mortalities.

Metabolic syndrome is a clustering of interrelated risk factors for cardiovascular disease and diabetes. These factors include hyperglycemia, hypertension, dyslipidemia, central adiposity and nonalcoholic fatty liver disease [21]. We have previously shown that rats fed a high-carbohydrate, high-fat diet mimic many signs of human metabolic syndrome [22,23]. Therefore, to determine the responses of chia seed as a rich source of ALA on the signs of metabolic syndrome, we investigated metabolic changes, cardiovascular and hepatic structure and function, lipid content and fatty acid profiles in the liver, retroperitoneal adipose tissue and the heart of rats fed either corn starch diet, high-carbohydrate, high-fat diet or either diet containing 5% whole chia seed supplement. Liver and adipose tissue were selected for their major role in fatty acid metabolism, while the heart was selected because fatty acids modulate its functions. Corn starch is a slowly digestible carbohydrate [24] and served as a control for the high-carbohydrate, high-fat diet in this study where the primary carbohydrates are fructose and sucrose. Unlike fructose, corn starch does not increase blood glucose, plasma insulin or nonesterified fatty acid (NEFA) concentrations [22,24].

2. Materials and methods

2.1. Animals and diets

The experimental groups consisted of 48 male Wistar rats (8–9 weeks old, 338±4 g) supplied by The University of Queensland Biological Resources unit and individually housed at the University of Southern Queensland Animal House. All experimentation was approved by the Animal Experimentation Ethics Committee of the University of Southern Queensland under the guidelines of the National Health and Medical Research Council of Australia. The rats were randomly divided into four separate groups (n=12 each) fed corn starch (C), corn starch+chia seed (CC), high-carbohydrate, high-fat (H) and high-carbohydrate, high-fat+chia seed (HC). All experimental groups were housed in a temperature-controlled, 12-h light/dark cycle environment with *ad libitum* access to water and the group-specific rat diet. Measurements of body weight and food and water intakes were taken daily to monitor the day-to-day health of the rats. Feed conversion efficiency (%) was calculated as [mean body weight gain [g]/daily energy intake (kJ)]×100 [25].

All group-specific diets were custom prepared in our laboratory. Corn starch diet was prepared by thorough mixing of corn starch, powdered rat feed (meat-free rat and mouse feed; Speciality Feeds, Glen Forrest, WA, Australia), Hubble, Mendel and Wakeman salt mixture (MP Biochemicals, Seven Hills, NSW, Australia) and water, while the corn starch and part of water were replaced with condensed milk, fructose and beef tallow in the high-carbohydrate, high-fat diet (Table 1). The drinking water in all high-carbohydrate, high fat-fed rats was augmented with 25% fructose. Chia seed-supplemented diets were prepared by replacing an equivalent amount of water in the diet with the whole seed (Table 1). The fatty acid composition of all group-specific diets and chia seed is described in Table 1. Five percent of chia seed supplementation was administered for 8 weeks starting 8 weeks after the initiation of the corn starch or high-carbohydrate, high-fat diet.

Table 1
Fatty acid profile and energy density of chia seed, C, CC, H and HC diets

Constituent	Chia seed	Diets			
		C	CC	H	HC
Corn starch, g/kg	–	570.00	570.00	–	–
Powdered rat feed[a], g/kg	–	155.00	155.00	155.00	155.00
HMW salt mixture, g/kg	–	25.00	25.00	25.00	25.00
Fructose, g/kg	–	–	–	175.00	175.00
Beef tallow, g/kg	–	–	–	200.00	200.00
Condensed milk, g/kg	–	–	–	395.00	395.00
Water, ml/kg	–	250.00	200.00	50.00	–
Chia seed, g/kg	–	–	50.00	–	50.00
Macronutrient composition					
Total carbohydrate, g/kg	–	600.25	600.25	515.67	515.67
Total fat, g/kg	–	8.07	8.07	239.04	239.04
Total protein, g/kg	–	31.78	31.78	58.12	58.12
Total fiber, g/kg	–	7.44	7.44	7.44	7.44
Total vitamins, g/kg	–	0.32	0.32	0.32	0.32
Total minerals, g/kg	–	0.13	0.13	0.44	0.44
Ash, g/kg	–	0.63	0.63	0.00	0.00
Total moisture, g/kg	–	326.38	296.38	153.98	123.98
Total calculated energy density, kJ/g	18.6	11.2	12.2	17.9	18.7
Total extractable lipids, g/kg (n=3)	272.6±0.1	6.2±0.1	11.6±0.1	187.0±2.3	193.9±0.4
Fatty acid, g/100g of total fatty acid content (n=3)					
C14:0	2.8±0.4	10.0±0.9	15.6±0.2	3.7±0.1	4.7±0.1
C16:0	7.6±0.2	17.5±0.4	14.5±0.1	24.6±0.5	26.9±0.5
C18	3.4±0.6	0.6±0.0	4.9±0.1	24.2±0.2	21.9±0.4
C18:1n-9	8.7±0.4	34.5±2.3	24.7±0.4	0.9±0.5	3.4±0.6
C18:1*trans*-7	0.0±0.0	0.0±0.0	0.0±0.0	40.8±0.8	34.1±0.2
C18:2n-6	20.5±0.2	30.5±0.8	25.5±0.0	2.7±0.1	3.0±0.1
C18:3n-3	56.1±0.5	4.7±0.1	12.6±0.0	0.1±0.1	2.1±0.4
C20:0	0.3±0.1	0.0±0.0	0.4±0.0	0.2±0.1	0.3±0.1
Total SFA	14.1±0.5	28.3±1.4	35.5±0.3	53.1±0.7	54.9±1.0
Total MUFA	9.3±0.6	35.7±2.3	25.5±0.4	44.1±0.6	40.0±0.7
Total PUFA	76.7±0.8	36.0±0.9	39.1±0.1	2.8±0.0	5.1±0.3

For the dietary fatty acid compositions, each value is a mean±S.E.M. Number of repetitive experiments are indicated within parenthesis. HMW, Hubble, Mendel and Wakeman; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

[a] Meat-free rat and mouse feed (Speciality Feeds) contains the following (in g/kg of feed): carbohydrates, 707.07; proteins, 194.00; fat, 48.00; fiber, 48.00; total vitamins, 2.08 and total minerals, 0.85.

2.2. Systolic blood pressure

Systolic blood pressure was measured as previously described [22] under light sedation following intraperitoneal (ip) injection of Zoletil (tiletamine 15 mg/kg, zolazepam 15 mg/kg; Virbac, Peakhurst, NSW, Australia). Measurements were taken using an MLT1010 Piezo-Electric Pulse Transducer (ADInstruments, Sydney, Australia) and inflatable tail-cuff connected to an MLT844 Physiological Pressure Transducer (ADInstruments) and PowerLab data acquisition unit (ADInstruments).

2.3. Oral glucose tolerance and insulin tolerance tests

The oral glucose tolerance and the insulin tolerance tests were performed 2 days apart from each other at 16 weeks. For oral glucose tolerance after 10–12 h of overnight food deprivation, basal blood glucose concentrations were measured in blood taken from the tail vein using Medisense Precision Q.I.D glucose meter (Abbott Laboratories, Bedford, MA, USA). Fructose-supplemented drinking water in the H and HC groups was replaced with normal water for the overnight food deprivation period. The rats were given 2 g/kg body weight of glucose as a 40% solution via oral gavage. Tail vein blood samples were taken at 30, 60, 90 and 120 min following glucose administration.

For insulin tolerance, basal blood glucose concentrations were measured after 4–5 h of food deprivation as above. The rats were injected ip 0.75 IU/kg insulin-R (Eli Lilly Australia, West Ryde, NSW, Australia), and tail vein blood samples were taken at 0, 30, 60, 90 and 120 min. Rats were withdrawn from the test if the blood glucose dropped below 1.1 mmol/L, and 4 g/kg glucose was immediately administered by oral gavage to prevent hypoglycemia.

2.4. Echocardiography

Echocardiographic examination (Phillips iE33, 12MHz transducer) was performed at 16 weeks as previously described [22,26]. Measurements were taken in accordance with the guidelines of the American Society of Echocardiography using the leading-edge method [27]. Briefly, rats were anesthetized using intraperitoneal Zoletil (tiletamine 15 mg/kg and zolazepam 15 mg/kg ip; Virbac) and Ilium Xylazil (xylazine 15 mg/kg ip; Troy Laboratories, Smithfield, NSW, Australia) and positioned in dorsal recumbency. Electrodes attached to the skin overlying the elbows and right stifle facilitated the simultaneous recording of a lead II electrocardiogram. A short-axis view of the left ventricle (LV) at the level of the papillary muscles was obtained and used for direct acquisition of M-mode images of the LV for measurement of diastolic posterior wall thickness (LVPWd), LV internal systolic dimension and LV end-diastolic dimension (LVIDd).

2.5. Body composition measurements

Dual-energy X-ray absorptiometric (DXA) measurements were performed on the rats after 8 and 16 weeks of feeding, 2 days before rats were killed for pathophysiological assessments, using a Norland XR36 DXA instrument (Norland Corp., Fort Atkinson, WI, USA). DXA scans were analyzed using the manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 2.5.3/1.3.1; Norland Corp) as previously described [28]. The precision error of lean mass for replicate measurements, with repositioning, was 3.2%. Visceral adiposity index (%) was calculated as: ([retroperitoneal fat (g)+omental fat (g)+epididymal fat (g)]/[body weight (g)])×100 and expressed as adiposity percent [29].

2.6. Isolated heart preparation

The left ventricular function of the rats in all treatment groups was assessed using the Langendorff heart preparation. Terminal anesthesia was induced via ip injection of pentobarbitone sodium (Lethabarb, 100 mg/kgip). After heparin (Sigma-Aldrich Australia, Sydney, Australia) administration (200 IU) through the right femoral vein, blood (~5 ml) was taken from the abdominal aorta. Isovolumetric ventricular function was measured by inserting a latex balloon catheter into the LV of the isolated heart connected to a Capto SP844 MLT844 physiological pressure transducer and Chart software on a Maclab system (ADInstruments Australia and Pacific Islands, Bella Vista, NSW, Australia). All left ventricular end-diastolic pressure values were measured while pacing the heart at 250 beats/min using an electrical stimulator. End-diastolic pressures were obtained starting from 0 up to 30 mmHg. Diastolic stiffness constant (κ, dimensionless) was calculated as in previous studies [22,30]. $+dP/dt$ and $-dP/dt$ were calculated as the mean rate of contraction and relaxation, respectively, of at least 50 beats with the heart paced at 250 beats/min, and the end-diastolic pressure was maintained at approximately 10 mmHg.

2.7. Aortic contractility

Thoracic aortic rings (4 mm in length) were suspended in an organ bath chamber with a resting tension of approximately 10 mN. Cumulative concentration–response (contraction) curves were measured for noradrenaline (Sigma-Aldrich Australia); concentration–response (relaxation) curves were measured for acetylcholine (Sigma-Aldrich Australia) and sodium nitroprusside (Sigma-Aldrich Australia) in the presence of a submaximal (70%) contraction to noradrenaline [22].

2.8. Organ weights

The right and left ventricles were separated after perfusion experiments and weighed. Liver, retroperitoneal, epididymal and omental fat were removed following heart removal and blotted dry for weighing. Organ weights were normalized relative to the tibial length at the time of their removal (in mg/mm). Immediately after weighing, the LV, liver and retroperitoneal fat were stored at −20°C in a 50-ml polypropylene centrifuge tube for fatty acid analysis.

2.9. Fatty acid analysis

The extraction of tissue and dietary lipids was undertaken by manual solvent extraction using a 2:1 chloroform/methanol mixture with 0.1% butylated hydroxytoluene as an antioxidant. Approximately 1–5 g of retroperitoneal fat, heart and liver and 2 ml plasma were macerated into a 50-ml polypropylene centrifuge tube and were mixed on a rotating device for 40 min with 20 ml of chloroform/methanol solvent and then centrifuged at 2500 rpm for 5 min. The extraction procedure was repeated twice, combining all of the extracting solvent and subsequently washing with ddH_2O to remove all polar material. Chloroform was evaporated under a stream of nitrogen on a hot plate set at 60°C until the beakers reached constant weight. The beaker weights were recorded for the calculation of gravimetric extractable lipid content [31].

Approximately 15–20 mg of extracted lipid samples with 1 mg of heptadecanoic acid (C17) added as an internal standard was methylated in a clean 10-ml test tube. Saponified lipids were extracted with 2 ml heptane and then transferred into an autosampler vial for gas chromatography. Fatty acid methyl esters were analyzed on an Agilent J&W DB-23 column (60 m×0.25 mm×0.25 μm) (Agilent Technologies, Santa Clara, CA, USA) by a Shimadzu GC-17A equipped with a flame ionization detector. The injection and the detector temperatures were set at 250°C and 285°C, respectively. The column temperature was set at 100°C for 2 min, raised at 10°C/min to 180°C, held for 5 min, raised at 5°C/min to 240°C and held for 25 min. Carrier gas (helium) was passed at 110 kPa, with constant linear velocity of 15cm/s. A sample of 1 μL was injected with a split ratio of 25. A multi-acid standard mixture was used for checking the performance of the gas chromatography and as a recovery test for the sample preparation procedure. Quantitation of the fatty acids in all samples was based on a linear calibration equation obtained from the C17 standards. For identification purposes, a 28-fatty-acid methyl ester mixture standard (Nu-Check Prep. Inc, Elysian, MN, USA) was used for retention time (RT) calibration. A plot of carbon number versus log RT for the saturated series, 1° of unsaturation and 2° of unsaturation allowed a relationship to be developed for identification purposes.

All fatty acids were expressed as grams per 100 g of total recovered fatty acids. The n-3:n-6 ratio was derived using the following formula:

$$\text{n3:n6} = [\text{C18:3n-3} + \text{C20:3n-3} + \text{C20:5n-3} + \text{C22:5n-3} + \text{C22:6n-3}] / [\text{C18:2n-6} + \text{C18:3n-6} + \text{C20:2n-6} + \text{C20:3n-6} + \text{C20:4n-6} + \text{C22:2n-6} + \text{C22:4}]\text{n-6}$$

As the diets were rich in C18:1n-9, the stearoyl-CoA 9-desaturase (SCD) index was calculated as the ratio between C16:1n-7 and C16:0, and the ratio between C18:1n-9 and C18:0 was not considered.

2.10. Histology

Two rats per group were taken exclusively for histological analysis. Two slides were prepared per tissue specimen and two random, nonoverlapping fields per slide were taken to avoid biased analysis. Organs were also collected from rats used for perfusion studies. Immediately after removal, heart and liver tissues were fixed in 10% buffered formalin for 3 days and then dehydrated and embedded in paraffin wax as previously described [22]. Thin sections (7 μm) of LV and the liver were cut and stained with hematoxylin and eosin stain for determination of inflammatory cell infiltration. Liver sections were also stained with Milligan's trichrome stain to determine fibrosis. Collagen distribution was measured in the LV with picrosirius red stain. Laser confocal microscopy (Zeiss LSM 510 upright Confocal Microscope) with color intensity quantitatively analyzed using NIH-imageJ software (National Institute of Health, USA) was used to determine the extent of collagen deposition in selected tissue sections [22].

2.11. Plasma biochemistry

Briefly, blood was centrifuged at 5000×*g* for 15 min within 30 min of collection into heparinized tubes. Plasma was separated and transferred to Eppendorf tubes for storage at −20°C before analysis. Activities of plasma enzymes and analyte concentrations were determined using kits and controls supplied by Olympus using an Olympus analyzer (AU 400, Tokyo, Japan) as previously described [22]. Plasma CRP was estimated using a commercial kit (Kamiya Biomedical, Thousand Oaks, CA, USA) according to the manufacturer-provided standards and protocols using a Cobas-Mira automated analyser.

2.12. Statistical analysis

All data are presented as mean±S.E.M. Results were tested for variance using Bartlett's test and variables that were not normally distributed were transformed (using log 10 function) prior to statistical analyses. Data from C, CC, H and HC groups were tested by two-way analysis of variance. When interaction and/or the main effects were significant, means were compared using Newman-Keuls multiple-comparison post hoc test. Where transformations did not result in normality or constant variance, a Kruskal-Wallis nonparametric test was performed. A P value of <.05 was considered as statistically significant. All statistical analyses were performed using GraphPad Prism version 5.00 for Windows (San Diego, CA, USA).

3. Results

3.1. Dietary intake, body parameters and plasma biochemistry

Food intake was decreased in H and HC rats compared with C and CC groups (Table 2). Chia seed supplementation did not change water intake in either group, but food consumption was increased in CC rats (Table 2). Chia seed supplementation increased energy intake compared with C and H diet-fed rats (Table 2). This increased energy intake in the chia seed-supplemented rats corresponded with increased body weight gain and feed conversion efficiency in both groups without any change in abdominal circumference or total body fat mass. However, chia seed supplementation reduced the visceral adiposity index and increased bone mineral content in both groups without altering the body lean mass (Table 2). The reduction in visceral adiposity index was due to decreased retroperitoneal and omental but not epididymal fat deposition in HC rats. In CC rats, selective lowering of omental fat was observed (Table 2). Chia seed supplementation decreased the total lipid content in the retroperitoneal fat and liver but increased the content in the heart (Table 2). This lipid redistribution induced by chia seed supplementation was associated with increased plasma triglycerides and NEFA, although plasma total cholesterol concentrations were not affected (Table 2). Chia seed supplementation also reduced heart wet weights.

Furthermore, chia seed supplementation normalized plasma urea concentrations suppressed by H diet feeding and reduced lactate

Table 2
Dietary intakes, body composition and anthropometrics, organ wet weights, tissue fatty acid composition and plasma biochemistry in C, CC, H and HC diet-fed rats

Variable	C	CC	H	HC	*P*		
					Diet	Treatment	Interaction
Food intake, g/d (n=10)	33.9±0.9^{b}	42.1±2.1^{a}	22.9±0.3^{c}	22.8±0.4^{c}	<.0001	.0014	.0011
Water intake, ml/d (n=10)	31.4±0.4^{a}	31.7±0.8^{a}	19.7±0.3^{b}	19.1±0.4^{b}	<.0001	.7714	.3856
Chia seed intake, g/d (n=10)	0.0±0.0^{c}	2.1±0.1^{a}	0.0±0.0^{c}	1.1±0.0^{b}	<.0001	<.0001	<.0001
Energy intake, kJ/d (n=10)	400.8±9.3^{b}	503.1±18.1^{a}	452.5±12.4^{b}	507.7±13.6^{a}	.0475	<.0001	.0946
Body weight gain (8–16 weeks), % (n=10)	11.0±1.3^{b}	22.6±1.5^{a}	15.2±1.9^{b}	23.8±1.7^{a}	.1033	<.0001	.3593
Feed conversion efficiency, % (n=10)	2.7± 0.1^{c}	4.5± 0.3^{a}	3.3± 0.2^{b}	4.6± 0.2^{a}	.0801	<.0001	.1913
Bone mineral content, g (n=10)	12.4±0.2^{c}	13.3±0.3^{bc}	13.9±0.1^{b}	15.4±0.6^{a}	<.0001	.0017	.4018
Total body fat mass, g (n=10)	75.5±4.4^{b}	82.8±8.7^{b}	153.9±6.6^{a}	153.1±12.3^{a}	<.0001	.7049	.6371
Total body lean mass, g (n=10)	325.9±4.5^{b}	343.5±9.2^{ab}	345.9±8.9^{ab}	368.1±10.0^{a}	.012	.0237	.7865
Abdominal circumference, cm (n=10)	20.0±0.3^{b}	20.4±0.3^{b}	22.2±0.4^{a}	22.0±0.2^{a}	<.0001	.7475	.3369
Visceral adiposity index, % (n=10)	4.4±0.3^{c}	2.1±0.2^{d}	7.9±0.5^{a}	5.9±0.3^{b}	<.0001	<.0001	.4479
Tissue wet weights[e], mg/mm (n=10)							
Retroperitoneal fat	169.4±12.6^{c}	126.0±14.5^{c}	387.6±26.0^{a}	296.6±19.1^{b}	<.0001	.001	.2141
Epididymal fat	105.3±7.0^{b}	85.8±10.5^{b}	192.8±13.5^{a}	169.6±13.4^{a}	<.0001	.0697	.8742
Omental fat	102.8±11.4^{b}	69.5±6.5^{c}	175.0±10.3^{a}	147.4±5.7^{b}	<.0001	.0014	.7469
Liver	204.2±22.2^{b}	223.0±6.6^{b}	318.5±12.0^{a}	288.6±5.8^{a}	<.0001	.6741	.0723
Heart	22.3±0.3^{b}	22.7±0.8^{b}	25.6±1.0^{a}	23.4±0.4^{b}	.006	.2077	.0555
Tissue lipid content, mg/g (n=6)							
Retroperitoneal adipose tissue	396.2±25.3^{c}	453.9±12.9^{c}	632.3±22.4^{a}	527.1±19.4^{b}	<.0001	.2676	.0008
Liver	25.9±2.4^{b}	26.0±3.2^{b}	60.2±2.9^{a}	29.8±3.2^{b}	<.0001	<.0001	<.0001
Heart	29.8±0.7^{b}	38.4±2.7^{b}	36.7±2.2^{b}	47.5±4.4^{a}	.0106	.0029	.7012
Plasma	11.5±0.8	13.9±1.7	13.5±2.8	14.2±1.1	.5385	.3834	.6233
Plasma urea, mmol/L (n=10)	5.6±0.6^{a}	5.1±0.4^{a}	3.7±0.3^{b}	2.6±0.2^{b}	<.0001	.0549	.4616
Plasma LDH, U/L (n=10)	191.1±18.2^{c}	122.0±12.2^{c}	386.6±34.8^{a}	302.7±38.0^{b}	<.0001	.0097	.793
Plasma uric acid, μmol/L (n=10)	25.9±1.4^{b}	24.0±1.9^{b}	54.7±5.2^{a}	32.9±3.7^{b}	<.0001	.0013	.0059
Plasma CRP, μmol/L (n=10)	59.3±7.4^{b}	58.2±5.9^{b}	102.3±4.6^{a}	54.6±2.6^{b}	.0009	<.0001	.0001
Plasma total cholesterol, mmol/L (n=10)	1.3±0.1^{b}	1.4±0.1^{b}	2.0±0.1^{a}	1.8±0.1^{a}	<.0001	.6201	.1423
Plasma triglyceride, mmol/L (n=10)	0.4±0.0^{c}	0.6±0.1^{c}	1.0±0.1^{b}	1.6±0.2^{a}	<.0001	.0024	.1112
Plasma NEFA, mmol/L (n=10)	1.2±0.1^{c}	1.7±0.3^{bc}	2.2±0.1^{b}	4.7±0.3^{a}	<.0001	<.0001	<.0001
Plasma fatty acid, g/100g of total fatty acid content (n=6)							
C14:0	75.56±2.32	73.24±3.27	75.20±1.37	71.79±3.44	.7436	.3063	.8437
C14:1n-5	1.01±0.18^{a}	0.00±0.00^{b}	0.80±0.07^{a}	0.29±0.11^{b}	.7227	<.0001	.0359
C16:0	0.02±0.02^{b}	2.75±0.2^{a}	0.14±0.14^{b}	2.34±0.31^{a}	.5064	<.0001	.2306
C16:1n-7	14.99±1.95^{a}	9.24±1.21^{b}	13.85±0.71^{a}	7.59±0.89^{b}	.2891	.0001	.8442
C18:1n-9	0.00±0.00^{b}	2.25±0.34^{a}	0.00±0.00^{b}	2.25±0.28^{a}	1	<.0001	1
C18:1*trans*-7	3.20±0.13^{b}	2.71±0.34^{b}	3.73±0.19^{b}	8.75±1.07^{a}	<.0001	.0008	.0001
C18:2n-6	1.71±0.18^{a}	0.00±0.00^{b}	2.02±0.22^{a}	0.00±0.00^{b}	.2884	<.0001	.2884
C18:3n-6	0.00±0.00	1.32±0.50	0.00±0.00	1.12±0.48	.7759	.0022	.7759
C18:3n-3	1.45±0.16^{a}	2.98±0.56^{b}	1.62±0.13^{a}	2.22±0.93^{b}	.3456	.0023	.1435
C20:3n-3	0.00±0.00^{b}	1.79±0.43^{a}	0.00±0.00^{b}	1.89±0.17^{a}	.831	<.0001	.831
C20:5n-3	2.07±0.07^{b}	0.86±0.09^{c}	2.65±0.34^{a}	0.57±0.06^{c}	.4345	<.0001	.0267
C24:0	0.00±0.00^{b}	0.33±0.02^{a}	0.00±0.00^{b}	0.28±0.05^{a}	.3642	<.0001	.3642
C22:6n-3	0.00±0.00^{c}	0.64±0.08^{b}	0.00±0.00^{c}	0.91±0.09^{a}	.0364	<.0001	.0364
Total SFA	75.58±2.31	78.22±1.90	75.34±1.35	74.41±3.17	.3853	.7118	.4431
Total MUFA	19.19±2.22	14.19±1.42	18.38±0.89	18.88±1.96	.2676	.2009	.1217
Total PUFA	5.23±0.30	7.59±0.73	6.28±0.65	6.71±1.26	.9176	.101	.2482
n3:n6 ratio	2.15±0.21^{b}	9.10±2.41^{a}	2.15±0.15^{b}	10.99±4.26^{a}	.5748	.0001	.5748

Each value is a mean±S.E.M. Means with superscript lettersa,b,c,d without a common letter differ (P <.05). Number of repetitive experiments are indicated within parenthesis. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

[e] Normalized against tibial length.

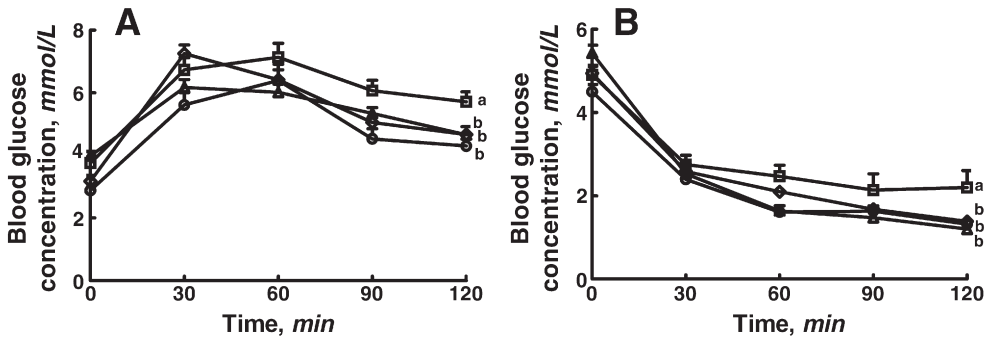

Figure	*P-value*		
	Diet	Chia	Diet x Chia
A	0.0055	0.1578	0.0058
B	0.0134	0.0286	0.0988

Fig. 1. Glucose (2 g/kg) (A) and insulin (0.75 IU/kg) (B) tolerance in C, CC, H and HC diet-fed rats. Data are shown as means±S.E.M. Means of values at 120 min without a common alphabet significantly differ. n=10/group.

dehydrogenase (LDH) activity as a marker of decreased metabolic activity, uric acid concentrations as a marker of fructose-induced oxidative stress and plasma CRP concentrations as a marker of inflammation (Table 1). In addition, chia seed-supplemented groups showed improved glucose and insulin tolerance (Fig. 1).

The plasma fatty acid profile suggests that the chia seed-supplemented rats resisted desaturation of C14:0 and C16:0 fatty acids by SCD (Table 2). The SCD index could not be calculated in C and H rats due to low concentrations of the saturated substrate. Both chia seed-supplemented groups had increased plasma concentrations of

Table 3
Changes in cardiovascular structure, function and fatty acid composition in C, CC, H and HC diet-fed rats

Variable	C	CC	H	HC	*P*		
					Diet	Treatment	Interaction
LVIDd, mm (n=10)	6.4±0.2^{b}	6.7±0.2^{b}	7.7±0.2^{a}	6.1±0.3^{b}	.1354	.0074	.0002
LVPWd, mm (n=10)	1.7±0.0^{b}	1.8±0.1^{b}	1.9±0.0^{ab}	2.1±0.1^{a}	.0011	.0408	.4841
Relative wall thickness (n=10)	0.4±0.01^{c}	0.5±0.01^{b}	0.6±0.02^{a}	0.4±0.01^{c}	.0006	.0006	<.0001
Fractional shortening, % (n=10)	60.8±1.4^{a}	64.0±1.9^{a}	50.5±2.1^{b}	59.2±2.2^{a}	.0004	.0038	.1617
Ejection fraction, % (n=10)	84.8±1.6	89.0±1.1	83.8±1.3	84.4±1.6	.0557	.0987	.2118
Ejection time, ms (n=10)	85.1±4.0	85.8±3.3	86.9±2.6	89.6±6.6	.528	.7011	.8212
Deceleration time, ms (n=10)	61.3±1.6^{c}	70.8±1.4^{b}	56.2±1.6^{c}	64.2±1.5^{a}	.0005	<.0001	.6264
LV developed pressure, mmHg (n=10)	64.7±6.1^{a}	51.3±4.5^{ab}	41.1±3.7^{b}	57.4±3.8^{a}	.0674	.7596	.003
+dP/dt, mmHg×s^{-1} (n=10)	1078.9±104.3^{a}	1009.1±62.8^{a}	753.6±37.3^{b}	1052.5±81.2^{a}	.0701	.1378	.0197
−dP/dt, mmHg×s^{-1} (n=10)	−613.5±66.2^{a}	−616.3±57.2^{a}	−373.9±21.9^{b}	−601.3±43.8^{a}	.0156	.0277	.0314
Diastolic stiffness (k), (n=10)	22.8±0.7^{c}	22.9±0.6^{c}	29.4±0.6^{a}	26.5±0.6^{b}	<.0001	.8741	<.0001
Estimated LV mass, g (n=10)	0.7±0.0^{b}	0.8±0.0^{b}	1.1±0.1^{a}	0.8±0.1^{b}	.0076	.1659	.0076
LV+septum wet weight[e], mg/mm (n=10)	19.0±0.4^{b}	19.4±0.7^{b}	21.4±1.0^{a}	19.2±0.3^{b}	.0847	.1503	.0466
Right ventricle wet weight[e], mg/mm (n=10)	3.2±0.2^{b}	3.3±0.2^{b}	4.2±0.2^{a}	4.2±0.2^{a}	<.0001	.8625	.7107
LV fibrosis, % surface area (n=4)	3.6±0.9^{b}	3.5±0.7^{b}	12.6±1.2^{a}	4.4±0.2^{a}	<.0001	.0003	.0004
Systolic blood pressure, mmHg (n=10)	123.7±1.8^{c}	138.3±3.3^{b}	150.7±3.6^{a}	153.2±5.6^{a}	<.0001	.0316	.1222
Fatty acids, g/100g of total fatty acid content (n=6)							
C14:0	42.49±3.73^{b}	53.44±3.72^{ab}	54.07±5.35^{ab}	58.05±1.18^{a}	.0458	.0635	.37
C14:1n-5	0.72±0.15^{a}	0.22±0.14^{b}	0.71±0.17^{a}	0.00±0.00^{b}	.3983	.0002	.4399
C16:0	1.22±0.13^{b}	8.45±0.70^{a}	2.26±1.49^{b}	6.75±0.34^{a}	.6996	<.0001	.1198
C16:1n-7	14.83±1.08^{a}	1.01±0.29^{c}	10.23±2.00^{b}	0.45±0.02^{c}	.0357	<.0001	.0932
C18:0	0.49±0.04^{a}	2.48±0.06^{b}	0.69±0.08^{c}	1.18±0.08^{d}	<.0001	<.0001	<.0001
C18:1n-9	0.53±0.05^{b}	11.28±0.65^{a}	0.31±0.07^{b}	11.90±0.65^{a}	.6695	<.0001	.3738
C18:1*trans*-7	12.96±1.00^{a}	2.05±0.09^{b}	11.41±1.57^{a}	1.58±0.04^{b}	.2914	<.0001	.5688
C18:2n-6	6.79±0.79	6.67±1.17	4.75±0.82	5.23±0.31	.0492	.8307	.7218
C18:3n-6	7.47±0.63^{a}	0.00±0.00^{b}	4.26±1.02^{a}	0.00±0.00^{b}	.0145	<.0001	.0145
C18:3n-3	0.00±0.00^{b}	0.60±0.15^{a}	0.0±0.0^{b}	0.41±0.07^{a}	.2646	<.0001	.2646
C20:4n-6	0.04±0.04^{a}	0.23±0.07^{b}	0.49±0.05^{c}	0.24±0.02^{b}	.0001	.543	.0002
C20:5n-3	11.31±0.95^{a}	6.19±0.34^{c}	8.92±0.95^{b}	6.44±0.05^{c}	.1385	<.0001	.0714
C22:5n-3	0.05±0.05^{b}	1.29±0.41^{a}	0.00±0.00^{b}	0.07±0.01^{b}	.006	.0048	.0103
C24:0	0.29±0.19^{c}	3.19±0.93^{a}	0.21±0.12^{c}	1.82±0.01^{b}	.1453	.0001	.1927
C22:6n-3	0.77±0.16^{b}	2.67±0.91^{c}	0.96±0.23^{b}	5.60±0.23^{a}	.0047	<.0001	.0111
Total SFA	44.53±3.60^{d}	67.73±1.43^{b}	57.33±5.98^{c}	67.95±0.87^{a}	.0847	.0001	.095
Total MUFA	29.04±1.79^{a}	14.56±0.89^{b}	22.66±3.55^{c}	13.93±0.63^{b}	.1046	<.0001	.1784
Total PUFA	26.43±2.18^{a}	17.71±1.07^{b}	20.01±2.55^{b}	18.12±0.3^{b}	.1053	.0072	.0682
n3:n6 ratio	0.86±0.06^{b}	2.05±0.05^{a}	1.28±0.28^{b}	2.27±0.15^{a}	.0645	<.0001	.5478
Stearoyl-CoA 9-desaturase index	12.43±0.94^{a}	0.11±0.02^{b}	13.47±2.94^{a}	0.07±0.00^{b}	.7493	<.0001	.7301

Each value is a mean±S.E.M. Means with superscript letters^{a,b,c,d} without a common letter differ (P <.05). Number of repetitive experiments are indicated within parenthesis. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

[e] Normalized against tibial length.

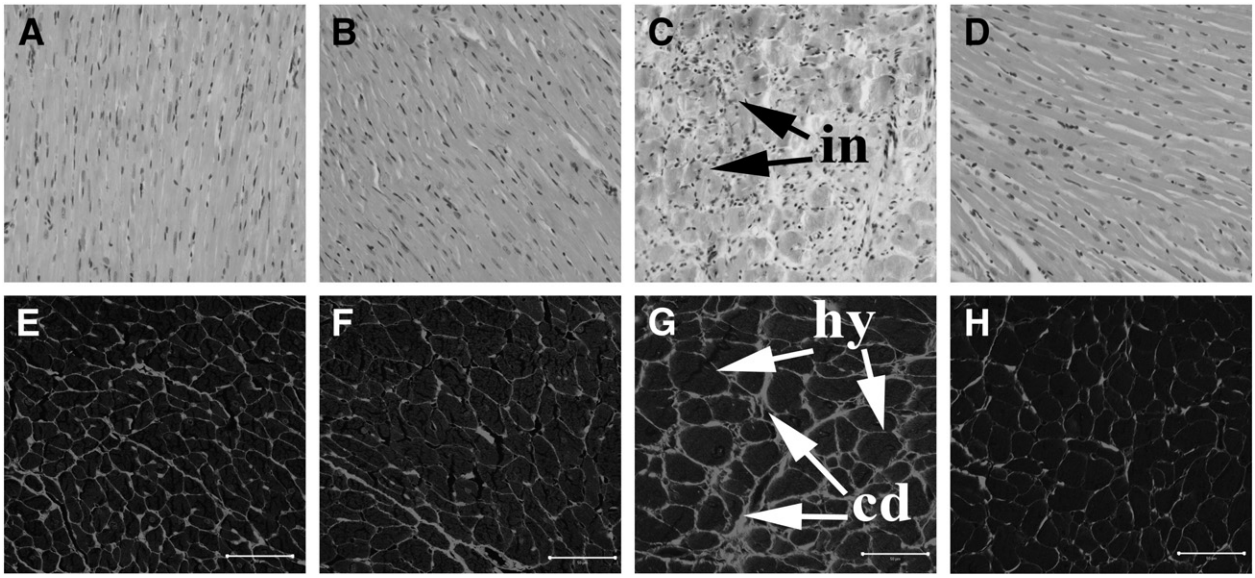

Fig. 2. Hematoxylin and eosin staining of LV (×20) showing inflammatory cells (marked as "in") as dark spots outside the myocytes; C (A), CC (B), H (C) and HC (D) diet-fed rats. Picrosirius red staining of left ventricular interstitial collagen deposition (×40) in C (E), CC (F), H (G) and HC (H) diet-fed rats. Collagen deposition is marked as "cd" and hypertrophied cardiomyocytes are marked as "hy."

C18:1n-9, but increased concentrations of C18:1*trans*-7 were observed only in HC group (Table 2). Both chia seed-supplemented groups had depleted plasma C18:2n-6 and C20:5n-3 but increased C18:3n-6, C18:3n-3, C20:4n-3 and C22:6 n-3 concentrations (Table 2). Although the total saturated and unsaturated fatty acids remained unchanged, the n3:n6 ratio was markedly enhanced in the plasma of the chia seed-supplemented groups (Table 2).

3.2. Cardiovascular changes

Compared with H rats, HC groups had smaller LV internal diameter with increased fractional shortening and deceleration time (Table 3). In addition, chia seed reduced LV wet weight and reduced estimated LV mass (Table 3). However, chia seed supplementation did not reduce blood pressure in either group.

After 16 weeks, H rats showed greater infiltration by inflammatory cells into the LV (Fig. 2C) as well as increased interstitial collagen deposition (Fig. 2G; Table 3) compared with C rats (Fig. 2A and E; Table 3). HC rats showed normalized inflammatory state (Fig. 2D) and markedly reduced collagen deposition (Fig. 2H; Table 3), but no changes in inflammatory cell infiltration or collagen deposition were seen in CC rats (Fig. 2B and F; Table 3). The reduction in LV fibrosis is consistent with reduced diastolic stiffness constant in HC diet-fed rats (Fig. 2D and H; Table 3).

Furthermore, H feeding diminished vascular responses in isolated thoracic aortic rings to noradrenaline, sodium nitroprusside and acetylcholine when compared with C rats (Fig. 3A-C). In isolated thoracic

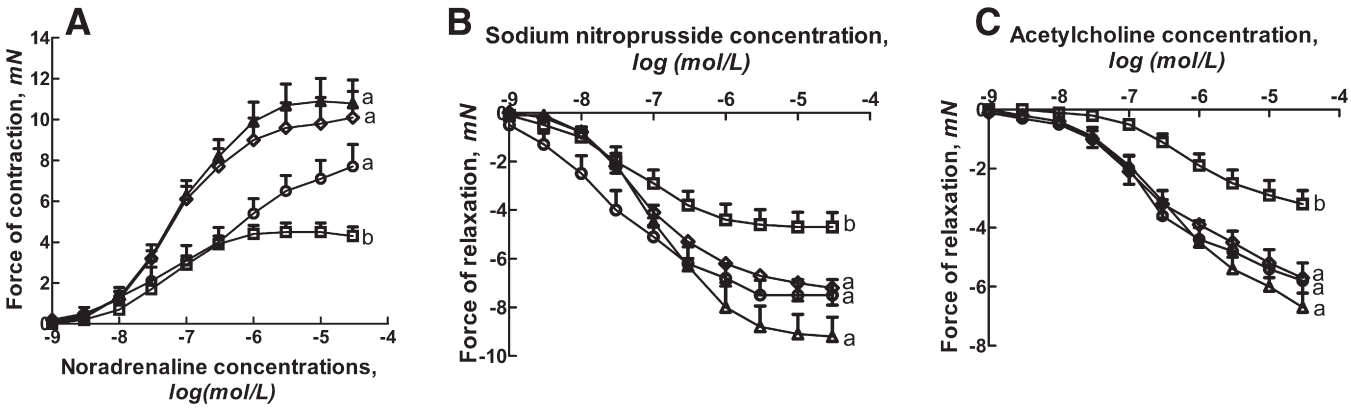

Figure	*P*-Value		
	Diet	Treatment	Interaction
A	0.0594	0.0002	0.2075
B	0.0016	0.005	0.5716
C	0.0074	0.011	0.2147

Fig. 3. Cumulative concentration–response curves for noradrenaline (A), sodium nitroprusside (B) and acetylcholine (C) in thoracic aortic rings from C, CC, H and HC diet-fed rats. Data are shown as means±S.E.M. Endpoint means without a common alphabet in each data set significantly differ. n=8–10/group.

aortic rings from HC rats, increased vasorelaxation was induced by sodium nitroprusside and acetylcholine (Fig. 3B and C) in addition to increased contractility to noradrenaline (Fig. 3A). Vascular responses in isolated thoracic aortic rings from CC rats remained unchanged compared with C rats (Fig. 3A-C).

Similar to the plasma fatty acid profile, chia seed-supplemented rats resisted SCD action on C14:0 and C16:0 fatty acids in the heart (Table 3). Both chia seed-supplemented groups had increased concentrations of C18:0, C18:1n-9 but decreased concentrations of C18:1*trans*-7 in the heart together with depleted C18:3n-6 and C20:5n-3 but increased C18:3n-3, 22:5n-3, C22:6n-3 and C24:0 concentrations (Table 3). As a result of decreased desaturation of C14:0 and C16:0, the total saturated fatty acid increased, and total unsaturated fatty acid content decreased (Table 3). However, the cardiac n-3:n-6 ratio was increased in the chia seed-supplemented groups (Table 3).

3.3. Hepatic structure and function

H diet feeding elevated plasma markers of liver function in comparison with C-fed rats (Table 4). HC diet feeding did not alter the elevated alanine transaminase (ALT) activity but decreased the aspartate transaminase (AST) activity (Table 4). ALP activity was increased in the HC group (Table 4). Creatine kinase (CK) activity decreased in both chia seed-supplemented groups, but none of the other enzymes were affected in CC group (Table 4).

C (Fig. 4A, E and I) and CC (Fig. 4B, F and J) groups showed negligible lipid accumulation, inflammatory cell infiltration or fibrosis in the liver. H feeding for 16 weeks increased the size of the fat vacuoles within the hepatocytes (Fig. 4C) with increased portal inflammatory cell infiltration (Fig. 4G) and portal fibrosis (Fig. 4K; Table 4). The HC diet-fed rats displayed normalized macrovesicular steatosis (Fig. 4D), portal inflammation (Fig. 4H) and fibrosis (Fig. 4I; Table 4).

Similar to cardiac and plasma fatty acid profile, chia seed-supplemented rats resisted stearoyl-CoA 9-desaturation of C14:0 and C16:0 fatty acids in the liver (Table 4). Both chia seed-supplemented groups had increased concentrations of C14:0, C16:0, C18:0 and C18:1n-9 but decreased concentrations of C18:1*trans*-7 and C18:2n-6 (Table 4). Although C20:4n-6 content in the liver remained unchanged across all groups, both chia seed-supplemented groups had depleted C18:3n-3 and C20:5n-3 but increased C22:5n-3, C22:6n-3 and C24:0 concentrations (Table 4). As a result of decreased desaturation of C14:0 and C16:0, the total saturated fatty acid increased and total unsaturated fatty acid content decreased (Table 4). However, the liver n-3:n-6 ratio was increased in the chia seed-supplemented groups (Table 4).

3.4. Retroperitoneal adipose tissue fatty acid composition

Similar to other tissues, chia seed-supplemented rats resisted stearoyl-CoA 9-desaturation of C14:0 and C16:0 fatty acids (Table 5) in the retroperitoneal adipose tissue. Both chia seed-supplemented groups had increased concentrations of C14:0, C16:0 and C18:1*trans*-7 but decreased concentrations of C18:2n-6 and C18:3n-6 (Table 5). C18:3n-3 was increased in HC group (Table 5) despite the extremely small amounts of fatty acids with chain length of more than 20 carbon atoms detected in the adipose tissue of all groups, making accurate quantification difficult. Consequently, the total saturated fatty acid increased and total unsaturated fatty acid content decreased, but the n-3:n-6 ratio in retroperitoneal fat was increased in the chia seed-supplemented groups (Table 5).

4. Discussion

The aim of this study was to assess the metabolic, cardiac and liver changes following 5% chia seed supplementation in high-carbohydrate, high-fat (H) diet-fed rats with low n-3 fatty acids.

Table 4
Changes in hepatic structure, function and fatty acid composition in C, CC, H and HC diet-fed rats

Variable	C	CC	H	HC	*P*		
					Diet	Treatment	Interaction
ALT, U/L (n=10)	31.0±1.8^{b}	31.6±3.0^{b}	46.2±3.8^{a}	45.4±4.9^{a}	.0002	.9777	.8452
AST, U/L (n=10)	76.0±3.5^{b}	75.0±3.2^{b}	104.8±5.9^{a}	76.4±8.1^{b}	.0099	.0118	.0183
ALP, U/L (n=10)	154.9±11.9^{b}	188.4±30.5^{b}	181.4±11.6^{b}	251.1±12.5^{a}	.0209	.0083	.3333
CK, U/L (n=10)	165.3±13.2^{b}	70.4±11.0^{c}	243.4±28.2^{a}	122.3±17.3^{bc}	.0013	<.0001	.4867
Liver fibrosis, % surface area (n=4)	1.6±0.2^{c}	1.8±0.1^{c}	7.4±0.5^{a}	3.6±0.2^{b}	<.0001	<.0001	<.0001
Fatty acid, g/100g of total fatty acid content (n=6)							
C14:0	6.69±0.98^{b}	14.16±1.30^{a}	3.10±0.59^{c}	9.92±1.56^{b}	.0031	<.0001	.7832
C14:1n-5	1.72±0.14^{a}	0.72±0.06^{b}	0.79±0.09^{a}	1.05±0.09^{c}	.0575	<.0001	.2051
C16	0.03±0.03^{b}	19.96±0.41^{a}	0.25±0.05^{b}	21.28±0.80^{a}	.1028	<.0001	.2363
C16:1n-7	25.40±1.10^{a}	3.74±0.75^{b}	23.30±0.75^{a}	1.31±0.32^{c}	.0088	<.0001	.8347
C18:0	0.00±0.00^{d}	1.04±0.14^{a}	0.32±0.07^{c}	0.64±0.05^{b}	.6316	<.0001	.0003
C18:1n-9	0.00±0.00^{c}	29.25±1.35^{b}	1.52±1.52^{c}	40.16±1.34^{a}	<.0001	<.0001	.001
C18:1*trans*-7	14.23±1.93^{a}	0.00±0.00^{b}	10.86±3.37^{a}	0.00±0.00^{b}	.3958	<.0001	.3958
C18:2n-6	26.58±4.73^{b}	8.60±0.96^{b}	44.26±4.93^{a}	7.18±0.29^{c}	.0289	<.0001	.0119
C18:3n-3	7.42±0.47^{a}	3.42±0.30^{c}	4.93±0.15^{b}	3.23±0.18^{c}	.0003	<.0001	.0011
C20:0	0.40±0.02^{c}	0.17±0.02^{b}	0.19±0.02^{b}	0.47±0.04^{a}	.1045	.356	<.0001
C20:4n-6	0.64±0.07	0.67±0.34	0.73±0.11	0.83±0.28	.5923	.7801	.8804
C20:5n-3	10.85±2.89^{a}	8.33±0.53^{b}	7.96±2.31^{b}	6.34±0.46^{c}	<.0001	.0001	.3082
C22:5n-3	0.16±0.07^{c}	2.81±0.19^{b}	0.05±0.03^{c}	1.56±0.08^{a}	<.0001	<.0001	<.0001
C24:0	0.39±0.13^{b}	4.11±0.68^{a}	0.06±0.06^{b}	3.64±0.77^{a}	.4495	<.0001	.894
C22:6n-3	0.24±0.15^{c}	2.16±0.16^{a}	0.03±0.03^{c}	1.38±0.13^{b}	.001	<.0001	.0381
Total SFA	10.26±3.20^{b}	39.46±1.40^{a}	3.91±0.61^{c}	35.96±1.85^{b}	.023	<.0001	.4843
Total MUFA	40.35±1.07^{ab}	33.75±1.83^{c}	36.65±1.86^{bc}	42.70±1.26^{a}	.1047	.8605	.0006
Total PUFA	49.38±3.93^{b}	26.79±0.82^{c}	59.43±2.08^{a}	21.34±1.16^{c}	.3362	<.0001	.0034
n3:n6 ratio	0.99±0.23^{b}	1.78±0.30^{a}	0.35±0.12^{c}	1.44±0.16^{ab}	.0329	.0003	.4911
Stearoyl-CoA 9-desaturase index^{d}	–	0.19±0.04	78.84±2.44	0.06±0.02	–	–	–

Each value is a mean±S.E.M. Means with superscript letters^{a,b,c,d} without a common letter differ (P <.05). Number of repetitive experiments is indicated within parenthesis. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

[d] Negligible C16:0 fatty acid was detected in C diet-fed group, therefore showing a very high stearoyl-CoA 9-desatururation index in this group.

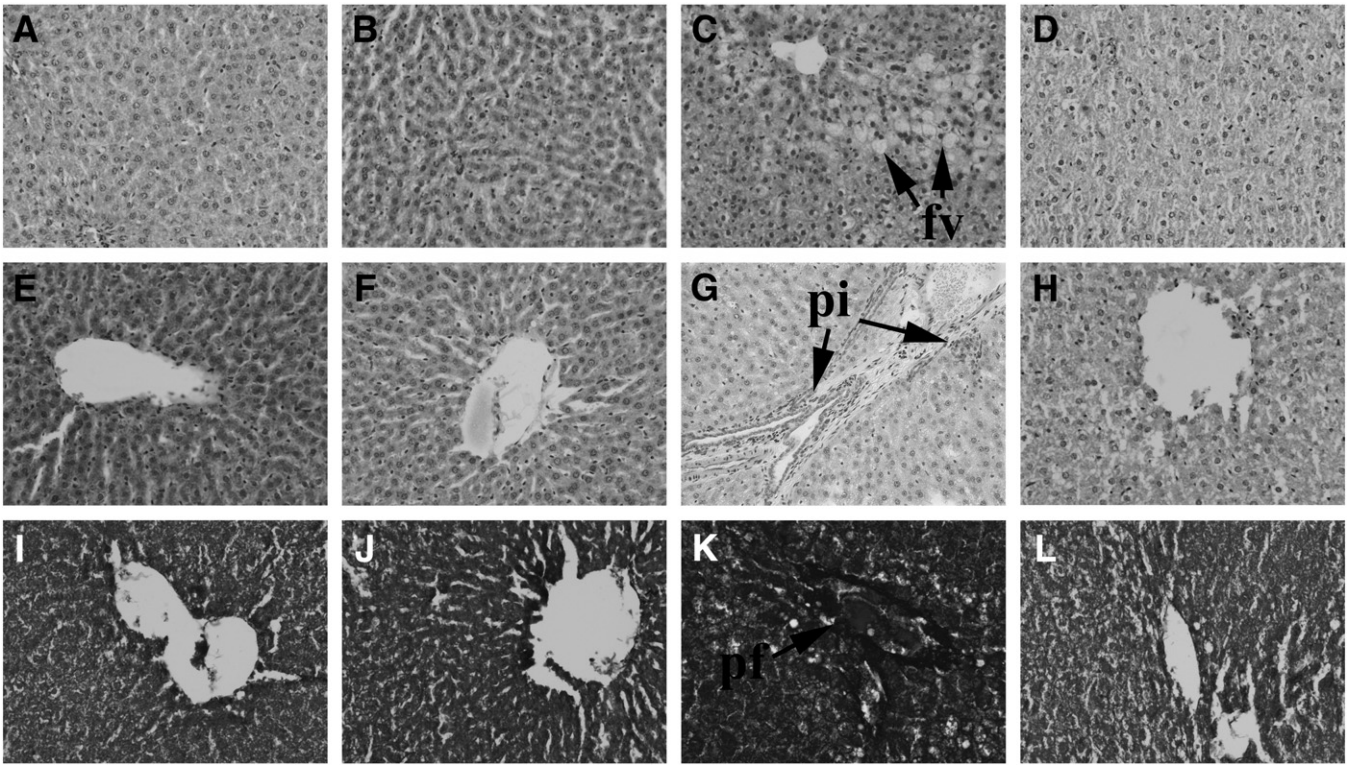

Fig. 4. Hematoxylin and eosin staining of hepatocytes (×20) showing hepatocytes with enlarged fat vacuole (marked as "fv") and inflammatory cells around the portal region (marked as "pi") (×20) from C (A, E), CC (B, F), H (C, G) and HC (D, H) diet-fed rats. Milligan's trichrome staining of the hepatic portal regions showing collagen (marked as "pf") (×20) in C (I), CC (J), H (K) and HC (L) diet-fed rats.

Rats fed H diet developed hypertension, impaired glucose and insulin tolerance, dyslipidemia, hepatic steatosis, cardiac fibrosis and functional deterioration, inflammation and abdominal obesity. With the exception of elevated blood pressure and some plasma markers of liver function, dietary chia seed supplementation attenuated structural and functional changes caused by H feeding. Chia seed supplementation caused lipid redistribution away from the abdominal cavity, suppressed stearoyl-CoA desaturase index and increased n-3:n-6 ratio in various tissues.

Chia seed-supplemented rats improved insulin and glucose tolerance, reduced visceral adiposity, decreased hepatic steatosis, reduced cardiac and hepatic fibrosis and inflammation without changes in plasma lipids or blood pressure. However, the most notable result from our study is the chia seed-induced lipid redistribution with lipid trafficking away from the visceral fat and liver with increased accumulation in the heart. Furthermore, the selectivity for different fatty acids for the unsaturated and the desaturated products of the 18 carbon length fatty acids was altered in different tissues by chia seed supplementation.

The relative abundance of the C18:1n-9 was increased in the heart and the liver of the chia seed-supplemented groups. The C18:1*trans*-7 was more selectively stored in the adipose tissue than the heart and the liver, and the C18:2n-6 or its elongated Δ^5 and Δ^6 desaturase products were depleted in all tissues suggesting that C18:2n-6 is

Table 5
Fatty acid composition of retroperitoneal fat and desaturase index from C, CC, H and HC diet-fed rats

Fatty acid, g/100g of total fatty acid content (n=6)	C	CC	H	HC	*P*		
					Diet	Treatment	Interaction
C14:0	0.00±0.00^{c}	1.75±0.08^{b}	0.00±0.00^{c}	2.55±0.11^{a}	<.0001	<.0001	<.0001
C14:1n-5	2.17±0.04^{b}	0.12±0.01^{c}	2.81±0.10^{a}	0.22±0.02^{c}	<.0001	<.0001	<.0001
C16:0	0.22±0.05^{b}	31.15±1.60^{a}	0.42±0.08^{b}	23.62±0.68^{c}	.0004	<.0001	.0003
C16:1n-7	31.48±1.06^{a}	2.44±0.27^{b}	22.40±0.21^{c}	1.47±0.46^{b}	<.0001	<.0001	<.0001
C18:0	0.22±0.03^{c}	0.47±0.09^{c}	0.62±0.02^{c}	1.10±0.10^{a}	<.0001	<.0001	.1143
C18:1*trans*-7	3.46±0.23^{c}	40.99±1.52^{b}	6.97±0.45^{d}	59.91±1.29^{a}	<.0001	<.0001	<.0001
C18:2n-6	51.18±0.96^{b}	10.78±0.80^{c}	60.76±0.47^{a}	6.51±0.49^{d}	.0013	<.0001	<.0001
C18:3n-6	0.08±0.04^{b}	0.33±0.21^{b}	5.18±0.08^{a}	0.09±0.01^{b}	<.0001	<.0001	<.0001
C18:3n-3	10.11±0.32^{a}	10.45±0.80^{a}	0.07±0.07^{c}	3.12±0.42^{b}	<.0001	.0021	.0106
C20:0	0.66±0.13^{a}	0.14±0.05^{c}	0.35±0.02^{bc}	0.54±0.04^{ab}	.5453	.0354	<.0001
Total SFA	1.10±0.14^{c}	33.78±1.44^{a}	1.48±0.13^{c}	28.09±0.50^{b}	.0025	<.0001	.0008
Total MUFA	37.21±0.86^{c}	43.98±1.37^{b}	32.40±0.43^{d}	62.03±1.23^{a}	<.0001	<.0001	<.0001
Total PUFA	61.70±0.92^{b}	22.18±0.78^{c}	66.12±0.48^{a}	9.85±0.93^{d}	<.0001	<.0001	<.0001
n3:n6 ratio	0.20±0.01^{c}	1.02±0.18^{a}	0.00±0.00^{c}	0.48±0.03^{b}	.0006	<.0001	.0776
Stearoyl-CoA 9-desaturase index	129.55±16.75^{a}	0.08±0.01^{c}	44.38±0.51^{b}	0.06±0.02^{c}	<.0001	<.0001	<.0001

Each value is a mean±S.E.M. Means with superscript letters^{a,b,c,d} without a common letter differ (P <.05). Number of repetitive experiments are indicated within parenthesis. SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

preferentially oxidized rather than stored. Trans-fatty acids only undergo partial β-oxidation and are not the preferred substrate [32]. However, C18:1*trans*-7 can be efficiently converted to conjugated linoleic acid by the action of stearoyl-CoA desaturase 1 (SCD-*1*) [33], the activity of which is inhibited by C18:3n-3-enriched chia seed. The fatty acid homeostasis in tissues of the chia seed-supplemented rats was maintained so that the C18:1*trans*-7 was preferentially stored in the adipose tissue; the relatively inert C18:1n-9 was stored in sensitive organs such as liver and heart and the C18:2, n-6, the parent fatty acid of the n-6 pathway, was preferentially oxidized. It is therefore plausible that the mechanism of down-regulation of the n-6 pathway by n-3 fatty acids involves increasing the transport and oxidation of C18:2n-6 into the mitochondria.

Our results are consistent with the inhibition of SCD-1 by ALA-rich chia seed. SCD-1 is a Δ^9 fatty acid desaturase that catalyzes the rate-limiting step in the production of monounsaturated from saturated fatty acids [34]. In addition to reduced MUFA synthesis, SCD-1 deficiency or treatment with SCD-1-targeted antisense oligonucleotides induced protection from obesity, cellular lipid accumulation and insulin resistance in mice [35–37]. Fructose, being more lipogenic than glucose, is a potent inducer of hepatic *Scd-1* [38–40], and this enzyme plays a pivotal role in fructose-induced lipogenesis [41]. In addition to metabolic effects elicited by dietary carbohydrates, fatty acids also modulate the transcriptional activation of *Scd-1* and other lipogenic genes [42, 43]. The binding of sterol regulatory element binding protein-1 (SREBP-1) to the SREBP response element of the *Scd-1* promoter is decreased by dietary *n-3* polyunsaturated fatty acid from fish oil [44]. In addition, increased plasma palmitoleate, a product of SCD-1, in humans has been independently associated with both hypertriglyceridemia and abdominal adiposity [45]. Saturated fat-enriched diet induced lipogenic genes in wild-type mice, with the induction of the *Scd-1* and *Cpt-1* gene [46]. On the contrary, in *Scd-1*-deficient mice, the high saturated fat diet does not induce lipogenesis; instead, mitochondrial fatty acid oxidation is increased [46]. However, inhibition of SCD-1 in a low-fat diet could potentially increase the diet intake to supplement the loss of unsaturated fatty acid due to inhibition for normal metabolic function. This effect is clearly seen in CC diet-fed rats where the dietary intake increases following chia seed supplementation.

In *Scd-1*-deficient mice, a lack of functional SCD-1 decreases the accumulation of hepatic triglycerides and cholesterol esters, down-regulates *de novo* fatty acid synthesis in the liver and reduces adiposity [37]. Furthermore, *Scd-1*$^{-/-}$ mice are resistant to high-carbohydrate and high-fat diet-induced liver steatosis [47]. *Scd-1*$^{-/-}$ mice have increased fatty acid β-oxidation in the liver [48], skeletal muscle [49] and brown adipose tissue [50] and up-regulated AMP-activated protein kinase pathway [48,49]. In addition to the regulation of lipid metabolism, SCD-1 is involved in the regulation of carbohydrate metabolism. *Scd-1*$^{-/-}$ mice have increased whole-body glucose tolerance and elevated insulin sensitivity in skeletal muscle and brown adipose tissue [51,52]. However, in the heart, the lack of *Scd-1* decreased mitochondrial fatty acid uptake and oxidation while increasing glucose transport and oxidation [53]. In leptin-deficient *ob*/*ob* mice, disruption of *Scd-1* gene improved cardiac function by correcting systolic and diastolic dysfunction without altering plasma triglyceride and NEFA concentrations [54].

To the best of our knowledge, this is the first report of lipid redistribution with a rich dietary source of any n-3 fatty acid associated with cardioprotection and hepatoprotection. In addition, we report an intricate pattern of fatty acid distribution in various tissues from rats fed a chia seed-supplemented diet that would probably lead to an improved lipid homeostatic condition. The results warrant further research on the use of chia seed as a complimentary therapy for treating some signs of metabolic syndrome.

Acknowledgments

We thank Mr. James Dorward, Chialife, Main Beach, Qld, Australia, for the supply of chia seeds, and Mr. Greg Jardine, Dr. Red Nutraceuticals, Mt. Nebo, Queensland, Australia, for financial support to allow this project to be undertaken. We thank Dr. Fiona Campbell, School of Veterinary Sciences, The University of Queensland, Australia, for the acquisition of echocardiographic images. H.P. and L.B. developed the original study aims; H.P. and S.K.P conducted the experiments and carried out the data analyses; data were interpreted by both H.P. and L.B. J.W. assisted with the identification and evaluation of fatty acids. L.W. assisted with dual-energy X-ray absorptiometry and provided nutritional advice. H.P. and L.B. prepared manuscript drafts, with all authors contributing to the final version. L.B. has been the corresponding author throughout the writing process. There are no conflicts of interest.

References

[1] Lee JH, O'Keefe JH, Lavie CJ, Harris WS. Omega-3 fatty acids: cardiovascular benefits, sources and sustainability. Nat Rev Cardiol 2009;6:753–8.
[2] Saremi A, Arora R. The utility of omega-3 fatty acids in cardiovascular disease. Am J Ther 2009;16:421–36.
[3] Yashodhara BM, Umakanth S, Pappachan JM, Bhat SK, Kamath R, Choo BH. Omega-3 fatty acids: a comprehensive review of their role in health and disease. Postgrad Med J 2009;85:84–90.
[4] Duda MK, O'Shea KM, Stanley WC. Omega-3 polyunsaturated fatty acid supplementation for the treatment of heart failure: mechanisms and clinical potential. Cardiovasc Res 2009;84:33–41.
[5] Anderson BM, Ma DW. Are all n-3 polyunsaturated fatty acids created equal? Lipids Health Dis 2009;8:33.
[6] Ayerza R, Coates W. Ground chia seed and chia oil effects on plasma lipids and fatty acids in the rat. Nutr Res 2005;25:995–1003.
[7] Alvarez-Chavez LM, Valdivia-Lopez MD, Aburto-Juarez MD, Tecante A. Chemical characterization of the lipid fraction of Mexican chia seed (*Salvia hispanica* L.). Int J Food Prop 2008;11:687–97.
[8] Ayerza R. The seed's protein and oil content, fatty acid composition, and growing cycle length of a single genotype of chia (*Salvia hispanica* L.) as affected by environmental factors. J Oleo Sci 2009;58:347–54.
[9] Ayerza Jr R, Coates W. Effect of dietary alpha-linolenic fatty acid derived from chia when fed as ground seed, whole seed and oil on lipid content and fatty acid composition of rat plasma. Ann Nutr Metab 2007;51:27–34.
[10] Chicco AG, D'Alessandro ME, Hein GJ, Oliva ME, Lombardo YB. Dietary chia seed (*Salvia hispanica* L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. Br J Nutr 2009;101:41–50.
[11] Vuksan V, Whitham D, Sievenpiper JL, Jenkins AL, Rogovik AL, Bazinet RP, et al. Supplementation of conventional therapy with the novel grain Salba (*Salvia hispanica* L.) improves major and emerging cardiovascular risk factors in type 2 diabetes: results of a randomized controlled trial. Diabetes Care 2007;30:2804–10.
[12] Nieman DC, Cayea EJ, Austin MD, Henson DA, McAnulty SR, Jin F. Chia seed does not promote weight loss or alter disease risk factors in overweight adults. Nutr Res 2009;29:414–8.
[13] Ratnayake WM, Galli C. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: a background review paper. Ann Nutr Metab 2009;55:8–43.
[14] Brouwer IA, Katan MB, Zock PL. Dietary alpha-linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: a meta-analysis. J Nutr 2004;134:919–22.
[15] de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.
[16] Lemaitre RN, King IB, Mozaffarian D, Kuller LH, Tracy RP, Siscovick DS. n-3 Polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the Cardiovascular Health Study. Am J Clin Nutr 2003;77:319–25.
[17] Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, et al. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr 1999;70:1001–8.
[18] Albert CM, Oh K, Whang W, Manson JE, Chae CU, Stampfer MJ, et al. Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. Circulation 2005;112:3232–8.
[19] Djousse L, Arnett DK, Carr JJ, Eckfeldt JH, Hopkins PN, Province MA, et al. Dietary linolenic acid is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the National Heart, Lung, and Blood Institute Family Heart Study. Circulation 2005;111:2921–6.

[20] Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation 2005;111:157–64.
[21] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009;120:1640–5.
[22] Poudyal H, Campbell F, Brown L. Olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high fat-fed rats. J Nutr 2010;140: 946–53.
[23] Poudyal H, Panchal S, Brown L. Comparison of purple carrot juice and β-carotene in a high-carbohydrate, high-fat diet-fed rat model of the metabolic syndrome. Br J Nutr 2010;104:1322–32.
[24] Zhang G, Hamaker BR. Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. Crit Rev Food Sci Nutr 2009;49:852–67.
[25] Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, et al. Anthropometrical parameters and markers of obesity in rats. Lab Anim 2007;41: 111–9.
[26] Brown L, Fenning A, Chan V, Loch D, Wilson K, Anderson B, et al. Echocardiographic assessment of cardiac structure and function in rats. Heart Lung Circ 2002;11:167–73.
[27] Sahn DJ, Allen HD. Real-time cross-sectional echocardiographic imaging and measurement of the patent ductus arteriosus in infants and children. Circulation 1978;58:343–54.
[28] Ward LC, Battersby KJ. Assessment of body composition of rats by bioimpedance spectroscopy validation against dual-energy X-ray absorptiometry. Scand J Lab Anim Sci 2009;36:253–61.
[29] Jeyakumar SM, Vajreswari A, Giridharan NV. Chronic dietary vitamin A supplementation regulates obesity in an obese mutant WNIN/Ob rat model. Obesity (Silver Spring) 2006;14:52–9.
[30] Brown L, Duce B, Miric G, Sernia C. Reversal of cardiac fibrosis in deoxycorticosterone acetate-salt hypertensive rats by inhibition of the renin-angiotensin system. J Am Soc Nephrol 1999;10(Suppl 11):S143–8.
[31] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509.
[32] Yu W, Liang X, Ensenauer RE, Vockley J, Sweetman L, Schulz H. Leaky beta-oxidation of a trans-fatty acid: incomplete beta-oxidation of elaidic acid is due to the accumulation of 5-*trans*-tetradecenoyl-CoA and its hydrolysis and conversion to 5-*trans*-tetradecenoylcarnitine in the matrix of rat mitochondria. J Biol Chem 2004;279:52160–7.
[33] Kuhnt K, Kraft J, Moeckel P, Jahreis G. *Trans*-11–18:1 is effectively Delta9-desaturated compared with *trans*-12–18:1 in humans. Br J Nutr 2006;95:752–61.
[34] Flowers MT, Ntambi JM. Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. Curr Opin Lipidol 2008;19:248–56.
[35] Jiang G, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, Wu M, et al. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. J Clin Invest 2005;115:1030–8.
[36] Miyazaki M, Kim YC, Gray-Keller MP, Attie AD, Ntambi JM. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. J Biol Chem 2000;275: 30132–8.
[37] Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, et al. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc Natl Acad Sci U S A 2002;99:11482–6.
[38] Parks EJ, Skokan LE, Timlin MT, Dingfelder CS. Dietary sugars stimulate fatty acid synthesis in adults. J Nutr 2008;138:1039–46.
[39] Koo HY, Wallig MA, Chung BH, Nara TY, Cho BH, Nakamura MT. Dietary fructose induces a wide range of genes with distinct shift in carbohydrate and lipid metabolism in fed and fasted rat liver. Biochim Biophys Acta 2008;1782:341–8.
[40] Miyazaki M, Dobrzyn A, Man WC, Chu K, Sampath H, Kim HJ, et al. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms. J Biol Chem 2004;279:25164–71.
[41] Flowers MT, Ntambi JM. Stearoyl-CoA desaturase and its relation to high-carbohydrate diets and obesity. Biochim Biophys Acta 2009;1791:85–91.
[42] Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu Rev Nutr 2005;25:317–40.
[43] Jump DB. N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. Curr Opin Lipidol 2008;19:242–7.
[44] Kim HJ, Miyazaki M, Ntambi JM. Dietary cholesterol opposes PUFA-mediated repression of the stearoyl-CoA desaturase-1 gene by SREBP-1 independent mechanism. J Lipid Res 2002;43:1750–7.
[45] Paillard F, Catheline D, Duff FL, Bouriel M, Deugnier Y, Pouchard M, et al. Plasma palmitoleic acid, a product of stearoyl-coA desaturase activity, is an independent marker of triglyceridemia and abdominal adiposity. Nutr Metab Cardiovasc Dis 2008;18:436–40.
[46] Sampath H, Miyazaki M, Dobrzyn A, Ntambi JM. Stearoyl-CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat. J Biol Chem 2007;282: 2483–93.
[47] Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, et al. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science 2002;297:240–3.
[48] Dobrzyn P, Dobrzyn A, Miyazaki M, Cohen P, Asilmaz E, Hardie DG, et al. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. Proc Natl Acad Sci U S A 2004;101:6409–14.
[49] Dobrzyn A, Dobrzyn P, Lee SH, Miyazaki M, Cohen P, Asilmaz E, et al. Stearoyl-CoA desaturase-1 deficiency reduces ceramide synthesis by downregulating serine palmitoyltransferase and increasing beta-oxidation in skeletal muscle. Am J Physiol Endocrinol Metab 2005;288:E599–607.
[50] Lee SH, Dobrzyn A, Dobrzyn P, Rahman SM, Miyazaki M, Ntambi JM. Lack of stearoyl-CoA desaturase 1 upregulates basal thermogenesis but causes hypothermia in a cold environment. J Lipid Res 2004;45:1674–82.
[51] Park SY, Cho YR, Finck BN, Kim HJ, Higashimori T, Hong EG, et al. Cardiac-specific overexpression of peroxisome proliferator-activated receptor-alpha causes insulin resistance in heart and liver. Diabetes 2005;54:2514–24.
[52] Rahman SM, Dobrzyn A, Lee SH, Dobrzyn P, Miyazaki M, Ntambi JM. Stearoyl-CoA desaturase 1 deficiency increases insulin signaling and glycogen accumulation in brown adipose tissue. Am J Physiol Endocrinol Metab 2005;288:E381–7.
[53] Dobrzyn P, Sampath H, Dobrzyn A, Miyazaki M, Ntambi JM. Loss of stearoyl-CoA desaturase 1 inhibits fatty acid oxidation and increases glucose utilization in the heart. Am J Physiol Endocrinol Metab 2008;294:E357–64.
[54] Dobrzyn P, Dobrzyn A, Miyazaki M, Ntambi JM. Loss of stearoyl-CoA desaturase 1 rescues cardiac function in obese leptin deficient mice. J Lipid Res 2010;51: 2202–10.