

A high fat diet in CF-1 mice: An experimental model for metabolic syndrome

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Abstract. Cardiovascular diseases account for the majority of deaths worldwide. Many of their risk factors have been identified but, for their continued study, research centering on new murine models is of interest. In this study, a high fat diet (HFD) and a normal diet (ND) (25 and 4.4% fat, respectively) were tested over a 40-day period to induce the same metabolic alterations in CF-1 mice in two separate experiments. The parameters measured for these effects corresponded to the weight of ingested food and water, to the weight of the mice and their selected organs (adipose tissue, gastrocnemius, liver and heart), to their biochemical profile (glycemia, blood uric nitrogen, uric acid, triglycerides, cholesterol, proteins and albumin) and to the percentage of fat in their livers. The biochemical profile of the CF-1 mice fed a diet high in fat but balanced in proteins (16.9%) showed statistically significant increases in glycemia, cholesterol and triglyceride levels. A statistically significant increase in the weight of adipose tissue was also observed. No statistically significant differences were observed in the muscular mass of either of the groups of mice, but a high percentage of fat was found in the liver. The results lead to the conclusion that CF-1 mice fed a HFD develop metabolic alterations that correspond to an equivalent metabolic syndrome. This is important in the evaluation of the effects of various interventions, such as food, exercise and molecules, on metabolic alterations in mice induced by the intake of a HFD.

Introduction

Cardiovascular diseases (CVDs) are the main cause of death worldwide (1). Several traditional CVD risk factors, such as

arterial hypertension, tobacco addiction, hypercholesterolemia, hypertriglyceridemia and diabetes mellitus, have been identified (2,3). The focus of the extensive research on CVD risk factors in the experimental field has been on the use of rodents, which allows an independent and easy study of the time period and the features under investigation. The most extensively used animals are rats, mice and hamsters. Diet is perhaps the most important factor in the development of research and the nutrition of experimental animals (4).

As the focus of our research is, in part, on traditional and emerging CVD risk factors and on strategies for the modification of these, it was important to have a murine model that exhibited these alterations, generated by a high fat diet (HFD). Our objective was therefore to establish a murine model that would develop the same metabolic alterations as those associated with CVD, such as hyperglycemia, hypercholesterolemia and hypertriglyceridemia.

Materials and methods

Two experiments were carried out over a period of 40 days. Each included a group of mice fed with a normal diet (ND) and a group fed a HFD. All mice were maintained under the same conditions. Experiments were conducted 6 months apart.

Animals and diets. Male CF-1 mice, 4 and 6 weeks old, (Instituto de Salud Pública, Santiago, Chile) were used in experiments 1 and 2, respectively. The animals were maintained at 22±2°C in a regular 12:12 h light-dark cycle (light from 08.00 to 20.00 h) and were administered food and water *ad libitum*, with intake measured daily. All animal manipulations were conducted in accordance with the Guidelines For the Use of Laboratory Animals of the University of Talca Bioethics Committee. The protocol was approved by the Universidad de Talca Institutional Animal Care and Use Committee following the recommendations of the Canadian Council on Animal Care (5).

At the end of the 40-day experimental period, mice were anaesthetized with an i.p. injection of a mixture of ketamine 10% (Ketostop; Drug Pharma-Invetec, Santiago, Chile) and xylazine 2% (Xylavet; Alfasan International B.V., Holland). Blood obtained from the aorta was used in order to determine

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Table I. Percentages of the components of the normal and high fat diets.

	Experiment 1		Experiment 2	
	Normal diet	High fat diet	Normal diet	High fat diet
Water (%)	6.25	5.82	6.90	6.80
Lipids (%)	4.44	25.30	5.30	24.30
Myristic acid (%)	4.98	1.70	5.10	1.20
Palmitic acid (%)	47.28	27.01	48.6	29.30
Linoleic acid (%)	15.00	28.01	15.80	27.06
Oleic acid (%)	21.11	32.10	22.01	30.20
Stearic acid (%)	8.19	8.91	8.68	12.14
Protein (%)	20.80	16.92	20.10	18.60
Carbohydrates (%)	57.31	42.56	55.70	40.40
Inorganic components (%)	7.20	5.40	7.90	5.90
Fibre (%)	4.00	4.00	4.10	4.00

Only those fatty acids which presented a percentage >1 are listed.

serum biochemistry parameters. Anaesthetized mice were sacrificed by exsanguination. The liver, gastrocnemius, white adipose tissue and heart were excised and stored at -80°C for later experiments.

In both experiments, the mice were fed the same chow diet for one week prior to the feeding of special diets. Following this acclimatization period, they were divided into two groups (n=6 and 8); one group was fed a ND (4.4% fat) (Champion S.A., Santiago, Chile) and the other a HFD based on the ND and supplemented with cow fat and sunflower oil from a local supermarket (25% fat). For component analysis, five pellets were selected at random from each diet and were pulverized. Then, the water, protein, carbohydrate, inorganic component and lipid content was measured according to the standardized Official Methods of Analysis of the Association of Official Analytical Chemists (6). Lipid content was determined by the Soxhlet method, protein by the Kjeldahl method, water by dehydration at high temperatures, and fiber by acid and base digestion and calcination. The lipid content of the fat fraction of the diet was determined by gas chromatography/mass spectrometry (Perkin Elmer Turbo Mass and Autosystem XL Gas Chromatograph). There were no significant differences between any of the components of the NDs or HFDs in experiments 1 and 2 (Table I). Elucidation patterns of the applied standards demonstrated that the ND consisted of a mixture of fatty acids with a predominance of palmitic acid. In contrast, the HFD consisted predominantly of the fatty acids of 16 atoms of saturated and 18 atoms of monounsaturated and diunsaturated carbons (Table I). The remaining fatty acids of the diets presented low and variable percentages.

Biochemicals. All reagents for the determination of uric acid, total cholesterol, HDL-cholesterol (HDL-c), triglycerides, blood uric nitrogen, glutamic pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT) were obtained from Roche S.A. (Santiago, Chile). Analyses were conducted using the Hitachi 717 (Japan).

Biochemical analyses were carried out in all of the mice, with the exception of those blood was impossible to extract prior to their sacrifice.

Statistical analysis. Statistical analysis of the data was performed using the Student's t-test (SPSS) (15).

Results

Food intake and body weight. During the acclimatization phase and at the start of the experimental period, there were no differences between the food intake and body weight of the mice. At the end of both experimental periods, no statistical difference was found between the food intake of the ND and HFD groups in experiment 1 (ND 5.4±4.4, HFD 5.0±3.3 g/day) or 2 (ND 6.8±0.8, HFD 7.8±2.6 g/day); however, there was a difference in the body weight (final weight - initial weight) of the ND and HFD mice in experiments 1 (ND 14.1±0.3 g, HFD 16.5±0.6 g, p<0.01) and 2 (ND 8.6±1.2 g, HFD 11.2±2.5 g, p=0.09).

At the end of both experiments, the epididymal fat pad weight of the mice fed a HFD was higher than in those fed a ND (Table II). No statistical differences in the gastrocnemius, liver or heart weight were observed in either group in either of the experiments (Table II).

Biochemical blood analysis. In both experiments, mice fed a HFD presented significant increases in serum levels of glucose, total cholesterol and triglycerides (Table III). The GPT and GOT, determined only in experiment 2, showed a significant increase in GOT activity in the HFD group (Table III).

Hepatic lipids. A significant percent increase in the hepatic lipid level of the mice fed a HFD in comparison with those fed a ND was observed in both experiments: experiment 1, ND (n=3) 2.3±0.5%, HFD (n=4) 4.2±0.2%, p<0.001; experiment 2, ND (n=6) 3.5±1.0%, HFD (n=5) 5.2±1.1%, p=0.02.



SPANDIDOS PUBLICATIONS Weight of the organs and tissues of the mice fed a normal versus high fat diet.

	Experiment 1			Experiment 2		
	Normal diet (n=7)	High fat diet (n=8)	P-value	Normal diet (n=6)	High fat diet (n=5)	P-value
Tissues						
Epididymal fat pad (g)	0.40±0.03	0.90±0.09	<0.001	0.57±0.09	1.31±0.32	<0.050
Gastrocnemius (g)	0.90±0.04	1.00±0.04	NS	0.60±0.03	0.60±0.02	NS
Organs						
Liver (g)	6.50±0.32	5.90±0.24	NS	5.91±0.19	6.38±0.45	NS
Heart (g)	0.50±0.03	0.50±0.02	NS	0.53±0.04	0.65±0.05	NS

NS, not significant.

Table III. Biochemical parameters of the mice fed a normal versus high fat diet.

	Experiment 1			Experiment 2		
	Normal diet (n=5)	High fat diet (n=5)	P-value	Normal diet (n=6)	High fat diet (n=6)	P-value
Glutamic oxalacetic transaminase (UI/l)	ND	ND	-	125.3±11.01	524.5±169.30	<0.05
Glutamic pyruvic transaminase (UI/l)	ND	ND	-	75.7±8.68	419.1±221.59	NS
Uric acid (mg/dl)	6.3±2.29	6.6±1.13	NS	ND	ND	-
Blood uric nitrogen (mg/dl)	21.8±4.08	25.2±5.72	NS	21.8±2.12	24.2±3.62	NS
Glycemia (mg/dl)	316.8±13.43	452.2±43.54	<0.010	304.1±12.02	406.3±40.01	<0.05
Total cholesterol (mg/dl)	89.0±7.94	206.6±8.66	<0.001	108.9±10.55	174.1±11.79	<0.01
HDL-cholesterol (mg/dl)	ND	ND	-	115.4±9.68	126.0±11.22	NS
Triglycerides (mg/dl)	79.7±15.19	234.4±38.77	<0.050	128.9±17.07	203.2±28.36	<0.05

ND, not determined; NS, not significant.

Discussion

It is known that diabetes, dyslipidemia and arterial hypertension are classic cardiovascular risk factors (7-12). For the study of atherosclerosis, knockout mice are generally used for ApoE (13,14) and the LDL receptor (15). However, some murine models of metabolic syndrome have been described (4,16,21). Our objective was to create a non-genetically modified murine model that presented the metabolic alterations, such as an increase in glycemia, cholesterol and triglycerides, observed with high frequency in the adult populations of both developed and developing countries.

High fat diet. The study of obesity and its effects on experimental animals, mainly rats and mice, has generated different diets, achieved by altering the types and concentrations of nutrients used (4,16-18). One of the generated models is the

HFD (4,16,17). In the present study, a 25% lipid diet was used, which consisted of a lower proportion of palmitic acid and a higher proportion of linoleic and oleic acids than the ND.

The percent composition of lipids in the diets used in this study was similar to those used by other researchers (18-20), though the diet differed from those commonly used (21-23). Some of the components used in its preparation were similar to those used in other studies, for example sunflower oil (24-26) and animal butter (27,28). Palmitic acid, the main fatty acid present in diets, has been associated with the development of dyslipidemia and insulin resistance (29,30). Other fatty acids present in diets are oleic and linoleic acid, which have been observed to improve insulin sensitivity in skeletal muscle, help reduce abdominal obesity and decrease dyslipidemia (31). Meanwhile, it has been reported that linoleic acid selectively encourages a pro-inflammatory environment in human endo-

thelial cells (32), stimulating lipid peroxidation and decreasing levels of HDL-c (33).

Metabolic alterations. CF-1 mice fed a HFD, in addition to presenting an increase in epididymal adipose tissue, presented a significant increase in glycemia, total cholesterol and triglyceridea, all of which are CVD risk factors.

Adipose and hepatic tissue. Along with the increase in fat in the liver, we found that the GPT and GOT enzymes, which are markers of hepatic steatosis, were increased. Studies, like the one developed by Axen *et al*, show that the consumption of a HFD can result in an increase in visceral adipose tissue, as well as in an increase in lipid content at the hepatic level (24).

Hyperglycemia. Wilkes *et al* (25) demonstrated, using Sprague Dawley rats, that the consumption of a diet rich in fat based on sunflower oil increased the basal uptake of glucose, but decreased the capitation of glucose stimulated by insulin in the skeletal muscle. This might be explained by the alteration caused by this type of diet in the composition and/or functionality of the GLUT-4 glucose transporter at this level (34). On the other hand, it has been demonstrated that the presence of unsaturated fatty acids in diets damages insulin secretion, which is not the case with fatty acids that present a high insulinotropic potential (27). As sunflower oil was one of the ingredients used in the current study's HFD, it is possible that the above-mentioned finding explains, at least in part, the hyperglycemia observed in our experiments.

Dyslipidemia. Lin *et al* (35), working with Wistar male rats and golden Syrian hamsters, found that a diet high in cholesterol and associated with n-3 polyunsaturated fatty acids (PUFA), but not with n-6 PUFA, increased the concentration of very low density lipoproteins, hypertriglyceridemia and hypercholesterolemia. The HFD administered in the current study consisted of high quantities of linoleic acid (n-3 PUFA). This might explain the hypertriglyceridemia and hypercholesterolemia observed. Other authors, studying the effects on Bio F₁B hamsters of fish oil-supplemented diets with or without cholesterol, found in the first case a higher frequency of dyslipidemia (28).

One of the lipid parameters of major recent relevance is HDL-c (36). Normal levels are associated with reduced risk in cardiovascular patients with MS (37). However, human and mouse lipoparticle levels are not comparable. In humans, LDL-c is predominant; in mice, HDL-c predominates (38). Perhaps this disparity explains why no differences have been found in our studies between animals fed with a HFD and a ND.

In summary, CF-1 mice fed a HFD for 40 days exhibited increased visceral fat, glucose, cholesterol, triglyceride, transaminase and liver lipid content. We did not measure arterial pressure or insulinemia, yet it is probable that the CF-1 murine model closely resembles metabolic syndrome in humans. This model, with the alterations described above, will allow for future study of the effects of various manipulations, such as food (e.g. apples, which have provided some interesting preliminary results), exercise, extracts or molecules, with eventual pharmacologic activity.

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