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ORIGINAL CONTRIBUTION

Changes in cardiac energy metabolic pathways in overweighed rats fed a high-fat diet

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Abstract
Background Heart produces ATP through long-chain fatty acids beta oxidation.
Purpose To analyze whether in ventricular myocardium, high-fat diet may modify the expression of proteins associated with energy metabolism before myocardial function was affected.
Methods Wistar Kyoto rats were divided into two groups: (a) rats fed standard diet (control; $n = 6$) and (b) rats fed high-fat diet (HFD; $n = 6$). Proteins from left ventricles were analyzed by two-dimensional electrophoresis, mass spectrometry and Western blotting.
Results Rats fed with HFD showed higher body weight, insulin, glucose, leptin and total cholesterol plasma levels as compared with those fed with standard diet. However, myocardial functional parameters were not different between them. The protein expression of 3-ketoacyl-CoA thiolase, acyl-CoA hydrolase mitochondrial precursor and enoyl-CoA hydratase, three long-chain fatty acid β-oxidation-related enzymes, and carnitine-*O*-palmitoyltransferase I was significantly higher in left ventricles from HFD rats. Protein expression of triosephosphate isomerase was higher in left ventricles from HFD rats than in those from control. Two α/β-enolase isotypes and glyceraldehyde-3-phosphate isomerase were significantly increased in HFD rats as compared with control. Pyruvate and lactate contents were similar in HFD and control groups. Expression of proteins associated with Krebs cycle and mitochondrial oxidative phosphorylation was higher in HFD rats.
Conclusions Expression of proteins involved in left ventricle metabolic energy was enhanced before myocardial functionality was affected in rats fed with HFD. These findings may probably indicate higher cardiac energy requirement due to weight increase by HFD.

Keywords Fat diet · Energy metabolism · Heart · Proteomics

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Introduction

Heart has high-ATP requirements that are produced using long-chain fatty acid β-oxidation as main metabolic pathway [1]. However, dietary guidelines recommend a high-carbohydrate/low-saturated-fat/low-cholesterol diet to prevent heart disease [2]. Depressed left ventricular function and ventricle hypertrophy have been associated with dietary lipid intake and elevated plasma insulin levels [3, 4]. In this regard, the expression of several proteins associated with the main energy metabolic pathways was reported changed in the rat left hypertrophied ventricle compared to that in non-hypertrophied ventricles [5].

In both animals and humans, weight gain and insulin resistance seem to alter cardiac metabolism [6]. In obesity, lipid accumulation within the myocardium was observed, and it was attributed to an imbalance between fatty acid uptake and oxidation [7]. However, many studies do not support that concept since in hearts with altered functionality related to obesity and insulin resistance, cardiac fatty

acid β-oxidation rates seem to be elevated [8, 9]. However, the consequences of body weight increase and insulin resistance produced by HFD intake on substrate selection in the heart have not been extensively investigated and less in an integrated form. Therefore, it could be interested to analyze, in an integrated form, whether the expression of proteins related to the main metabolic pathways could be affected by HFD and insulin resistance before detrimental structural and/or functional changes in the myocardium could be detected.

Adipokines, released from adipose tissue, take part in the regulation of lipid and glucose metabolism. High-fat meal affects the secretion of adipokines, including leptin levels, which can alter fatty acid and glucose oxidation in the heart [10]. Taken together, our aim was to analyze the level of expression of proteins involved in the main energy metabolic pathways in left ventricles from rats fed a HFD before functional heart modifications could be detected. In addition, the possible association of these changes with insulin resistance and circulating leptin levels was also analyzed.

Materials and methods

Animals and experimental procedure

The study was conducted in male Wistar rats ($n = 12$; 250 g; Harlan Ibérica SL, Barcelona, Spain), according to the guidelines for ethical care of experimental animals of the European Union. The experimental protocol was approved by the institutional review committee of Complutense University. Animals were divided into two groups: rats fed a standard diet for 7 weeks were used as control group ($n = 6$) (Harlan Teklad #Ref 2014, WI, USA) and rats fed a high-fat diet (HFD) (33.5 % fat; Harlan Teklad #TD; 03307) for 7 weeks ($n = 6$). Table 1 shows the composition of both diets. Body weight was measured once a week. Food and water intake were determined throughout the experimental period. Cardiac hemodynamic, metabolic and molecular parameters were determined 7 weeks after the study began.

Table 1 Composition of standard and high-fat diets

	Standard diet (g/kg)	High-fat diet (g/kg)
Crude protein	143.0	233.5
Carbohydrates	480.0	347.5
Fat	40.0	350.0
Minerals and vitamins	29.1	69.0

The contents of fatty acid profile in high-fat diet were (% total fat) 40 % saturated, 50 % monounsaturated and 10 % polyunsaturated. Standard diet kcal/g = 2.9, high-fat diet kcal/g = 5.4

Arterial pressure and cardiac function

Systolic arterial pressure was indirectly measured by tail-cuff plethysmography (Narco Bio-Systems, TX, USA). At the end of the experimental period, rats were anesthetized with ketamine and xylazine (90 and 5 mg/kg i.p., respectively) and a catheter (Scisense FT211B, ON, Canada) was inserted into the left ventricle through the right carotid artery as reported [11, 12]. The catheter was connected to data acquisition system (PowerLab/800; ADI), and signals were monitored and digitally stored for analysis with the software Chart for Windows. Left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), the first derivative of left ventricular pressure increase over time ($+dP/dt$) and the first derivative of left ventricular pressure decrease over time ($-dP/dt$) were determined. $+dP/dt$ and $-dP/dt$ are isovolumic phase indices of left ventricular function. After heart parameters, animals were killed. Hearts were weighed, and left ventricles were isolated and immediately frozen and stored at -80 °C. Isolation and manipulation of hearts were always performed under sterile conditions.

Metabolic parameters

At the end of the study, plasma concentrations of cholesterol, glucose, insulin (R&D Systems, MN, USA) and leptin (DRG GmbH instrument, Germany) were measured using specific quantitative sandwich enzyme immunoassays according to the manufacturer's instructions. Peripheral insulin sensitivity was evaluated based on the homeostasis model assessment (HOMA).

Two-dimensional electrophoresis (2-DE)

Frozen left ventricles were homogenized in a buffer containing 8 mol/L urea, 2 % CHAPS w/v, 40 mmol/L dithiothreitol, 0.2 % Bio-Lyte™ ampholyte (Bio-Rad) and 0.01 % w/v bromophenol blue. As reported, samples (250 μg protein/sample) were loaded on immobilized gradient IPG strips (pH 3–10), and isoelectric focusing was performed using a Protean IEF cell system (Bio-Rad) [13]. In the second dimension, the proteins from the strips were resolved on 10 % SDS–PAGE gels using a Protean II XL system (Bio-Rad), and the gels were silver stained using Plus One Silver Staining kit (GE Healthcare).

Image acquisition and analysis and mass spectrometry

The silver-stained gels were scanned in a UMAX POWERLOOK III Scanner operated by the software Magic Scan V 4.5. Intensity calibration was carried out using an intensity step wedge prior to gel image capture. Image

analysis was carried out using PD Quest 6.2.1 and Quantity One 4.2.3 (Bio-Rad). Each spot intensity volume was processed by background subtraction. For calculations, two gels were developed for each left ventricle, and the mean of the two densitometric values for each spot was used for the statistical analysis.

In a previous work, most of the here studied proteins were identified by MS, in the rat left ventricle proteome [5]. Spots from three different gels were obtained and analyzed. After silver staining was removed and digested with sequencing grade trypsin (Promega). For MS analysis, 1 μL of purified extracts was mixed with 1 μL of α-cyano 4-hydroxy-transcinnamic matrix (Sigma) in 50 % acetoniltrile, and 1 μL of this mixture was spotted onto a Maldi plate and analyzed in a 4700 Proteomic Analyzer (Applied Biosystem) operating in reflector positive mode as previously reported [13]. For protein identification, Mascot database 1.9 (http://www.matrixscience.com) was used as algorithm to match the peptides obtained by mass spectrometry. MS identifications were accepted based on a tripartite evaluation that takes into account significant molecular weight search (Mowse) scores, spectrum annotation and observed versus expected migration on the 2-DE gel.

Western blot analysis

Protein expression of triosephosphate isomerase and carnitine-*O*-palmitoyltransferase I (CPTI) was analyzed by Western blot. As reported, homogenized left ventricle cells were solubilized in Laemmli buffer containing 2-mercaptoethanol. The obtained proteins were separated on denaturing SDS/15 % (w/v) polyacrylamide gels. Equal amount of proteins (20 μg/lane), estimated by bicinchoninic acid reagent (Pierce), was loaded. To verify that equal amount of left ventricle proteins was loaded in the gel, parallel gels with the same samples were run and stained with Coomassie blue. Proteins were then blotted onto nitrocellulose (Immobilion-P; Millipore), and the blots were blocked overnight at 4 °C with 5 % (w/v) nonfat dry milk in TBS-T (20 mmol/L Tris/HCl (pH 5.2), 137 mmol/L NaCl and 0.1 % Tween 20), as reported [14]. Membranes were incubated with monoclonal antibodies against either triosephosphate isomerase (1:500; sc-30145, Santa Cruz Biotechnology) or CPTI (1:1,000; sc-20670, Santa Cruz Biotechnology). After that, membranes were incubated with HRP (horseradish peroxidase)-conjugated anti-(rabbit, mouse or goat IgG) antibodies at a dilution of 1:2,000. The proteins were then detected by enhanced chemiluminescence (ECL®, Amersham Biosciences) and evaluated by densitometry (Quantity One; Bio-Rad Laboratories). Prestained protein markers (Sigma) were used for molecular mass determinations.

Detection of pyruvate and lactate

The amount of pyruvate and lactate content in the left ventricles was quantified using a Pyruvate assay Kit (BioVision, USA) and a Lactate assay Kit (BioVision, USA), respectively. For this purpose, 80 μg of each homogenized left ventricle was used to assay pyruvate and lactate concentration using the colorimetric assay following the manufacturer's instructions.

Statistical analysis

Results are expressed as mean ± SEM. To determine statistical differences between HFD rats and controls, Mann–Whitney test was used. Pearson's correlation analysis was used to determine associations between levels of protein expression in the rat left ventricle proteome and HOMA index, rat body weight and insulin, leptin, glucose and total cholesterol plasma levels. Statistical determinations were performed using the SPSS 12.0 program (SPSS Inc.). A p value <0.05 was considered statistically significant.

Table 2 Biochemical and cardiac hemodynamics parameters from control and high-fat diet rats

Parameters	Control $N = 6$	HFD $N = 6$
Cardiac hemodynamic parameters		
SBP (mmHg)	158.8 ± 13.9	158.6 ± 9.4
HR (bpm)	394.6 ± 29.6	379.6 ± 15.3
HRW (g/100 g bw)	0.3 ± 0.01	0.2 ± 0.01
LVSP (mmHg)	130.6 ± 16.3	146.0 ± 7.7
LVEDP (mmHg)	3.6 ± 0.3	3.7 ± 0.5
dP/dt_{max} (mmHg/s)	9,145.2 ± 333.8	8,262.3 ± 187.7
dP/dt_{min} (mmHg/s)	−6,710.2 ± 622.5	−7,321.3 ± 161.4
Biochemical parameters		
Body weight (g)	411.0 ± 13.4	557.3 ± 7.7*
Glucose (mg/dL)	88.4 ± 4.0	107.0 ± 2.2*
Total cholesterol (mg/dL)	48.1 ± 0.03	59.4 ± 2.4*
Insulin (μg/dL)	0.6 ± 0.1	1.0 ± 0.05*
HOMA index	3.1 ± 0.3	6.5 ± 0.3*
Leptin (pg/mL)	7,597.5 ± 1,245.0	27,795 ± 5,502.1*

Results are represented as mean ± SEM * $p < 0.05$ with respect to control rats

SBP systolic blood pressure, *HR* heart rate (expressed as beats per minute), *HRW* heart relative weight, expressed as g of heart/100 g of body weight, *LVSP* left ventricular systolic pressure, *LVEDP* left ventricular end-diastolic pressure, dP/dt_{max} positive first derivate of pressure with respect to time, dP/dt_{min} negative first derivate of pressure with respect to time, *HOMA* homeostasis model assessment

Results

Circulating and heart hemodynamic parameters

As previously reported, 7 weeks after HFD, rats showed a significantly higher body weight compared to controls (Table 2). Moreover, plasma glucose, total cholesterol, insulin and leptin levels were higher in HFD rats than in controls (Table 2). HOMA index was also increased in HFD rats with respect to controls (Table 2).

Seven weeks after HFD, rats did not show changes in heart relative weight, heart rate, LVSP, LVEDP, $+dP/dt$ or $-dP/dt$ with respect to those in control rats (Table 2). Systolic blood pressure was also similar between HFD and control rats (Table 2).

Expression of proteins related to fatty acid and glucose metabolism in left ventricles

A 2-DE for each rat was performed. In 2-DE gels, the left ventricle proteins were identified based on a previous work from our group [5]. In this previous work, all the determined proteins were identified by MS and MS/MS and the matched peptides and Mascot accession numbers were shown in it [5]. Mitochondrial malate dehydrogenase and pyruvate dehydrogenase were further identified by mass spectrometry for this study (Fig. 1). As Table 3 shows, protein expression of 3-ketoacyl-CoA thiolase, acyl-CoA hydrolase mitochondrial precursor and enoyl-CoA hydratase enzymes involved in β-fatty acid oxidation cycle was significantly increased in left ventricles from HFD rats with respect to that in controls (Table 3). Moreover, CPTI

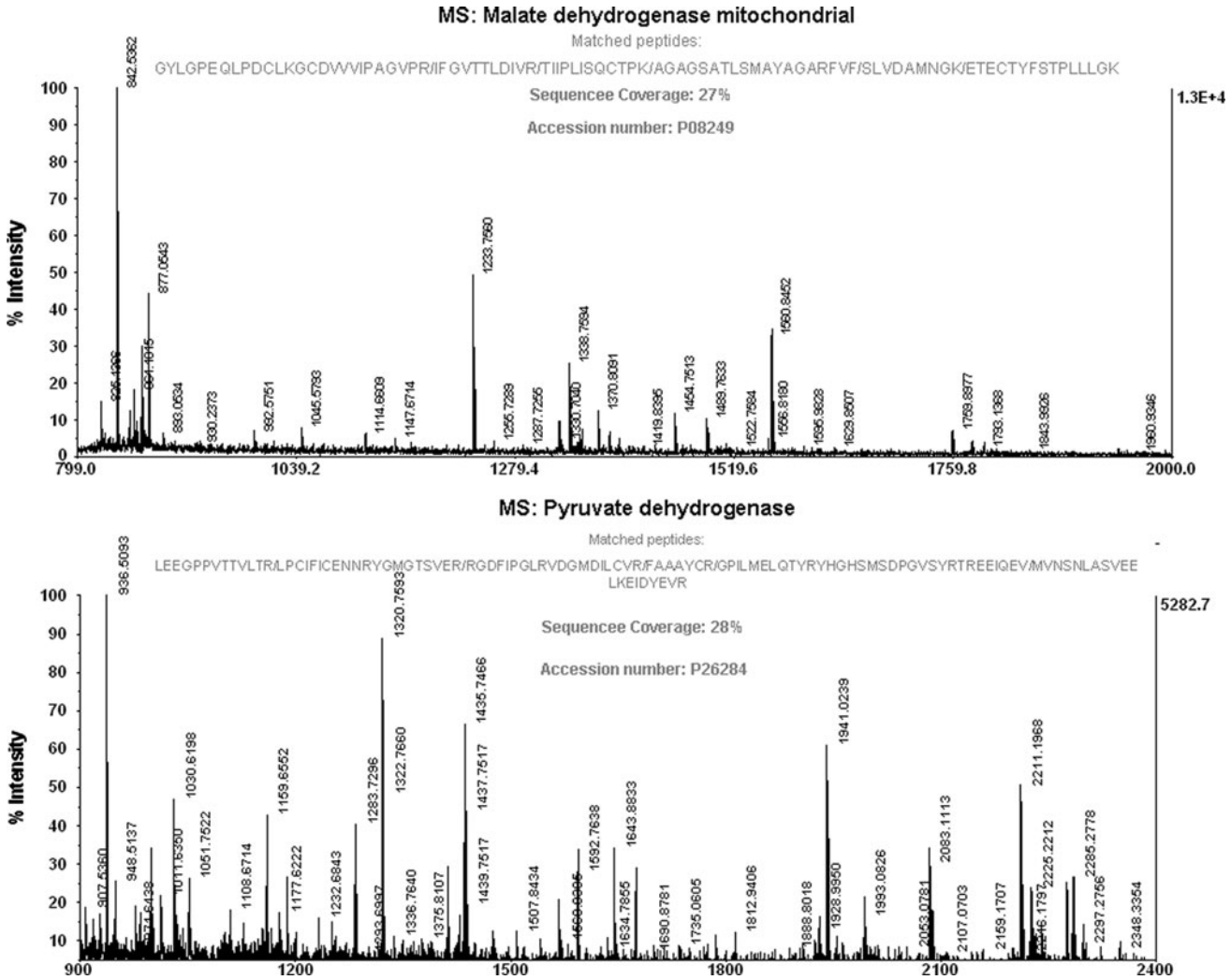

Fig. 1 Mass spectrometry to identify proteins. Spectra of mitochondrial malate dehydrogenase and pyruvate dehydrogenase are shown. Matched peptides and sequence coverage are shown in the figure for each specific protein, and they were analyzed using the Mascot database (http://www.matrixscience.com) for the identity of each protein

Table 3 Results of the left ventricle proteins involved in fatty acid β-oxidation and glycolysis identified in 2-DE

Analyzed proteins	Control $N = 6$	HFD $N = 6$	p value
Proteins involved in fatty acid β-oxidation			
3-ketoacyl-CoA thiolase	0.87 ± 0.29	2.43 ± 0.43*	0.016
Acyl-CoA thioester hydrolase mitochondrial precursor	1.92 ± 0.89	6.96 ± 0.24*	0.009
Enoyl-CoA hydratase	5.15 ± 1.14	22.11 ± 5.44*	0.034
Proteins involved in glycolysis			
Triosephosphate isomerase	2.96 ± 1.14	13.51 ± 3.84*	0.027
Fructose-1,6-bisphosphate aldolase	4.96 ± 0.54	5.67 ± 1.77	0.999
α/β-Enolase			
Isotype 1	1.15 ± 0.48	9.37 ± 2.26*	0.006
Isotype 2	1.8 ± 0.45	7.70 ± 1.03*	0.006
Glyceraldhyde-3-phosphate dehydrogenase	19.63 ± 3.04	49.76 ± 6.65*	0.006

Results are represented as mean ± SEM. A p value <0.05 was considered statistically significant

expression, determined by Western blot, was also higher in left ventricles from HFD than in controls (Fig. 2).

Protein expression of fructose 1,6-biphosphate aldolase and triosephosphate isomerase, two glycolytic key step enzymes, was determined. As Table 3 shows, while fructose 1,6-biphosphate aldolase expression was not different between HFD rats and control rats, triosephosphate isomerase was significantly higher in left ventricles from HFD as compared with controls. The higher expression of triosephosphate isomerase in left ventricles from HFD rats was further assessed by Western blotting (Fig. 2).

Two α/β-enolase isotypes were also identified in the left ventricles, and they were significantly increased in HFD as compared with controls (Table 3). The protein expression of glyceraldehyde-3-phosphate isomerase was also increased in left ventricles from HFD with respect to controls (Table 3).

Pyruvate and lactate content in left ventricles was determined. Both experimental groups showed similar pyruvate and lactate content in the left ventricles (Fig. 2). However, pyruvate dehydrogenase expression was increased in left ventricles from HFD rats compared to controls (Table 3).

Proteins involved in tricarboxylic acid cycle and mitochondrial oxidative phosphorylation

Protein expression of both isocitrate dehydrogenase and mitochondrial malate dehydrogenase, enzymes involved in tricarboxylic acid cycle, was significantly higher in left ventricles from HFD rats than in control rats (Table 4).

Proteins involved in mitochondrial oxidative phosphorylation were also analyzed. As Table 4 shows, the level of expression of several proteins involved in mitochondrial oxidative phosphorylation, that is, NADH dehydrogenase, α and β subunits of flavoprotein mitochondrial precursor and ubiquinol-cytochrome C reductase, was higher in left ventricles from HFD rats than from controls. The expression of ATP synthase beta subunit was not different between left ventricles from HFD rats and controls (Table 4). Creatine kinase M chain expression was also higher in left ventricles from HFD rats with respect to controls (Table 4).

Association between rat body weight and changes in energy metabolism proteins in left ventricle

Pearson's analysis showed that rat body weight was positively associated with the level of expression of the following proteins that had been shown differences in left ventricles between control and HFD rats: acyl-CoA thioester hydrolase mitochondrial precursor, α/β-enolase isotypes 1 and 2, glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, NADH dehydrogenase, α subunit of flavoprotein mitochondrial precursor, ubiquinol-cytochrome C reductase (Table 5). The other proteins that were differently expressed between HFD and control rats did not show statistical differences with the rat body weight (Table 5).

Association between circulating parameters and level of expression of proteins in left ventricles

There was no association between the level of expression of any of the energy metabolism-related proteins that had demonstrated differences in left ventricles between HFD rats and controls with circulating levels of glucose, insulin and with HOMA index (Table 5). Total cholesterol plasma levels were only positively associated with left ventricle expression of beta subunit of flavoprotein mitochondrial precursor (Table 5). A positive correlation was also observed between circulating leptin levels and both α subunit of flavoprotein mitochondrial precursor and acyl-CoA thioester hydrolase mitochondrial precursor was also observed (Table 5).

Discussion

This study showed that the expression of several proteins associated with different pathways related to energetic

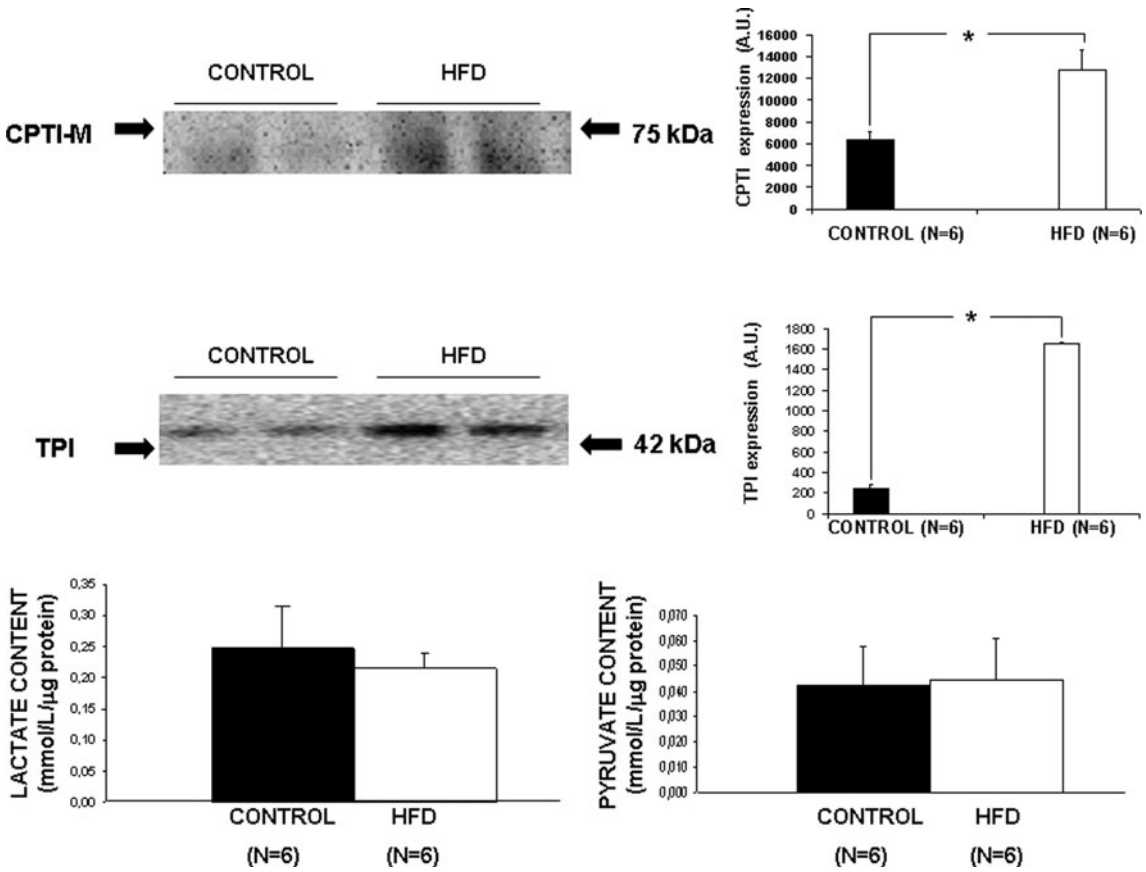

Fig. 2 In the *upper panel* is shown representative Western blots of *left* ventricular expression of both carnitine-*O*-palmitoyltransferase I (CPTI) and triosephosphate isomerase (TPI) in rats fed high-fat diet (HFD) and controls. On the *right* is shown the densitometric analysis of all Western blots expressed as arbitrary densitometric units (AU). At the *bottom* is shown lactate and pyruvate content in left ventricles from control and HFD rats. Results are represented as mean ± SEM. $^{*}p < 0.05$ with respect to controls

metabolisms mainly fatty acid β-oxidation, glycolysis, tricarboxylic acid cycle and mitochondrial oxidative phosphorylation, were modified in left ventricles from HFD rats that also showed higher body weight further to insulin resistance as compared with those from rats fed with standard diet. The changes in the expression of these energy metabolism-related proteins preceded cardiovascular derangement since myocardial functionality and ventricle growth were not found modified.

The main fuel for cardiac muscle is fatty acid β-oxidation. Intracellular control of fatty acid β-oxidation begins at the level of fatty acid uptake by mitochondria through CPTI, which transfers fatty acid from acyl-coenzyme A to long-chain acylcarnitine, that then it is transported into mitochondria [15, 16]. In HFD rats, CPTI was increased in left ventricle compared to that in controls. It was accompanied of higher left ventricular expression of three enzymes involved in the fatty acid β-oxidation. Taken together, these results suggest that fatty acid β-oxidation could be favored in left ventricle of HFD rats. These findings may be in accordance with clinical observations using positron emission tomography showing that obese women hearts have an increased fatty acid uptake [9].

The increase of fatty acid β-oxidation in left ventricle should be associated with reduction in carbohydrate oxidation. In this regard, experimental studies in perfused hearts have shown that accelerated fatty β-acid oxidation contributes to development of diabetic cardiomyopathies by inhibiting glucose oxidation [17]. Moreover, in the setting of obesity was proposed that high rates fatty acid oxidation contributes to insulin resistance by direct inhibition of glucose metabolism [18, 19]. In the present study, it was not observed association between circulating insulin levels and HOMA index with the level of expression of proteins related to fatty acid β-oxidation. Moreover, in left ventricles from HFD rats, glycolytic-related proteins such as triosephosphate isomerase, α/β-enolase isotypes and glyceraldehyde-3-phosphate dehydrogenase were overexpressed as compared with left ventricles from control rats. Taken together, in left ventricles from HFD rats, the overexpression of the glycolytic-related proteins could occur trying to increase glucose degradation since insulin

Table 4 Results of the left ventricle proteins involved in tricarboxylic acid cycle and mitochondrial oxidative phosphorylation analyzed in 2-DE

Analyzed proteins	Control $N = 6$	HFD $N = 6$	p value
Proteins involved in tricarboxylic acid cycle			
Pyruvate dehydrogenase	2.94 ± 0.74	8.77 ± 0.75*	0.006
Malate dehydrogenase mitochondrial	16.85 ± 2.92	28.56 ± 2.95*	0.037
Isocitrate dehydrogenase	2.5 ± 0.27	10.80 ± 0.82*	0.009
Proteins involved in mitochondrial oxidative phosphorylation			
NADH dehydrogenase	2.17 ± 0.54	7.86 ± 1.00*	0.006
Electron transfer flavoprotein α-subunit mitochondrial precursor	5.65 ± 1.63	28.18 ± 3.85*	0.006
Electron transfer flavoprotein β-subunit mitochondrial precursor	3.39 ± 0.86	22.19 ± 2.63*	0.025
Ubiquinol-cytochrome C reductase	3.15 ± 0.93	18.36 ± 2.70*	0.014
Mitochondrial precursor ATP synthase beta chain	76.18 ± 27.07	64.82 ± 8.96	0.522
Creatine kinase M chain	4.39 ± 0.95	15.42 ± 1.64*	0.006

Results are represented as mean ± SEM. A p value <0.05 was considered statistically significant

resistance may reduce glucose uptake diminishing glucose availability into the cell. However, Pearson's analysis did not show association between circulating glucose levels and changes in the expression of triosephosphate isomerase, α/β-enolase isotypes and glyceraldehyde-3-phosphate dehydrogenase.

Another possibility is that the protein expression of glycolytic-related enzymes was increased in left ventricles from HFD rats as response of the increased expression of fatty acid β-oxidation enzymes. In this regard, an increased glucose uptake and glucose oxidation to prevent high fatty acid oxidation in mice hearts were reported [20]. A paradoxical observation of the present study was that, although in left ventricles from HFD rats the expression of glycolytic enzymes was increased, total net content of pyruvate, the glycolytic end-product, was not different with respect to that found in control left ventricles. Several possibilities may be raised to explain it. First, it could be plausible that accelerated pyruvate degradation through either lactate generation or pyruvate degradation toward acetyl-CoA may be favored in left ventricles from HFD rats. However, lactate content in ventricles from HFD rats was not different with respect to that from control rats, which it may discard an increased anaerobic pyruvate degradation. However, the protein expression of pyruvate dehydrogenase, one of the three enzymes included in the pyruvate dehydrogenase complex that mediates pyruvate oxidation to yield acetyl-CoA, was increased in left ventricles from HFD rats. Moreover, the expression level of proteins involved in tricarboxylic acid cycle and in mitochondrial oxidative phosphorylation was increased in left ventricles from HFD rats as compared with controls. Taken together, these observations may suggest and reflect an increased level of energy demand by the left ventricles of HFD rats. Accordingly, the protein expression of creatine M kinase was higher in left ventricles from HFD than that from controls. Creatine M kinase is one mechanism that the heart uses to maintain ATP levels when ATP demand exceeds ATP supply. In this regard, studies in obese mice have suggested increased myocardial oxygen consumption although cardiac efficiency was reduced [21]. In addition, energy balance studies have demonstrated unbalance in ATP synthesis in perfused livers from rats fed high-fat diet by significant increase in mitochondrial membrane potential and decrease in mitochondrial/cytosolic pH difference [22].

Therefore, it could be plausible that left ventricles of HFD rats require higher ATP synthesis favoring the here observed higher expression of different energy metabolism-related proteins as defensive response.

Few studies have been focused on the effect of leptin on heart metabolism. Indeed, most of the leptin effects in heart metabolism have been inferred from studies with leptin deficiency/resistance genetic experimental models. In this regard, in isolated hearts, leptin increases both fatty acid oxidation and glucose uptake [23, 24]. Moreover, in ob/ob mice, a genetic model associated with leptin lacking, significant increase of fatty acid oxidation and reduction in both glucose oxidation and mitochondrial oxidative capacity have been reported [19, 25]. As previously it was reported, circulating plasma levels of leptin were significatively higher in HFD rats with respect to controls [12]. However, leptin plasma levels were only associated with changes in the left ventricular expression of both acyl-CoA thioester hydrolase mitochondrial precursor and the electron transfer flavoprotein α subunit mitochondrial precursor. These findings may suggest a limited role for leptin as responsible for the changes observed in the expression of most of the energy metabolic-related proteins in left ventricle of HFD rats.

The level of expression of a number of energy metabolism-related proteins was modified in left ventricle from HFD rats. However, heart functionality remains preserved. Therefore, it may suggest that modification in the expression level of these proteins was not the consequence of alterations in myocardial functionality. Therefore, the

Table 5 Pearson's correlation analysis between the proteins showing significative differences between left ventricles from HFD and control rats and body weight, glucose, insulin, HOMA index, total cholesterol and leptin levels

Protein	Body weight		Glucose circulating levels		Insulin circulating levels		HOMA index		Total cholesterol plasma levels		Leptin circulating levels	
	Pearson's coefficient	*p*	Pearson's coefficient	*p*	Pearson's coefficient	*p*	Pearson's coefficient	*p*	Pearson's coefficient	*p*	Pearson's coefficient	*p*
3-Ketoacyl-CoA thiolase	0.506	0.054	0.005	0.988	0.108	0.714	0.133	0.650	0.077	0.794	0.475	0.086
Acyl-CoA thioester hydrolase mitochondrial precursor	0.653	0.008*	0.114	0.699	0.294	0.308	0.343	0.230	0.520	0.057	0.543	0.045*
Enoyl-CoA hydratase	0.331	0.293	−0.095	0.781	−0.185	0.586	−0.175	0.606	0.528	0.095	0.038	0.912
Triosephosphate isomerase	0.431	0.124	0.190	0.534	−0.091	0.767	0.036	0.907	0.147	0.632	0.178	0.561
α/β-Enolase isotype 1	0.520	0.032*	0.197	0.465	0.000	0.999	0.097	0.721	0.287	0.282	0.206	0.443
α/β-Enolase isotype 2	0.606	0.010*	0.185	0.492	0.238	0.375	0.298	0.262	0.317	0.232	0.449	0.081
Glyceraldehyde-3-phosphate dehydrogenase	0.597	0.015*	−0.161	0.566	0.194	0.488	0.151	0.592	0.264	0.341	0.377	0.166
Isocitrate dehydrogenase	0.648	0.007*	0.405	0.134	0.090	0.749	0.267	0.336	0.091	0.748	0.491	0.063
Mitochondrial malate dehydrogenase	0.374	0.139	−0.059	0.828	0.203	0.450	0.200	0.457	0.086	0.752	0.053	0.847
Pyruvate dehydrogenase	0.485	0.057	−0.114	0.687	0.043	0.880	0.030	0.915	0.182	0.516	0.163	0.561
NADH dehydrogenase	0.622	0.008*	0.015	0.957	−0.037	0.892	0.025	0.927	0.170	0.529	0.294	0.269
Electron transfer flavoprotein α-subunit mitochondrial precursor	0.616	0.008*	0.202	0.454	0.060	0.825	0.162	0.550	0.180	0.506	0.521	0.038*
Electron transfer flavoprotein β-subunit mitochondrial precursor	0.379	0.201	0.265	0.381	0.282	0.631	0.062	0.499	0.737	0.004*	0.289	0.339
Ubiquinol-cytochrome C reductase	0.657	0.011*	−0.037	0.904	−0.111	0.350	0.268	0.375	0.366	0.219	0.537	0.058
Creatine kinase M chain	0.461	0.072	0.144	0.609	0.135	0.718	0.190	0.840	−0.149	0.597	0.317	0.249

p value <0.05 was considered statistically significant

observed modifications in the level of expression of the energy metabolism-related proteins in the left ventricle could be interpreted as an adaptative mechanism of cardiac metabolism to overweight. In this regard, a relevant observation was that body weight was the most strongly parameter associated with changes in the level of expression of energy metabolism-related proteins in left ventricle. It also supports the idea that overweight affects heart energy requirements. In this regard, weight gain results in alterations in both renin–angiotensin system and adrenergic pathways which in turn both of them may contribute to modify the expression of proteins associated with energy metabolism [26, 27]. However, further studies are needed to elucidate such hypothesis.

Based on the present results, it is difficult to know the mechanism(s) by which left ventricles of HFD rats showed altered the expression levels of several metabolic-related enzymes. Peroxisome proliferator-activated receptors (PPARs), nuclear receptors, are known transcription regulators of gene-encoding proteins associated with energetic metabolism even in the heart. In this regard, PPAR β/γ is essential for the adult heart to maintain mitochondrial capacity and oxidative metabolism [28]. Moreover, PPAR β/γ and mainly PPAR-α have been associated with increased fatty acid oxidation in the myocardium [29, 30]. Moreover, several reports have identified PPARs as mediators in the adaptive response to fasting particularly on the increase in the expression of enzymes related to long-chain β-acid oxidation. As example, carnitine palmitoyltransferase I gene is activated by fatty acid via PPAR-α in cardiac myocytes [31]. Moreover, dietary fatty acids and their derivatives are PPAR ligands. Further studies analyzing the involvement of PPARs in the here-reported metabolic changes induced by HFD are guaranteed.

In summary, the present results demonstrate that before myocardial functionality was affected by high-fat diet and overweight, the expression of proteins involved in energy production in the left ventricle was modified. Although some studies have suggested to circulating fatty acids levels and insulin resistance as factors that affect cardiac election of the energetic substrates, the here-reported results suggest that independently of circulating cholesterol, glucose, insulin levels and even insulin resistance, proteins involved in different energy metabolic pathways were overexpressed in left ventricle of HFD rats. A better knowledge of the molecular mechanisms affected by high-fat diet may help us to prevent its pathological consequences in the myocardium.

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Conflict of interest Authors declare no conflicts of interest.

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