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**AN EXPERIMENTAL MODEL OF WESTERN DIET IN FEMALE WISTAR RATS  
LEADS TO CARDIAC HYPOXIA RELATED TO A STIMULATED  
CONTRACTILITY**

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**33 Abstract**

34 Previous studies in Western diet (WD)-fed male rats have highlighted a link between the  
35 stimulation of cardiac contractility, mitochondrial adaptations and a pro-inflammatory fatty  
36 acid profile of phospholipids in the heart. Our objectives were to determine 1) if WD-fed female  
37 Wistar rats and obese humans display a similar pro-inflammatory profile in their cardiac  
38 phospholipids and 2) if this lipid profile is associated with deleterious effects on the heart of  
39 the female rodents. Female Wistar rats were fed WD for 5 weeks or a laboratory chow as a  
40 control. Ionic homeostasis, redox status, inflammation markers, and fatty acid composition of  
41 phospholipids were analysed in the heart., WD increased the abdominal fat mass without  
42 modifying the body weight of female rats. As previously found in males, a WD induced a shift  
43 in membrane fatty acid composition toward a pro-inflammatory profile in the female rats, but  
44 not in obese humans. It was associated with an increased COX2 expression suggesting an  
45 increased pro-inflammatory eicosanoid production. Signs of increased intracellular calcium  
46 strongly supported a stimulation of cardiac contractility without any induction of apoptosis. The  
47 heart of WD-fed rats exhibited a hypoxic state as a higher HIF1- $\alpha$  expression was reported. The  
48 expressions of antioxidant enzymes were increased, but the redox reserves against reactive  
49 oxygen species were lowered. In conclusion, as previously observed in males, we suppose that  
50 cardiac abnormalities are magnified with severe obesity in female rats, leading to hypoxia and  
51 intense oxidative stress which could ultimately induce cell death and heart failure.

52

**53 Key points :**

- 54 - WD increases myocardial calcium and induces cardiac hypoxia probably through a  
55 stimulation of contractility in female rats
- 56 - These phenomena are associated with a strong decrease in the GSH/GSSG ratio
- 57 - The capacity to fight against oxidative stress-induced cardiomyocyte death is thus  
58 reduced by WD

59

**60 Key-words:** Heart - Western diet - Inflammation - Hypoxia - Oxidative stress

61

**62 Running title:** Western diet and cardiac homeostasis

## 63 **Introduction**

64 According to the World Health Organisation, overweight (body mass index (BMI) over 25) and  
65 obesity (BMI over 30) were responsible for four million deaths worldwide in 2017. Indeed,  
66 excess in body fat triggers a panel of chronic diseases such as type 2 diabetes, some kinds of  
67 cancer, sleep apnea, cognitive decline, and CVD including atrial fibrillation, stroke,  
68 atherosclerosis, acute myocardial infarction, and heart failure [27]. Overweight and obesity are  
69 thus considered as an epidemic which rages worldwide and affects all the age groups from  
70 childhood to old age, inducing huge health care costs [35]. This epidemic continues to expand  
71 drastically notably in developing countries where the vast majority of overweight and obese  
72 children reside [11].

73 In order to alleviate the deleterious consequences of overweight and obesity on cardiovascular  
74 diseases, modifications of several facets of the life style, such as reduction of calorie intake and  
75 promotion of daily physical activity are required [16]. However, long-term life style  
76 modifications could be difficult to achieve and individual variability for the response to a  
77 dietary intervention is high. It is thus crucial to determine the aetiology of CVD to select the  
78 most efficient therapeutic approach.

79 It is now well established that abdominal obesity is tightly associated with the development of  
80 CVD [7]. It is now accepted that the function of adipose tissue is not limited to fat storage. This  
81 tissue is also recognized as an endocrine organ able to produce numerous hormones or  
82 adipokines such as leptin, adiponectin, resistin, etc. [30]. Leptin plays a crucial role in the  
83 induction of satiety and its blood level increases proportionally to body fat mass [9]. However,  
84 obese people develop leptin resistance, leading to an over consumption of calories [25], an  
85 uncontrolled accumulation of adipose tissue notably in the abdominal area. This excess of  
86 abdominal fat in morbid obesity is known to be an important cause of heart failure and death  
87 [15].

88 To study and better understand the deleterious effects of obesity on the cardiovascular system,  
89 the rat has been recognized as a good model. Indeed, rats fed with a Western diet (high fat diet  
90 or high fat/high sucrose diet) develop abdominal obesity [12, 19]. We recently observed in male  
91 Wistar rats that the *in vivo* cardiac mechanical function was increased by an experimental model  
92 of Western diet (WD) [21, 22]. This stimulation of contractility was confirmed in isolated  
93 mitochondria by an activation of oxidative phosphorylation [23]. Experiments in isolated  
94 perfused hearts indicated that the phenomenon originated rather from an extra cardiac

95 regulation and it was associated with an improper coronary flow [21, 22]. Such effects were  
96 associated with an enrichment of cardiac phospholipids with the pro-inflammatory arachidonic  
97 acid at the detriment of C18:2 n-6 [21, 22]. These changes in membrane polyunsaturated fatty  
98 acids (PUFA) composition led us to hypothesize that a possible pro-inflammatory state of the  
99 heart was responsible for some of the observed changes in cardiac function. This hypothesis  
100 was elaborated in male Wistar rats. The large majority of the studies dealing with obesity of the  
101 rats is performed in male rats since these animals gain weight more easily than females when  
102 they are fed with WD [6]. Hence, little is known about obesity in female rats. In particular, it is  
103 not known if WD is deleterious for the heart of these animals.

104 This study aimed at determining 1) if WD-fed female Wistar rats and obese humans display a  
105 similar pro-inflammatory fatty acid profile as that observed in the cardiac phospholipids of  
106 WD-fed male Wistar rats and 2) if this lipid profile is associated with deleterious effects on  
107 heart function of the female rodents. In this purpose, we fed female rats either with an  
108 equilibrated diet or with WD which is known to favour abdominal obesity. The heart and blood  
109 were then rapidly collected and several analyses were performed. We were thus able to gather  
110 data about the morphology of the heart, its ionic homeostasis, its redox status and aptitude to  
111 scavenge reactive oxygen species (ROS), its subcellular oxidative stress and its inflammatory  
112 state. Moreover, the fatty acid composition of cardiac phospholipids was determined in the  
113 whole heart crude membrane extract of these female rats, but also in the whole phospholipids  
114 of the right atrium of lean and obese humans.

115

116

## 117 **Materials and Methods**

### 118 *Animals, diets and organ collection*

119 Twenty-four 8-week old female Wistar rats (Janvier Labs, Le Genest-Saint-Isle, France) were  
120 housed three per cage under controlled lighting, humidity and temperature conditions. After  
121 one week of acclimatization under laboratory chow, the animals were randomly allocated to  
122 two diet groups for 5 weeks. The first group of rats were fed a Western diet (WD) containing  
123 23, 23.3 and 44.5 % of fat, protein and carbohydrate, respectively. The carbohydrate content  
124 was composed of 22% of sucrose. The second group of animals received the laboratory chow

125 (UAR-A04, Safe, Augy, France) composed of 3.1, 16.1, 43.5 and 3.2% of fat, protein, starch  
126 and sucrose. The fatty acid composition of the two diets is presented in Table 1.

127 After the 5-week experimental diets, the animals were anaesthetized with 4% isoflurane and  
128 exsanguinated via blood collection from the abdominal aorta. Cervical dislocation was  
129 performed and the heart was rapidly harvested. The atria were discarded and the ventricles were  
130 opened with scissors. A maximum of the residual blood was eliminated with paper towel. The  
131 hearts were then freeze-clamped at the temperature of liquid nitrogen. No more than 1 min  
132 elapsed between the end of the exsanguination and the heart freezing. Plasma and adipose  
133 tissues were stored at -80°C until further analyses.

134 All the experiments followed the European Union recommendations for the care and use of  
135 laboratory animals for experimental and scientific purposes. All the animal work was approved  
136 by the local board of ethics for animal experimentation (Comité d'éthique pour  
137 l'expérimentation animale, Auvergne) and notified to the research animal facility of our  
138 laboratory (authorization N° 2018122009381153). The research complied with the ARRIVE  
139 guidelines on animal research [26].

140

#### 141 *Ethical considerations for human samples*

142 The protocol was approved by the review board "Comité de Protection des Personnes Sud-  
143 Ouest et Outre-Mer III" of Bordeaux, France (authorisation number 2018/17) and conformed  
144 with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical  
145 Research Involving Human Subjects. Biopsies were collected after written informed consent  
146 was obtained from the patients concerned. The trial was registered in ClinicalTrials.gov under  
147 the identifier: NCT03690076.

148

#### 149 *Collection of human atrial biopsies*

150 Fresh, human atrial biopsies were collected from 10 patients while they were undergoing open-  
151 chest cardiac surgery at the Gabriel Montpied University Hospital, Clermont-Ferrand, France.  
152 All the patients were having such an intervention for the first time. All the individuals were  
153 tested to determine the effect of obesity on the fatty acid composition of atrial phospholipids.  
154 In the lean group (4 male of 5 patients), 4 patients had a BMI ranging between 20 and 24.9 and  
155 one was slightly overweight (BMI = 26.5). In the obese group (4 male of 5 patients), the 5

156 patients had a BMI ranging between 30 and 34.9 (class 1 obesity). The experiments were full-  
157 blind studies. For valve surgery and/or coronary revascularisation, the heart has to be stopped  
158 and extracorporeal circulation is necessary. Introduction of a cannula of a heart-lung bypass  
159 machine in the right atrium necessitates the removal of a piece of right atrium. These tissues  
160 (0.3 g of wet weight), collected for our study, were immediately freeze-clamped in liquid  
161 nitrogen and stored at  $-80^{\circ}\text{C}$  until biochemical analyses.

162 The clinical characteristics of the lean and obese patients involved in the present study are  
163 presented in a previous publication [18].

164

#### 165 *Preparation of isolated mitochondria*

166 Mitochondria were extracted from frozen rat ventricles as previously described [18].  
167 Myocardium was powdered in liquid nitrogen and approximately 300 mg of tissue were used  
168 to prepare the mitochondria. The myocardium powder was homogenized with a Polytron  
169 blender (0.4 s, rheostat = 3) in 5.4 ml/g of tissue in a cold isolation buffer A composed of  
170 mannitol 220 mM, sucrose 70 mM, MOPS 5 mM and EGTA 2 mM, pH 7.4 at  $4^{\circ}\text{C}$ , fatty acid-  
171 free serum albumin 0.2%. The homogenate was transferred into a Potter-Elvehjem, carefully  
172 rinsed with 10.8 ml/g of the tissue of buffer A and further homogenized with the tissue grinder  
173 (350 RPM, 3 strokes). After centrifugation ( $800 \times g$ , 10 min,  $4^{\circ}\text{C}$ ), the supernatant containing  
174 subsarcolemmal mitochondria was kept on ice. The pellet was resuspended in a cold isolation  
175 buffer B composed of KCl 100 mM, MOPS 50 mM and EGTA 2 mM, pH 7.4 at  $4^{\circ}\text{C}$ , fatty  
176 acid-free serum albumin 0.2%. A protease (subtilisin 0.02%) was added at ice-cold  
177 temperatures for 1 min to digest myofibrils and the whole was then homogenized with a tissue  
178 grinder (300 rpm, 3 to 4 strokes). Immediately afterward, subtilisin action was stopped by  
179 adding an ice-cold isolation buffer B (30 ml) and centrifuging ( $8000 \times g$ , 10 min,  $4^{\circ}\text{C}$ ). The  
180 supernatant was discarded and the pellet was resuspended in isolation buffer B. The  
181 homogenate was then centrifuged ( $800 \times g$ , 10 min,  $4^{\circ}\text{C}$ ), and the resulting supernatant, with  
182 intermyofibrillar mitochondria, was collected and filtered with the other supernatant.  
183 Mitochondria were then washed by centrifugation ( $8000 \times g$ , 10 min,  $4^{\circ}\text{C}$ ). The pellet of  
184 mitochondria was suspended in cold isolation buffer C composed of KCl 100 mM, MOPS 50  
185 mM and EGTA 1 mM, pH 7.4 at  $4^{\circ}\text{C}$ , re-centrifuged ( $8000 \times g$ , 10 min,  $4^{\circ}\text{C}$ ) and, finally,  
186 resuspended at a concentration of approx. 15 mg/ml.

187

188 *Western Blot Analysis*

189 Tissues were ground three times in a mini bead beater in the presence of a lysis buffer made  
190 up of 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) 50 mM, sodium chloride  
191 150 mM, ethylene diamine tetraacetic acid (EDTA) 10 mM, anhydrous sodium tetrabasic  
192 pyrophosphate 10 mM,  $\beta$ -glycerophosphate 25 mM, sodium fluoride 100 mM and anhydrous  
193 glycerol 1.086 M supplemented with phosphatase inhibitors (Sigma Aldrich, Saint-Quentin-  
194 Fallavier, France). Successive centrifugations were performed and the supernatants collected.  
195 Protein concentration was determined using a bicinchoninic acid assay kit (Thermo Fisher  
196 Scientific, Asnières-sur-Seine, France). For protein immunoblotting, 25  $\mu$ g of protein were  
197 separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and  
198 transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were then  
199 immunoblotted with the appropriate antibody to detect calcium-calmodulin ATPase-II  
200 beta/gamma/delta (CaMKII, 47 kDa, 1:1000, GeneTex #GTX52374), calcium-calmodulin  
201 ATPase-II beta/gamma/delta phosphorylated at threonine 287 (CaMKIIP, 47 kDa, 1:1000,  
202 GeneTex #GTX523424), carnitine palmitoylCoA transferase 1 (CPT1 $\beta$ , 87 kDa, 1  $\mu$ g/ml,  
203 Abcam ab134988); caspase 3 (17, 19, 35 kDa, 1:1000, Cell Signaling #9662); cyclooxygenase  
204 2 (COX2, 74 kDa, 1:1000, Cell Signaling #12282), glutathione peroxidase-1 (GPX1, 22 kDa,  
205 1 $\mu$ g/ml, Abcam #ab22604), hypoxia-inducible factor-1 $\alpha$  (HIF1 $\alpha$ , 120 kDa, 1:1000, Cell  
206 Signaling #14179), I $\kappa$ B $\alpha$  (IkB $\alpha$ , 39 kDa, 1:1000, Cell Signaling #9242), NADPH  
207 oxidase-1 (NOX1, 65 kDa, 0.5  $\mu$ g/ml, Abcam #ab131088), nitrotyrosine (1  $\mu$ g/ml,  
208 ThermoFisher Scientific #32-1900), pyruvate dehydrogenase kinase 4 (PDK4, 42 kDa, 0.5  
209  $\mu$ g/ml, ThermoFischer Scientific PAS-79800); sirtuin 1 (SIRT1, 120 kDa, 1:1000, Cell  
210 Signaling #9475); sirtuin 3 (SIRT3, 28 kDa, 1:500, Santa Cruz sc-365175); sodium/proton  
211 exchanger 1 (NHE1, 91 kDa, 1:500, GeneTex #GTX85047), superoxide dismutase 1 (SOD1,  
212 18 kDa, 1:5000, Abcam #ab13498), and superoxide dismutase 2 (SOD2, 22 kDa, 1:1000, Cell  
213 Signaling #13194). Antibody binding was detected using horseradish peroxidase (HRP)-  
214 conjugated secondary antibodies and the ECL Western Blotting Substrate (Thermo Fisher  
215 Scientific, Asnières-sur-Seine, France).

216 Immunoblots were visualized using a chemiluminescence imaging system (MF ChemiBIS,  
217 DNR bio-imaging systems, Jerusalem, Israel) and quantified using Multi Gauge V3.2 software.



218 A mix was prepared by combining an equivalent volume of each protein extract which was  
219 prepared for each animal of the two groups. This mix was deposited on each immunoblot gel.  
220 Its chemiluminescence value was then used as a reference which allowed to normalize the  
221 results for each animal and for each gel.

222 The assessments were performed on myocardial tissue or isolated mitochondria as indicated in  
223 the text. Myocardial and mitochondrial proteins were referred to total proteins determined by  
224 densitometry because of their intergroup stability.

225 Protein carbonylation (OxyBlot Protein oxidation kit, #s7150) was measured according to the  
226 manufacturer's instructions (Merck Millipore, Saint Quentin Fallavier, France).

227

#### 228 *Gene expression*

229 Gene expression of ion channels, interleukin-1 $\beta$  (*Il-1 $\beta$* ), and tumor necrosis factor-alpha (*Tnf-*  
230  $\alpha$ ) was measured in the cardiac homogenates. RNA extraction was performed using TRIzol<sup>®</sup>  
231 (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions.  
232 Chloroform was added (0.2 mL/mL of TRIzol<sup>®</sup>), and samples were mixed and centrifuged  
233 (12,000 g, 15 min, 4 °C). The aqueous phase containing total RNA was collected, mixed with  
234 isopropanol to precipitate RNA and centrifuged (12,000 g, 15 min, 4 °C). The resulting pellet  
235 was washed with ethanol 70% (v/v), dried and suspended in water. The RNA amount and  
236 integrity were determined by measuring the ratio of optical density at 260 and 280 nm and  
237 agarose gel migration. For each sample, 2  $\mu$ g of total RNA were used to perform reverse  
238 transcription. The resulting products were used for real-time quantitative polymerase chain  
239 reaction (RT-qPCR) to appraise gene expression. Gene expression was determined using  
240 specific primers (Table 2) and Rotor-Gene SYBR Green PCR master mix on a Rotor-Gene Q  
241 System (Qiagen, Courtaboeuf, France). Finally, mRNA quantification was calculated using the  
242 delta delta CT method.  $\beta$ -actin was used as the housekeeping gene due to its intergroup stability.

243

#### 244 *Lipid analysis*

245 Total lipids were extracted from cardiac tissues as it is usually performed in our laboratory [34].  
246 Lipid extracts (from approximately 50 mg of myocardium) were prepared using 4 ml  
247 chloroform-methanol (2:1 v/v) and 1 ml 0.9% NaCl. After phospholipid and neutral lipid  
248 separations on Sep-Pack cartridges, fatty acid methyl esters were prepared and separated by gas

249 chromatography as previously described [14]. Addition of an internal standard (C21:0) before  
250 the extraction protocol of each sample allowed the determination of the total amounts of fatty  
251 acids of phospholipids and neutral lipids.

252

253

#### 254 *Other biochemical determinations*

255 Total DNA was extracted according to Chomczynski & Sacchi [4] and assayed with a Nanodrop  
256 Lite spectrophotometer (Thermo Fisher Scientific, Courtaboeuf, France) at a wavelength of  
257 260 nm. 8-hydroxy-2-deoxyguanosine was assayed with a commercial kit (8-hydroxy 2  
258 deoxyguanosine ELISA Kit, ab201734, Abcam, Amsterdam, The Netherlands) according to the  
259 manufacturer's recommendations. The results were normalized to the total DNA content.  
260 Collagen was measured with the Total Collagen Assay kit (1x QZBTOTCOL2) from  
261 QuickZyme (Leiden, The Netherlands) according to the manufacturer's instructions.  
262 Malondialdehyde (MDA) was determined using the Lipid Peroxidation (MDA) Assay Kit  
263 (Colorimetric) (ab233471, Abcam, Amsterdam, The Netherlands). Plasma 3 $\beta$ -hydroxybutyrate  
264 was assayed using the Sigma Aldrich kit (MAK041) according to the manufacturer's  
265 recommendations after filtration on UFC5010 Amicon Ultra-0.5 Centrifugal Filter Unit  
266 (Millipore, Molsheim, France). Total, reduced and oxidized glutathiones (Glutathione  
267 GSH/GSSG Assay Kit, MAK440) were measured according to the manufacturer's instructions  
268 (Merck KGaA, Darmstadt, Germany). The activity of citrate synthase was measured according  
269 to standard practice in our laboratory [33].

270

#### 271 *Statistical analysis*

272 Results are presented as mean  $\pm$  S.E.M. All the results were contrasted across the two groups  
273 by one-way analysis of variance when normality and homoscedasticity were observed.  
274 Normality and homoscedasticity were determined using the Shapiro-Wilk W and Bartlett tests.  
275 If normality or homoscedasticity was not observed, a Mann-Whitney U/Wilcoxon Rank-Sum  
276 test for two samples was performed. When required, group means were contrasted with a  
277 Tukey/Kramer test. A probability ( $p$ ) less than 0.05 was considered significant. Statistical  
278 analyses were performed using the NCSS 2010 software.

279

## 280 **Results**

### 281 *General data*

282 Biometric and morphologic data of the animals are summarized in Table 3. The WD did not  
283 alter the animal weight, but strongly increased the mass of subcutaneous (+58%) and peri  
284 ovarian (+208%) adipose tissues. Conversely, the heart weight was not modified.

285

### 286 *Cardiac composition*

287 The WD reduced the myocardial deoxyribonucleic acid (DNA) and protein contents by 19%  
288 (Fig. 1). Despite the magnitude was very similar, the decrease was significant only for the  
289 protein content ( $p < 0.001$ ) and suggested the occurrence of an oedema.

290 The cardiac collagen content was also lower by 31% ( $p < 0.001$ ) in WD compared to LC group,  
291 even after normalization to the protein content (-13%,  $p < 0.01$ ). Apoptosis was measured by  
292 determining caspase 3 and cleaved caspase 3 expressions. No difference was observed between  
293 the two groups for these two parameters (data not shown). Similarly, the ratio between cleaved  
294 caspase 3 and caspase 3 was similar in the control and WD groups ( $0.97 \pm 0.07$  and  $1.03 \pm 0.05$ ,  
295 NS), suggesting that WD ingestion for five weeks did not favour or prevent cardiac apoptosis.

296

### 297 *Ionic exchangers*

298 As our data suggested the occurrence of an oedema and thus a sodium accumulation in the WD  
299 group, the gene expression of two important sodium-related exchangers was investigated.

300 The sodium/proton exchanger-1 (*Nhe1*) gene expression tended to be lower with the WD (-  
301 13%,  $p = 0.097$ , Fig. 2), but the content of the protein was not significantly altered (data not  
302 shown). Gene expression of the calcium/sodium exchanger-1 (*Ncx1*) was higher in the WD  
303 group compared to the control group (+30%,  $p < 0.001$ ).

304 Plasma lactate ( $1.28 \pm 0.03$  and  $1.29 \pm 0.04$  mM in the LC and WD groups, respectively, NS)  
305 and 3 $\beta$ -hydroxybutyrate ( $82 \pm 5$  and  $96 \pm 12$   $\mu$ g/ml in the LC and WD groups, respectively,  
306 NS), two energy substrates entering into the cell through the monocarboxylate carrier-1 which  
307 co-transport a proton and tends to acidify the intracellular compartment, were not altered by  
308 the different diets.

309 These changes in exchanger gene expressions suggest impairment of the ionic homeostasis.

310

### 311 *Intracellular calcium and cardiac activity*

312 It is known that increased intracellular calcium is responsible for a stimulation of cardiac  
313 mechanical activity. The expression of CaMKII protein, a key regulator of channels mediating  
314 cardiac excitability and conduction tended to be reduced in the WD group (-19%,  $p = 0.065$ )  
315 but the expression of its phosphorylated form was not altered (Fig. 3). PDK4 protein expression  
316 was significantly lower with the WD (-30%,  $p < 0.05$ ), suggesting a stimulation of glucose  
317 utilization. Such a stimulation is characteristic of an activation of cardiac mechanical activity.  
318 This occurred without any change in mitochondrial density as it was indicated by the unaltered  
319 citrate synthase activity and carnitine palmitoylCoA transferase 1 $\beta$  (CPT1 $\beta$ ) expression.

320

### 321 *Redox status*

322 As illustrated in Figure 4, total glutathione tended to be lower in the WD group (-26%), but the  
323 difference was not significant. By contrast, reduced glutathione (GSH) was significantly  
324 smaller (-52%;  $p < 0.05$ ) and the oxidized form of glutathione (GSSG) tended to be higher with  
325 WD (+32%). Consequently, the GSH to GSSG ratio was strongly lowered in the WD group (-  
326 68%,  $p < 0.001$ ), indicating a reduced redox potential.

327 To determine if this decrease in redox potential was related to oxygen or substrate deficiency.  
328 we evaluated myocardial content in neutral lipids, but did not find any significant difference  
329 ( $2.5 \pm 0.2$  and  $3.4 \pm 0.6$  mg of fatty acids/g of myocardium in the LC and WD groups,  
330 respectively). By contrast, HIF-1 $\alpha$  expression tended to be higher with the WD (+42%,  $p <$   
331  $0.086$ ), suggesting a disequilibrium between oxygen supply and utilization in this group.

332

### 333 *Subcellular ROS production and oxidative stress*

334 Total myocardial malondialdehyde (MDA) content, an index of lipid peroxidation, was  
335 unchanged by the WD ( $1.4 \pm 0.1$  vs.  $1.7 \pm 0.2$  nmoles/g of proteins for the LC and WD groups,  
336 respectively). NOX-1 expression was not altered (data not shown), suggesting no oxidative  
337 stress in the vascular compartment. Similarly, SIRT1 expression (data not shown) and 8-  
338 hydroxy-2-deoxyguanosine content ( $71 \pm 2$  vs.  $76 \pm 5$  ng/100  $\mu$ g DNA for the LC and WD

339 groups, respectively) were not affected, outlining the absence of oxidative stress at the nucleus  
340 level. Finally, SOD1 protein expression was unchanged (data not shown) suggesting that the  
341 cytosolic compartment did not display any oxidative stress.

342 Conversely, the redox changes had noticeable effects on the mitochondrial oxidative status (Fig.  
343 5). SOD2 protein expression tended to be higher with the WD (+12%,  $p = 0.08$ ) which suggests  
344 an increased ability to scavenge superoxide anions. Furthermore, GPX1 expression was higher  
345 (+17%,  $p < 0.05$ ) indicating an increased capacity to scavenge peroxides. As a result, protein  
346 carbonylation and SIRT3 protein expression tended to be reduced with the WD (-34% with  $p =$   
347 0.15, -65% with  $p = 0.11$ , respectively). Finally, nitrotyrosination of mitochondrial proteins was  
348 not affected by the WD.

349

### 350 *Inflammation*

351 In our study, the WD led to higher COX2 protein and *Tnf- $\alpha$*  gene expressions compared to the  
352 control group (+82%,  $p < 0.05$  and +22%,  $p < 0.01$  respectively, Fig. 6). However, I $\kappa$ B $\alpha$  protein  
353 level was not affected.

354

### 355 *Fatty acid composition of cardiac phospholipids*

356 We determined if the WD affected the fatty acid composition of whole heart crude membrane  
357 fraction in the female rats (Table 4).

358 Membrane saturated fatty acids (SFAs) were slightly increased with the WD (+3%,  $p < 0.05$ )  
359 and this was mainly due to an increase in C18:0 (+14%,  $p < 0.01$ ) as other SFAs were  
360 underrepresented in WD compared to LC. This was the case for C16:0 (-14%,  $p 0.001$ ), C17:0  
361 (-50%,  $p < 0.001$ ) and C20:0 (-9%,  $p < 0.01$ ).

362 Monounsaturated fatty acids (MUFAs) total amount was not modified by the diets. However,  
363 some MUFAs were increased by the WD. This was the case for C15:1 (+59%,  $p < 0.001$ ) and  
364 C17:1 (+17%,  $p < 0.05$ ). C16:1 n-7, C18:1 n-9, and C22:1 n-11 levels were decreased (-44%, -  
365 22, and -34% respectively with  $p < 0.001$  for the three fatty acids).

366 PUFAs of the n-6 family were slightly reduced by the WD (-5%,  $p < 0.05$ ). The main highlights  
367 of this class of fatty acids were a strong decrease in C18:2 n-6 (-45%,  $p < 0.001$ ) and a high

368 increase of the pro inflammatory arachidonic acid (C20:4 n-6, +23%,  $p < 0.001$ ) and its upper  
369 metabolites (C22:4 n-6 and C22:5 n-6, +68% and +192%, respectively,  $p < 0.001$  for both).

370 PUFAs of the n-3 series were increased (+10%,  $p < 0.01$ ). This was true for C22:5 n-3 and  
371 C22:6 n-3 (+26 and +8%,  $p < 0.001$  and  $p < 0.01$ , respectively), but the anti-inflammatory  
372 eicosapentaenoic acid (C20:5 n-3), which is a substrate for COX 2, was strongly decreased (-  
373 92%,  $p < 0.001$ ).

374 These results show that the WD activated desaturases and elongases to favour the accumulation  
375 of long chain n-6 and n-3 PUFAs.

376

### 377 *Class I obesity and fatty acid composition of atrial phospholipids of human individuals*

378 Since the WD given to our male and female rats was high in SFAs and MUFAs and low in  
379 PUFAs compared to the laboratory chow diet, we evaluated the effect of class I obesity on the  
380 fatty acid composition of human atrial phospholipids (Table 5) to verify if similar effects occur.

381 Surprisingly, only few differences were observed in the fatty acid profile of cardiac  
382 phospholipids between obese and lean individuals. Only C15:0 and C22:1 n-11 were  
383 significantly higher in obese individuals (+48 and +127%, respectively,  $p < 0.05$  for both fatty  
384 acids). A trend toward a lower proportion of C18:2 n-6 was observed in the obese people (-  
385 14%, NS), but C20:4 n-6 level was similar in the two groups. It is then unlikely that desaturases  
386 and elongases were activated in class I obesity. Moreover, the level of the anti-inflammatory  
387 eicosapentaenoic acid was not affected by obesity.

388

## 389 **Discussion**

390 The objectives of the study were to determine 1) if WD-fed female Wistar rats and obese  
391 humans (mainly males) display a similar pro-inflammatory fatty acid profile as that observed  
392 in the cardiac phospholipids of WD-fed male Wistar rats and 2) if this lipid profile is associated  
393 with deleterious effects on heart function of the female rodents.

394 We showed that the WD used in the present study triggered an early fat deposit in the female  
395 rat without any increase in body mass. At the cardiac level, it was associated with an oedema  
396 and an increase in myocardial calcium which were probably involved in the stimulation of  
397 cardiac mechanical activity. These effects were accompanied by a hypoxic state, as suggested

398 by the higher HIF-1 $\alpha$  expression. The resulting lower redox potential was responsible for higher  
399 expressions of SOD2 and GPX1 which contributed to alleviate mitochondrial oxidative stress.  
400 Evidences for early stages of inflammation were also suggested by the higher expressions of  
401 COX2 and *Tnf- $\alpha$*  with the WD. Surprisingly, we failed to demonstrate an activation of the  
402 NF $\kappa$ B pathway as the expression of I $\kappa$ B $\alpha$  was unchanged by the WD. These observations were  
403 associated to large modifications of the PUFAs profile of cardiac phospholipids as higher pro-  
404 inflammatory arachidonic acid and lower anti-inflammatory eicosapentaenoic acid proportions  
405 were observed in the female rats. Contrasting with our previous data in male rats [21, 22], these  
406 modifications in membrane lipid composition were not observed in the atria of obese humans,  
407 maybe because the composition of the human diet was not controlled as that of the WD.

408

409 *Stimulation of cardiac mechanical work, PUFA profile of cardiac phospholipids and*  
410 *inflammation cascade*

411 We recently observed in WD-fed male rats a higher abdominal fat deposit and *in vivo* left  
412 ventricle developed pressure compared to animals receiving a standard diet [21, 22]. For  
413 technical reasons, it was not possible to evaluate cardiac mechanical activity in the present  
414 study, but our data supported increased intracellular calcium level in the hearts of the WD-fed  
415 female rats, without any sign of myocardial apoptosis. A physiological increase in intracellular  
416 calcium stimulates the cardiac mechanical function through an activation of the  
417 excitation/contraction coupling [8] and mitochondrial energy metabolism [28]. The decrease in  
418 CaMKII expression suggests a desensitization of the calcium signal due to an increased  
419 intracellular calcium. This higher intracellular calcium concentration was associated with a  
420 lower PDK4 expression which is known to increase pyruvate dehydrogenase activity [37] and  
421 glucose oxidation. Yet, stimulation of mechanical activity of the heart has been found to  
422 increase glucose oxidation without modifying fatty acid utilization by Neely *et al.* [24]. Thus,  
423 our data strongly suggest that the short term WD also increased the cardiac mechanical activity  
424 of the female rats.

425 Further supporting a plausible increase in intracellular and matrix calcium, we have previously  
426 observed a stimulation of mitochondrial energy production in the same atrial human samples  
427 than those used for the present study [18]. A physiological elevation of matrix calcium  
428 stimulates oxidative phosphorylation [20]. This effect allows the production of a sufficient  
429 amount of energy to sustain an increased cardiac mechanical activity. We previously reported

430 a similar metabolic stimulation of mitochondria which were isolated from the left ventricle of  
431 WD-fed male rodents [23].

432 The higher cardiac mechanical function was associated with several changes in the fatty acid  
433 composition of membrane phospholipids. Hence, we observed a decrease in C18:2 n-6  
434 proportion in favour of C20:4 n-6 and a reduction of C20:5 n-3 in favour of C22:5 and C22:6  
435 n-3 in the cardiac phospholipids of WD-fed female rats. These changes were similar to those  
436 previously observed in WD-fed male rats [21, 22]. These elongase and desaturase activations  
437 induced by WD whatever the gender of the rodents were probably due to the high contents of  
438 saturated and monounsaturated fats in the diet and occurred in order to maintain a healthy  
439 unsaturation degree and fluidity of the cardiac membranes. We suspected that the higher pro  
440 inflammatory C20:4 n-6 and lower anti-inflammatory C20:5 n-3 were responsible for an  
441 activation of the inflammation process which was characterized by a huge production of pro-  
442 inflammatory eicosanoids. Indeed, COX2 expression was increased by 82% with WD and the  
443 ratio between C20:4 n-6 and C20:5 n-3 in membrane phospholipids increased from  $153 \pm 22$  in  
444 the control group to  $995 \pm 207$  in the WD group ( $p < 0.01$ ). A direct link between a probable  
445 overproduction of pro-inflammatory eicosanoids and the increased *in vivo* contractility remains  
446 to be characterized but our previous study [21] strongly suggested that the increased *in vivo*  
447 contractility depended on the increased arachidonic acid proportion and pro-inflammatory  
448 eicosanoid overproduction. On the contrary, in the perfused heart, myocardial contractility was  
449 not increased by the WD when the coronary flow was strictly similar between WD and the  
450 control groups [22]. This indicates that the WD-induced changes in the fatty acid profile of  
451 cardiac phospholipids did not alter cardiomyocyte contractility. It is possible that the pro-  
452 inflammatory eicosanoid production was blunted in the *ex vivo* situation and therefore the  
453 contractility was not increased. However, we cannot exclude the involvement of an extra  
454 cardiac effect via hormonal and/or a nervous regulations. Moreover, as myocardial  $I\kappa B\alpha$   
455 expression was not modified with WD, the NF $\kappa$ B pathway was probably not activated.  
456 However, *Tnf- $\alpha$*  gene expression was significantly increased indicating that the hearts of the  
457 WD-fed rats were probably only recently engaged in the inflammation pathway and then that  
458 the low-grade inflammation which is known to occur during obesity was not responsible for the  
459 substantial changes of cardiac homeostasis that we observed.

460 We had the opportunity to compared our observations to the fatty acid composition of human  
461 atrial phospholipids in lean and moderately obese people. The membrane lipid composition of  
462 moderately obese patients resembled to that of lean subjects. However, two fatty acids differed



463 in their proportions. The first fatty acid was pentadecylic acid (C15:0), a saturated fatty acid  
464 which is present in dairy fats [31]. The two rodent diets being devoid of dairy fats, C15:0 was  
465 not detected in the cardiac phospholipids of the control diet- and WD-fed rats. In humans, its  
466 proportion was higher in obese subjects than in lean ones, suggesting that obese people from  
467 our study had consumed more dairy products than lean patients. The second fatty acid differing  
468 in lean and obese patients was cetoleic acid (C22:1 n-11), a minor component of some fish oils.  
469 C22:1 n-11 was decreased in rat cardiac phospholipids by the WD, because this diet contained  
470 a lower proportion of fish oil than the control diet. In humans, it was increased by obesity. As  
471 no food reports were included in this study, we cannot confirm if obese people ate more sea  
472 food than their lean littermate.

473 The atrial phospholipids of obese human did not apparently display the pro inflammatory  
474 profile noticed in the ventricle phospholipids of WD-fed male and female rats. The rat pro  
475 inflammatory profile was characterized by lower linoleic acid and higher arachidonic acid  
476 proportions. In the right atrium of obese subjects, a tendency toward a lower linoleic acid  
477 proportion (-14%) was noticed, but arachidonic acid proportion was unchanged. Two main  
478 reasons may explain inconsistencies between rat and human tissues. Firstly, the diet of obese  
479 people might not contain as much saturated and monounsaturated fatty acids as WD of our rats.  
480 This would partly prevent elongase and desaturase activation. Secondly, the development stage  
481 of obesity differed between the two species. Contrasting with the human study, the body weight  
482 was unchanged in female rats in spite of the WD ingestion. The increased elongase and  
483 desaturase activities and the elevated COX2 expression that were observed in the left ventricles  
484 of rats could have also occurred in human right atria earlier during the development of obesity.  
485 This hypothesis is supported by our previous study showing differences between WD-fed rats  
486 according to their body weight gain [21]. Unfortunately, it was not possible to collect human  
487 right atria at an early stage of obesity.

488

#### 489 *Other mechanisms susceptible in explaining the early changes in cardiac homeostasis*

490 Some of our previous data [22] suggested that the stimulating agent acting *in vivo* after the WD  
491 intake was supplied via the blood through hormonal regulation or the nervous system according  
492 to a neuro-humoral activity. Obesity, notably excess fat deposit, leads to leptin overproduction  
493 and adrenergic stimulation [10] via an effect of leptin on the ventromedial hypothalamus [29].  
494 Although we did not evaluate circulating leptin in the present study, the observed accumulation

495 of peri ovarian and subcutaneous fats in the WD-fed female rats is a strong argument suggesting  
496 a leptin overproduction. Adrenergic stimulation activates the adenylate cyclase, leading to the  
497 synthesis of cyclic AMP, increasing myofibril calcium level and thus inducing positive  
498 inotropic and chronotropic effects via activation of cardiomyocyte  $\beta$ 1 adrenoceptors [5].  
499 Adrenergic stimulation also activates the  $\beta$ 2 adrenoceptors of the coronary vessels, promoting  
500 their dilatation, an increased coronary flow, and an enhanced oxygen and substrate supplies to  
501 the cardiomyocytes [2], that contribute to sustain cardiac mechanical activity. The stimulated  
502 cardiac mechanical activity observed after WD ingestion can be related to the activation of the  
503 adrenergic system. However, we had not the possibility to evaluate blood adrenaline and  
504 norepinephrine levels in our study.

505 The development of the oedema in the heart of the WD group indicated sodium overload in the  
506 cardiomyocytes which can result from an energy deficiency. We demonstrated a drop in the  
507 GSH to GSSG ratio in the WD group compared to the LC one. GSH is consumed by the GPX1  
508 enzyme to detoxify different kind of peroxides. The reaction leads to the formation of the  
509 oxidized GSSG which is regenerated into GSH by means of the glutathione reductase enzyme  
510 and NADPH. The amount of GSH depends on NADPH availability which is closely related to  
511 NADH level [32]. Consequently, the GSH to GSSG ratio probably evolves in parallel with the  
512 NADH to  $\text{NAD}^+$  ratio and thus the redox potential. The lower GSH to GSSG ratio suggested  
513 thus a lower redox potential which probably results from an intense oxidation of NADH in the  
514 respiratory chain to produce ATP in order to sustain the stimulated cardiac mechanical activity  
515 in the WD group. The magnitude of the energy necessary for contractile machinery probably  
516 overpasses ATP production in the WD group leading to dysfunctions of several ionic transport  
517 systems. The Na/K-ATPase represents a good candidate for the perturbations observed in the  
518 WD group, considered a dysfunction of this transport system due to energy deficiency should  
519 retain sodium in the cardiomyocytes and should favour water entry into these cardiomyocytes  
520 and thus the oedema.

521

#### 522 *Possible mechanisms responsible for heart failure occurring with severe obesity*

523 The trend for a higher HIF-1 $\alpha$  expression in the WD group further supports the energy  
524 deficiency hypothesis in our study. The increase in HIF-1 $\alpha$  expression was important (42%) all  
525 the more so due to the short duration of the WD ingestion (5 weeks only). The induction of the  
526 HIF-1 $\alpha$  suggested an insufficient oxygen supply. The pro inflammatory lipid profile is a good

527 candidate to explain a deficit in coronary flow. Indeed, COX2 and eicosanoid synthases favour  
528 the production of numerous vasoactive agents which can increase or decrease the coronary flow.  
529 In the present study, we did not evaluate these agents, nor obtain a direct measurement of the  
530 *in vivo* coronary flow. However, a previous study performed in WD-fed male rats with a similar  
531 pro inflammatory lipid profile as that observed in female rats of the present study showed a  
532 WD-related decrease in contractility when the hearts were perfused at fixed pressure. Upon  
533 these conditions, the coronary flow depends on the dilatation state of the coronary vessels which  
534 could be decreased by the vasoactive agents produced from the pro inflammatory lipid profile.  
535 The lower contractility was thus attributed to a decreased coronary flow or an heterogeneous  
536 coronary flow with low-flow zones. An oxygen disequilibrium resulting from the stimulated  
537 myocardial contractility and a too low coronary flow could be responsible for the increased  
538 HIF1- $\alpha$  expression.

539 Another hypothesis could involve angiotensin 2. A recent study has reported that blood  
540 angiotensin 2 is strongly increased after a WD [17]. Given its vasoconstriction properties, this  
541 substance could participate to the increased HIF1- $\alpha$  expression. However, we had not the  
542 opportunity to determine circulating angiotensin 2 in the present study.

543 It was also demonstrated that an increase of HIF-1 $\alpha$  expression triggered an adaptation towards  
544 hypoxic conditions and cellular repairs [13]. In agreement with this, the capacities to scavenge  
545 superoxide anion and hydrogen peroxides were improved, which could facilitate cellular  
546 survival, as the expression of the mitochondrial enzymatic defences (SOD2 and GPX1) was  
547 increased. Supporting this, the mitochondrial oxidative stress evaluated by the level of protein  
548 carbonylation, tended to be lower with WD (-34%,  $p = 0.15$ ). However, we could speculate that  
549 oxidative stress could increase with time as the ionic disequilibrium observed after the WD  
550 feeding can be a source of mitochondrial ROS. It was shown that excess calcium within the  
551 mitochondrial matrix triggers ROS release from these organelles [3]. The ROS release from a  
552 mitochondria can even induce a ROS release from neighbouring mitochondria, a phenomenon  
553 which is called ROS-induced ROS release [38]. The decreased GSH to GSSG ratio, indicated  
554 either an increased ROS detoxification, a reduced NADH production from the intermediary  
555 metabolism due to oxygen deficiency, and/or an increased NADH utilization in the respiratory  
556 chain.

557 In the present study, although the system was unbalanced, no oxidative stress and no cellular  
558 death occurred, probably because body fat deposition was still in an early phase. However, with

559 severe obesity, many physiological processes may be dysregulated, leading to the saturation of  
560 cellular capacities to scavenge intracellular ROS, cell death and heart failure.

561

562 *Limitations of the study*

563 Android obesity is characterized by abdominal fat accumulation in males and it is recognized  
564 as being deleterious for cardiovascular health [1]. In the present study, the WD-fed female rats  
565 displayed both peri ovarian adiposity and subcutaneous fat accumulation. Peri ovarian adiposity  
566 consists of fat accumulation in the abdominal area and it resembles to android obesity.  
567 However, gender differences may exist in the consequences of the different fat deposits on  
568 health. Consequently, it was unlikely that we could incriminate one fat depot specifically in the  
569 deleterious effect on the heart in female rats.

570 The fatty acid composition of human biopsies was determined in the right atrium whereas it  
571 was measured in the left ventricles of Wistar rats. We had the opportunity to perform a  
572 preliminary study in humans by using atrial tissue obtained during an open chest surgery. This  
573 piece of tissue is collected to allow the establishment of an extracorporeal circulation. In our  
574 study, we used this tissue to perform our measurements because the collection of human left  
575 ventricle is ethically almost impossible. Right atrium and left ventricle have distinct functions  
576 and could display different membrane lipid profile, but we speculated that obesity has the same  
577 effect on membrane phospholipids. So analysing atrial phospholipids is also limitation of our  
578 study. In addition, atrial biopsies were collected from either lean or obese male and female. Yet,  
579 the membrane lipid profiles of these biopsies were compared to the cardiac tissue of control-  
580 and WD-fed rats which were females. A gender difference may exist concerning the fatty acid  
581 profile of atrial phospholipids. However, the studies that we performed earlier in male rats [21,  
582 22] indicate that an experimental model of WD with a very similar diet profile, triggered similar  
583 changes in the cardiac lipid profile as those observed in the female rats of the present study. In  
584 humans, this might be probably different. Finally, the fatty acid profile of cardiac phospholipids  
585 is highly related to the quantity and nature of fatty acids found in the diet. The ingested lipids  
586 may differ between individuals and this should result in divergent fatty acid profiles of atrial  
587 phospholipids. The fact that men and woman were mixed in a same group could introduce  
588 variability and prevent the occurrence of significant results, especially since the size of our  
589 groups was low for a human study (n=5).

590

## 591 *Conclusion*

592 Ingestion of a WD rapidly induces an important stress which overwhelms the organism. The  
593 increase in intracellular calcium is intense, since cardiac mechanical activity is strongly  
594 increased. Although the fatty acid composition of cardiac phospholipids adopted an  
595 inflammatory profile, it remains to determine whether the increased intracellular calcium is due  
596 to the changes in membrane lipids and to the induction of the inflammation process. Obese  
597 humans displayed a similar activation of cardiac function, although the membrane lipid  
598 composition of the right atrium is more similar in obese and lean humans.

599 Due to a disequilibrium between the stimulation of cardiac function and the oxygen supply in  
600 the rat, an energy deficiency settles in the myocardium and triggers a strong decrease in the  
601 redox potential. The reduced GSH to GSSG ratio observed in the present study proves that the  
602 capacities to scavenge ROS is strongly decreased in that situation. In the conditions of our  
603 experiment, the disequilibrium had no deleterious cardiac consequences except the  
604 development of an oedema, because the obesity state was not too severe. However, with severe  
605 obesity, an important oxidative stress would occur and numerous cardiomyocytes would die.  
606 This is detrimental to the heart and would explain the well-known heart failure resulting from  
607 severe obesity.

608

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617

## 618 **Declaration of Competing Interest**

619 The authors declare that there is no conflict of interest.

620

## 621 **Abbreviation list**

622 ACE: angiotensin converting enzyme; AMP: adenosine monophosphate; Anova: analysis of  
623 variance; ARRIVE guideline: “animal research: reporting of in vivo experiments”; ASMS  
624 team: alimentation et santé musculo-squelettique; ATP: adenine triphosphate; AU: arbitrary  
625 unit; BMI: body mass index; CaMKII: calcium-calmodulin ATPase-II beta/gamma/delta;  
626 CaMKIIP: calcium-calmodulin ATPase-II beta/gamma/delta phosphorylated at threonine 287;  
627 COX2: cyclooxygenase 2; CPT1b: carnitine palmitoylCoA transferase 1 $\beta$ ; CVD:  
628 cardiovascular disease; DNA: deoxyribonucleic acid; EDTA: ethylene diamine tetraacetic acid;  
629 EGTA: ethylene glycol tetraacetic acid; F: forward; GPX1: glutathione peroxidase 1; GSH:  
630 reduced glutathione; GSSG: oxidized glutathione; HIF1 $\alpha$ : hypoxia-inducible factor 1-alpha;  
631 HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HRP: horse radish peroxidase;  
632 IkBa: IkappaB- $\alpha$ ; IL-1 $\beta$ : interleukin 1-beta; KCl: potassium chloride; LC: laboratory chow;  
633 MDA: malondialdehyde; M: mix; MOPS: 3-(N-morpholino)propanesulfonic acid; MUFA:  
634 monounsaturated fatty acid; NaCl: sodium chloride; NADH: reduced nicotinamide adenine  
635 dinucleotide; NAD<sup>+</sup>: oxidized nicotinamide adenine dinucleotide; NADPH: reduced  
636 nicotinamide adenine dinucleotide phosphate; NCSS: number cruncher statistical software;  
637 NCX1: sodium/calcium exchanger 1; nd: not detected; NHE1: sodium/proton exchanger 1;  
638 NOX1: NADPH oxidase 1; NS: not significant; n-6/n-3: ratio between n-6 and n-3  
639 polyunsaturated fatty acids; p: probability; PDK4: pyruvate dehydrogenase kinase 4; Periov.:  
640 periovarian; PUFA: polyunsaturated fatty acid; PVDF: polyvinylidene difluoride; R: reverse;  
641 RNA: ribonucleic acid; ROS: reactive oxygen species; RPM: rotations per minute; RT-qPCR:  
642 reverse transcription quantitative real-time polymerase chain reaction; SDS-PAGE: sodium  
643 dodecyl sulfate-polyacrylamide gel electrophoresis; SEM: standard error of the mean; SF>A:  
644 saturated fatty acid; SIRT 1: sirtuin 1; SIRT3: sirtuin 3; SOD1: superoxide dismutase 1; SOD2:  
645 superoxide dismutase 2; Subcut.: subcutaneous; TNF- $\alpha$ : tumor necrosis factor alpha; WD:  
646 Western diet;

647

648

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652

## 653 **Contribution statement**

654 J.-P. R., C. J., F. C., V. P., B. M., A. T., and L. D. contributed to the technical part of the study.  
655 L. D. performed the statistical analyses. A. T., F. C., and L. D. contributed to the study  
656 conception and design, analysis and interpretation of data. L. D. wrote the manuscript. A. T., F.  
657 C., and J.-P. R. revisited it critically and gave final approval of the version to be published.  
658 The authors declare that all data were generated in-house and that no paper mill was used.  
659

**660 Figure legend**

661 **Fig. 1** Some aspects of myocardial morphology after the Western diet feeding. The results  
662 represent the average of 12 rats per group. A, B and C: Myocardial deoxyribonucleic acid  
663 (DNA), proteins and collagen content normalized to the cardiac fresh weight ; D: myocardial  
664 collagen content normalized to the protein content. LC: rats fed with the laboratory chow; WD:  
665 rats fed with the Western diet ; p: probability. Probability lower than 0.05 was considered  
666 significant.

667

668 **Fig. 2** Influence of the Western diet on the sodium/proton exchanger-1 (*Nhe1*) and  
669 sodium/calcium exchanger-1 (*Ncx1*) gene expressions. The results represent the average of 12  
670 rats per group. A: *Nhe1* gene expression expressed in arbitrary units (AU); B: *Ncx1* gene  
671 expression expressed in arbitrary units (AU); LC: rats fed with the laboratory chow; WD: rats  
672 fed with the Western diet ; p: probability. Probability lower than 0.05 was considered  
673 significant.

674

675 **Fig. 3** Influence of the Western diet on myocardial activity. The Western Blots have been  
676 performed individually for each rat and the quantitative results represent the average of 12 rats  
677 per group. A: calcium-calmodulin ATPase-II phosphorylated at threonine 287, calcium-  
678 calmodulin ATPase-II, pyruvate dehydrogenase kinase-4 and carnitine palmitoylCoA  
679 transferase-1 $\beta$  expressions as well as citrate synthase activity; B: calcium-calmodulin ATPase-  
680 II phosphorylated at threonine 287, calcium-calmodulin ATPase-II, pyruvate dehydrogenase  
681 kinase-4 and carnitine palmitoylCoA transferase-1 $\beta$  representative immunoblots; C:  
682 corresponding protein content by densitometry; AU: arbitrary units; LC: laboratory chow; WD:  
683 Western diet; M: Mix; p: probability. Probability lower than 0.05 was considered significant.

684

685 **Fig. 4** Influence of the Western diet on some parameters of the redox potential. The means  
686 represent a consortium of 12 animals per group. A: myocardial content of total glutathione; B:  
687 myocardial content of reduced glutathione (GSH); C: myocardial content of oxidized  
688 glutathione (GSSG); D: ratio between reduced and oxidized glutathione; E: hypoxia-inducible  
689 factor (HIF-1) alpha (HIF-1 $\alpha$ ) expression; F: HIF-1 $\alpha$  representative immunoblots; G:  
690 corresponding protein content by densitometry; AU : arbitrary units; LC: rats fed with the



691 laboratory chow; WD: rats fed with the Western diet; M: Mix; p: probability. Probability lower  
692 than 0.05 was considered significant.

693

694 **Fig. 5** Effects of the Western diet on some aspects of mitochondrial oxidative stress. The means  
695 represent a consortium of 12 rats per group. A: sirtuin-3 (SIRT3), superoxide dismutase-2  
696 (SOD2), glutathione peroxidase-1 (GPX1), carbonylated proteins (Prot. Carb.) and  
697 nitrotyrosinated proteins ( Prot. Nitr.) expressions of the myocardium; B: representative  
698 immunoblots of these proteins; C: corresponding protein content (Prot. Cont.) by densitometry;  
699 AU: arbitrary unites; LC: laboratory chow; WD: Western diet; M: Mix; p: probability.  
700 Probability lower than 0.05 was considered significant.

701

702 **Fig. 6** Influence of the Western diet on some parameters of the inflammation process. The  
703 results represent the average of 12 rats per group. A: myocardial cyclooxygenase-2 (COX2)  
704 expression; B: COX2 representative immunoblots; C: corresponding protein content by  
705 densitometry; D: myocardial interleukin-1 $\beta$  (*Il-1 $\beta$* ) gene expression; E: myocardial tumor  
706 necrosis factor- $\alpha$  (*Tnf- $\alpha$* ) gene expression; F: myocardial I $\kappa$ B $\alpha$  (I $\kappa$ B $\alpha$ ) expression; G:  
707 I $\kappa$ B $\alpha$  representative immunoblots; H: corresponding protein content by densitometry; AU:  
708 arbitrary unites; LC: laboratory chow; WD: Western diet; M: Mix; p: probability; NS: not  
709 significant. Probability lower than 0.05 was considered significant.

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- 836

837 Table 1. Fatty acid composition of the total lipids of the laboratory chow and Western diet

Fatty acid	LC	WD
C14:0	0.53	1.34
C16:0	18.8	25.32
C18:0	2.54	12.11
Total SFA	22.3	39.06
C16:1n-7	0.71	2.28
C18:1n-7	1.59	3.59
C18:1n-9	21.22	37.91
C20:1n-9	0.8	0.44
Total MUFA	24.36	44.37
C18:2n-6	47.54	15.29
C20:2 n-6	0.12	0.28
Total n-6 PUFA	47.75	15.7
C18:3n-3	3.84	0.65
C18:4n-3	0.13	0.02
C20:5n-3	0.54	nd
C22:6n-3	0.83	nd
Total n-3 PUFA	5.43	0.69
Total PUFA	53.18	16.39
n-6/n-3	8.79	22.75

838 The results are expressed in proportion of total fatty acids measured as peak area. LC: laboratory  
839 chow; WD: Western diet; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids;  
840 PUFA: polyunsaturated fatty acids; n-6/n-3: ratio between n-6 PUFA and n-3 PUFA; nd: not  
841 detected.

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844 Table 2. Real-time PCR primers used in this study.

Gene	Sequence (5'-3')
<b>TNF-<math>\alpha</math></b> (NM_012675.3)	(F) GCCTCTTCTCATTCCTGCTC (R) GAGCCCATTTGGGAACCTTCT
<b>IL-1<math>\beta</math></b> (NM_031512.2)	(F) AAATGCCTCGTGCTGTCTGA (R) GGTGTGCCGTCTTTCATCAC
<b>NHE1</b> (NM_012652)	(F) AACGGCTGCGGTCCTATAAC (R) CGAGACATGGTGGGTGAGTC
<b>NCX1</b> (NM_001270779.1)	(F) TGTTGTAAATGAGCTTGGTGG (R) TTACGGTAGAGGGAATCGGATG
<b><math>\beta</math>-Actin</b> (NM_031144.3)	(F) TCTGTGTGGATTGGTGGCTCTA (R) CTGCTTGCTGATCCACATCTG

845 (F): forward; (R): reverse

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849 Table 3. Effects of the Western diet on some aspects of rat biometrics

	LC	WD	Anova
Body weight (g)	294 ± 4	299 ± 5	NS
Subcut AT (g)	2.6 ± 0.2	4.1 ± 0.3	p < 0.001
Perov AT (g)	2.4 ± 0.4	7.4 ± 0.4	p < 0.001
Heart (g)	0.85 ± 0.01	0.85 ± 0.02	NS

850 The number of animals was 12 in each group. LC: Laboratory Chow; WD: Western Diet ;  
851 Subcut AT: subcutaneous adipose tissue; Periov AT: periovarian adipose tissue; Anova :  
852 Analysis of variance; p; probability; NS: not significant.

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856 Table 4 Fatty acid composition of myocardial phospholipids of laboratory chow- and Western  
857 diet-fed rats

	LC	WD	Anova
C14:0	0.25 ± 0.02	0.24 ± 0.02	NS
C16:0	15.90 ± 0.25	13.62 ± 0.37	p < 0.001
C17:0	0.42 ± 0.01	0.21 ± 0.01	p < 0.001
C18:0	23.71 ± 0.07	26.97 ± 0.45	p < 0.01
C20:0	0.22 ± 0.01	0.20 ± 0.01	p < 0.01
SFA	40.43 ± 0.42	41.46 ± 0.26	p < 0.05
C15:1	0.17 ± 0.01	0.27 ± 0.01	p < 0.001
C16:1 n-7	0.52 ± 0.03	0.29 ± 0.03	p < 0.001
C17:1	0.41 ± 0.01	0.48 ± 0.02	p < 0.05
C18:1 n-7	5.18 ± 0.19	5.61 ± 0.38	NS
C18:1 n-9	4.06 ± 0.06	3.17 ± 0.08	p < 0.001
C20:1 n-9	0.14 ± 0.01	0.12 ± 0.01	NS
C22:1 n-11	0.93 ± 0.04	0.61 ± 0.05	p < 0.001
MUFA	11.66 ± 0.24	10.68 ± 0.43	NS
C18:2 n-6	20.56 ± 0.23	11.34 ± 0.22	p < 0.001
C20:2 n-6	0.15 ± 0.01	0.24 ± 0.01	p < 0.001
C20:3 n-6	0.25 ± 0.01	0.24 ± 0.01	NS
C20:4 n-6	17.34 ± 0.16	21.30 ± 0.61	p < 0.001
C22:4 n-6	0.69 ± 0.03	1.16 ± 0.03	p < 0.001
C22:5 n-6	0.66 ± 0.03	1.93 ± 0.11	p < 0.001
n-6 PUFA	39.07 ± 0.54	37.16 ± 0.56	p < 0.05
C18:3 n-3	0.16 ± 0.01	0.09 ± 0.01	p < 0.001
C20:5 n-3	0.12 ± 0.01	0.01 ± 0.01	p < 0.001
C22:5 n-3	0.95 ± 0.04	1.20 ± 0.04	p < 0.001
C22:6 n-3	6.73 ± 0.09	7.28 ± 0.13	p < 0.01

n-3 PUFA	7.91 ± 0.09	8.70 ± 0.21	p < 0.01
PUFA	47.29 ± 0.41	46.37 ± 0.78	NS
n-6/n-3	5.03 ± 0.04	4.34 ± 0.07	p < 0.001

858 The number of experiments was 12 per group. The results are expressed in percents of total  
859 fatty acids. LC: Laboratory Chow; WD: Western Diet; SFA : total saturated fatty acids; MUFA:  
860 total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-6/n-3: ratio  
861 between n-6 PUFA and n-3 PUFA; Anova: Analysis of variance; p: probability; NS: not  
862 significant.

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864

865 Table 5 Effects of obesity on the fatty acid composition of atrial phospholipids of human  
 866 individuals

Fatty acid	Lean	Obese	Anova
C14:0	1.37 ± 0.06	1.55 ± 0.40	NS
C15:0	0.26 ± 0.03	0.38 ± 0.03	p < 0.05
C16:0	24.30 ± 0.43	25.74 ± 0.52	NS
C17:0	0.31 ± 0.02	0.31 ± 0.02	NS
C18:0	13.87 ± 1.14	15.02 ± 2.05	NS
C20:0	0.20 ± 0.02	0.24 ± 0.04	NS
SFA	41.25 ± 0.83	43.29 ± 1.82	NS
C15:1	0.51 ± 0.08	0.70 ± 0.13	NS
C16:1 n-7	2.82 ± 0.51	2.56 ± 1.19	NS
C17:1	0.56 ± 0.07	0.53 ± 0.13	NS
C18:1 n-7	2.20 ± 0.16	2.16 ± 0.25	NS
C18:1 n-9	15.37 ± 1.28	16.75 ± 3.85	NS
C20:1 n-9	0.25 ± 0.05	0.24 ± 0.03	NS
C22:1 n-11	1.49 ± 0.22	3.38 ± 0.65	p < 0.05
MUFA	23.41 ± 1.62	26.43 ± 4.61	NS
C18:2 n-6	14.44 ± 1.15	12.37 ± 0.58	NS
C20:2 n-6	0.19 ± 0.02	0.12 ± 0.01	NS
C20:3 n-6	0.75 ± 0.07	0.79 ± 0.08	NS
C20:4 n-6	11.94 ± 1.48	11.37 ± 1.54	NS
C22:4 n-6	0.62 ± 0.04	0.61 ± 0.07	NS
n-6 PUFA	30.23 ± 1.14	25.49 ± 2.16	NS
C18:3 n-3	0.33 ± 0.06	0.21 ± 0.04	NS
C20:5 n-3	0.35 ± 0.05	0.49 ± 0.22	NS
C22:5 n-3	0.70 ± 0.07	0.58 ± 0.07	NS
C22:6 n-3	2.15 ± 0.11	2.24 ± 0.59	NS
n-3 PUFA	3.60 ± 0.33	3.62 ± 0.82	NS

PUFA	$34.07 \pm 1.27$	$29.11 \pm 2.78$	NS
n-6/n-3	$7.93 \pm 0.40$	$8.08 \pm 1.54$	NS

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867

868 The number of experiments was 5 per group. The results are expressed in percents of total fatty  
869 acids. SFA : total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total  
870 polyunsaturated fatty acids; n-6/n-3: ratio between n-6 PUFA and n-3 PUFA; Anova: Analysis  
871 of variance; p: probability; NS: not significant.

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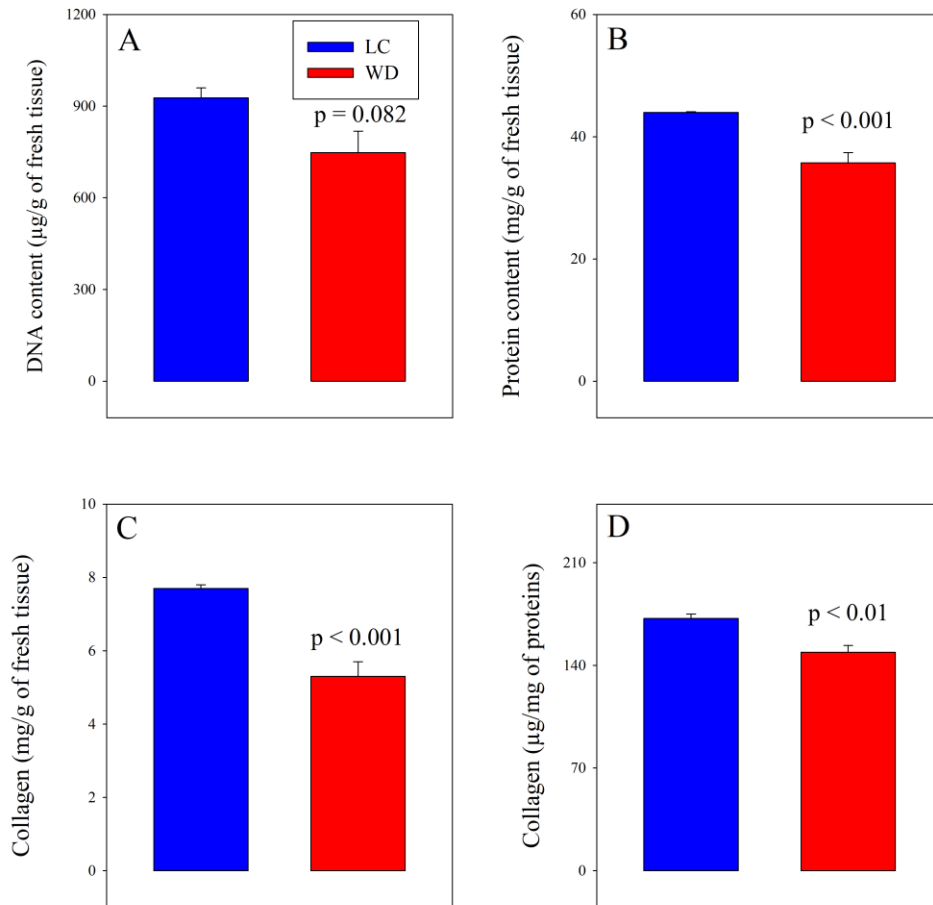
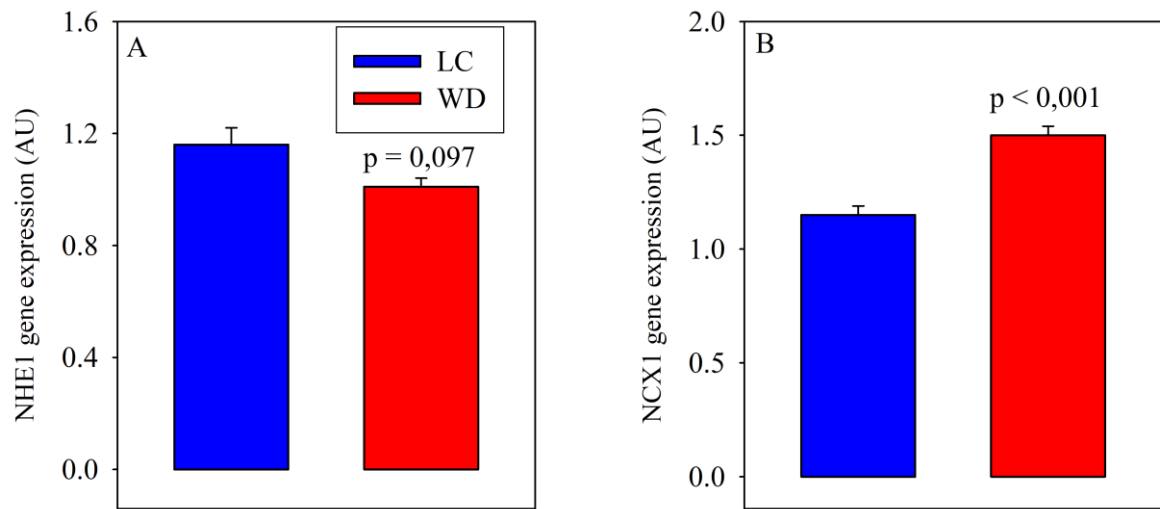


Figure 1

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Figure 2

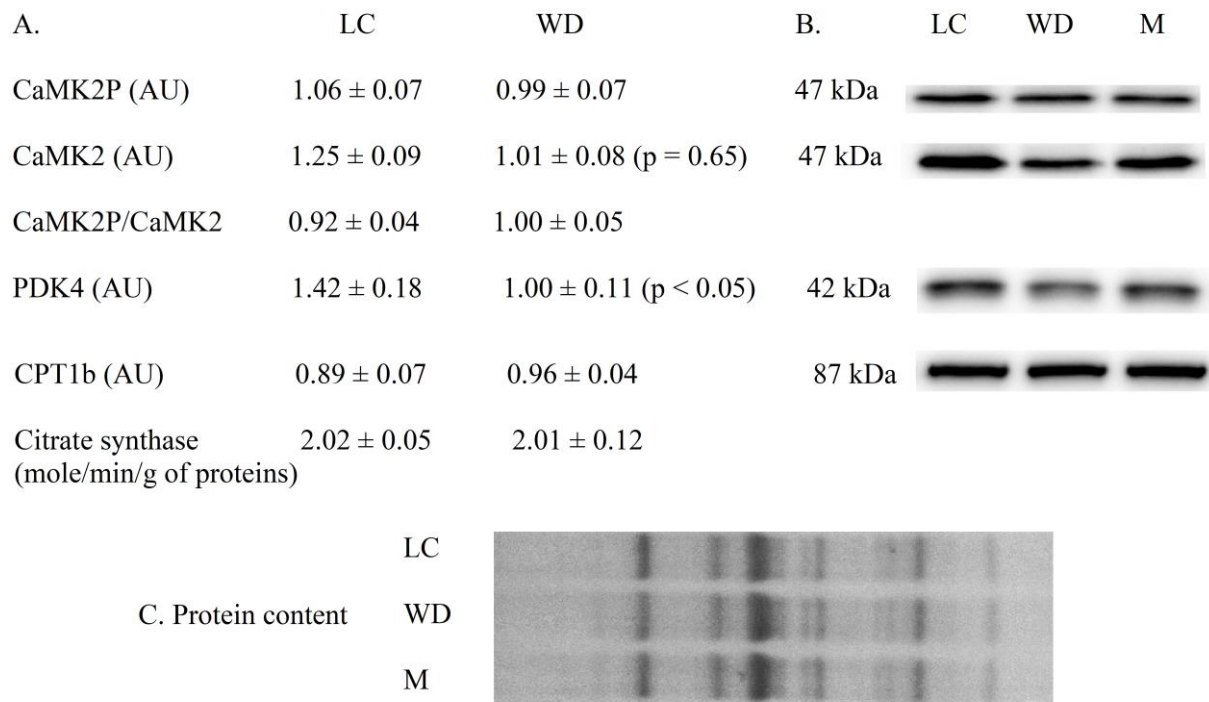


Figure 3

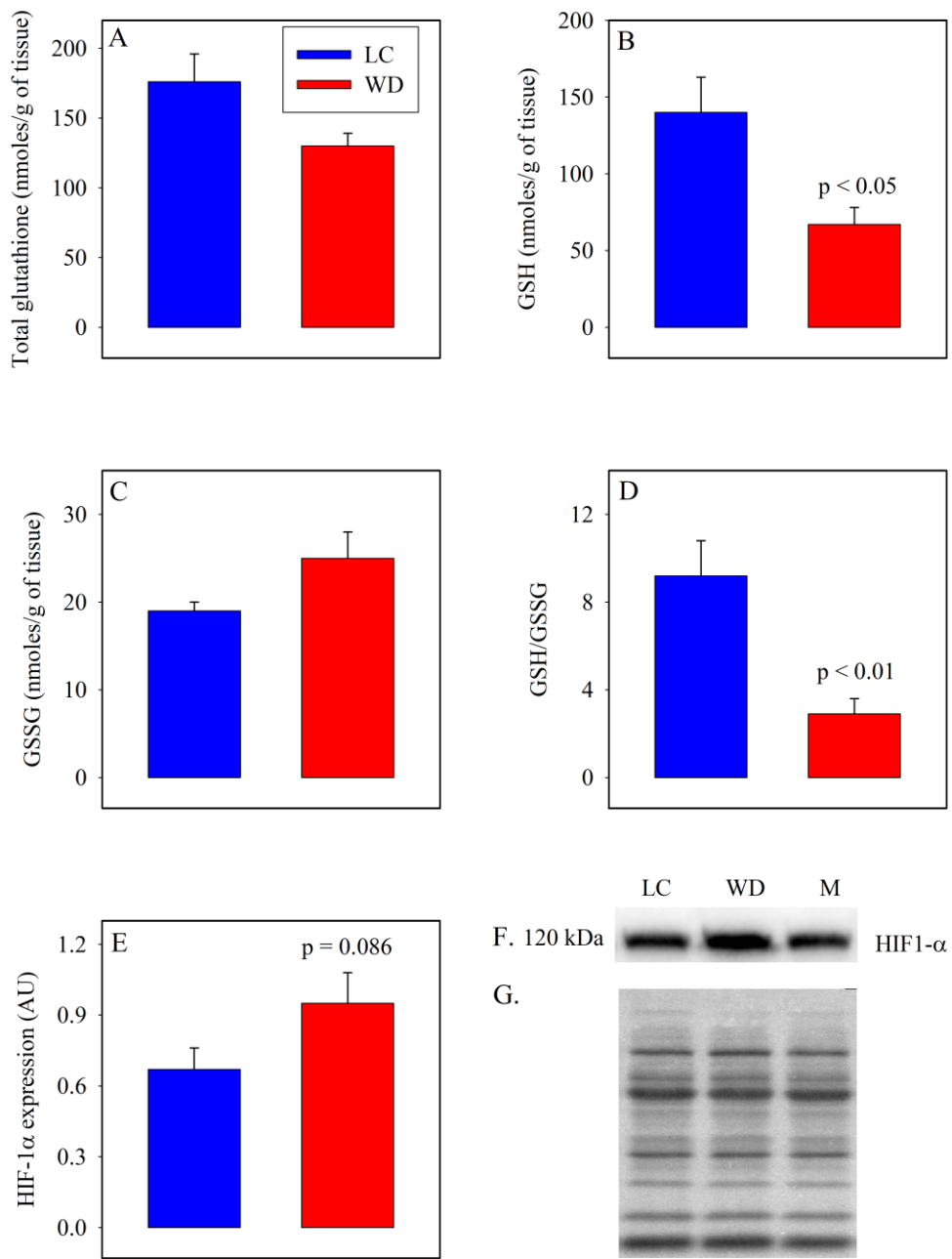


Figure 4

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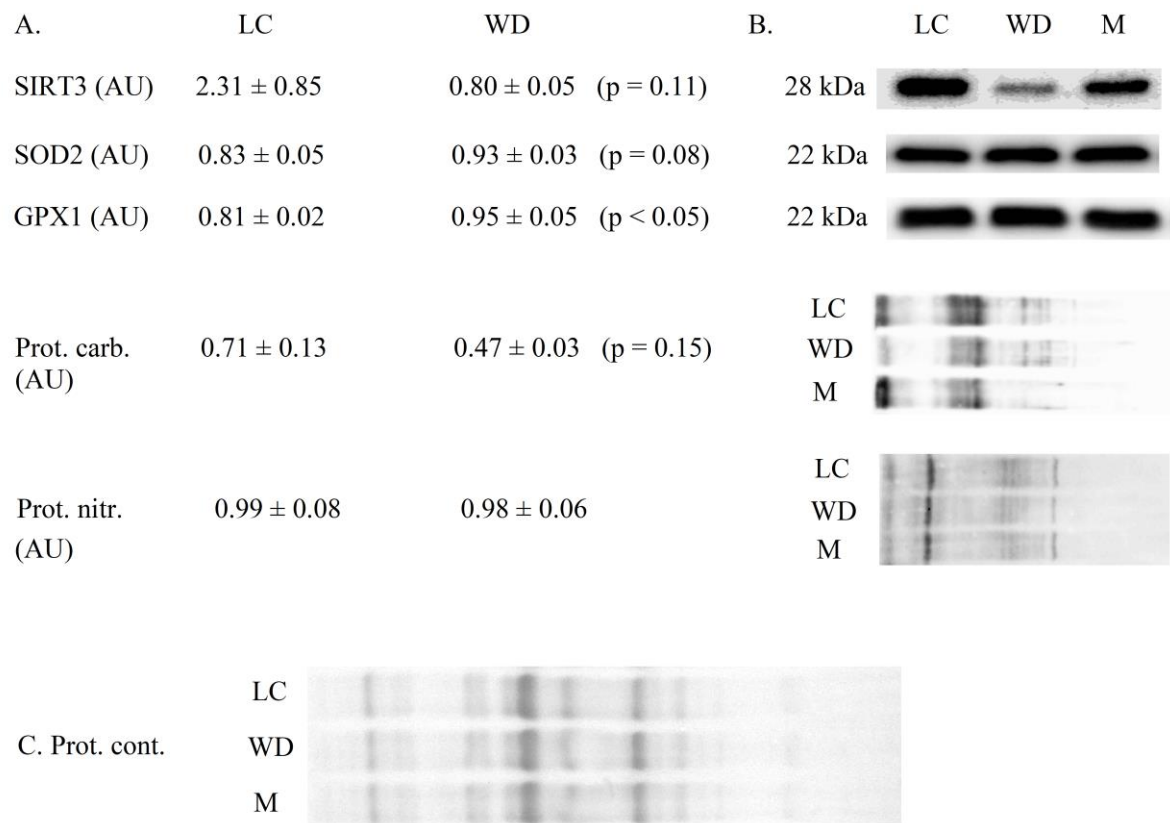


Figure 5

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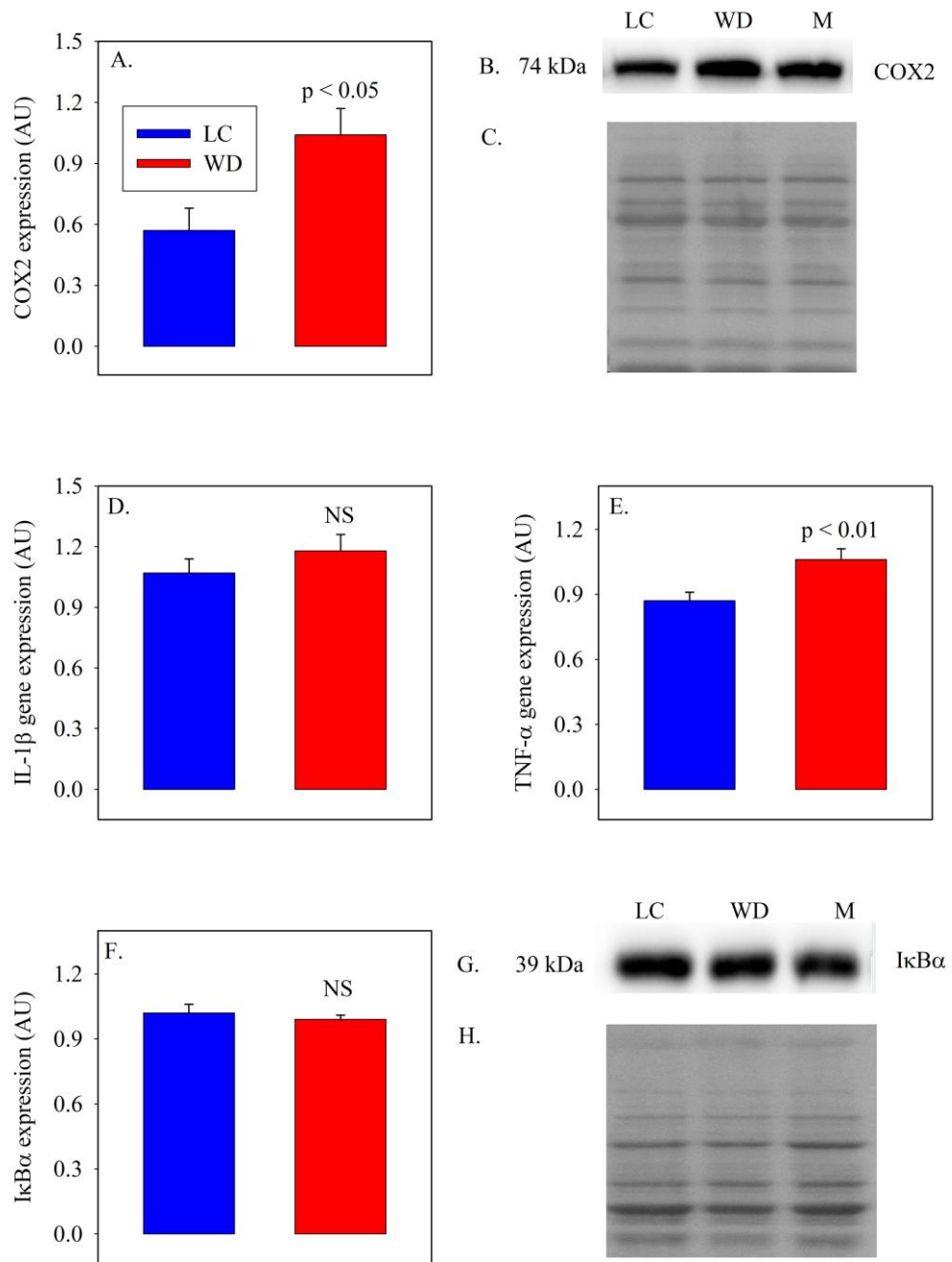


Figure 6