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The impact of obesity in the cardiac lipidomic and its consequences in the cardiac damage observed in obese rats

El impacto de la obesidad sobre el lipidoma cardiaco y sus consecuencias en el daño cardiaco en ratas obesas.

Abstract

Aims

To explore the impact of obesity on cardiac lipid profile in rats with diet-induced obesity. In addition, we evaluate whether or not the specific changes in lipid species are associated with cardiac fibrosis.

Methods

Male Wistar rats were fed either a high-fat diet (HFD, 33.5% fat) or standard diet (3.5% fat) for 6 weeks. Cardiac lipidomic analysis was performed by liquid chromatography tandem mass spectroscopy.

Results

HFD rats showed cardiac fibrosis and enhanced levels of cardiac superoxide anion (O_2^-), HOMA index, adiposity and plasma leptin and reduction in those of cardiac glucose transporter (GLUT 4) as compared with control animals. Cardiac lipidomic analysis showed a significant increase of triglycerides, especially those enriched with palmitic, stearic and arachidonic acid. An increase in levels of diacylglycerol (DAG) was also observed. No changes in cardiac levels of diacylphosphocholine or even a reduction in total levels of diacylphosphoethanolamine, diacylphosphoinositol and sphingolipids (SM) was observed in HFD as compared with control animals. After adjustment for other variables (oxidative stress, HOMA, cardiac hypertrophy), total levels of DAG were independent predictors of cardiac fibrosis while the levels of total SM were independent predictors of the cardiac levels of GLUT 4.

Conclusions

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These data suggest that obesity exerts an important impact on cardiac lipid composition, although it does not modulate the different species in a similar manner. Nonetheless, these changes are likely to participate in the cardiac damage in the context of obesity since total DAG levels can facilitate the development of cardiac fibrosis and SM levels predict GLUT4 levels.

Key words

Lipidomic, obesity, cardiac remodelling, fibrosis

Resumen

Objetivos

Explorar el impacto de la obesidad sobre el perfil lipídico cardiaco en ratas con obesidad inducida por dieta. Se evaluó, además, si estos cambios se asocian con fibrosis cardiaca.

Métodos

Ratas macho Wistar fueron alimentadas con una dieta con alto contenido en grasa (HFD; 33,5% grasa) o con una dieta estándar (3,5% grasa) durante 6 semanas. El análisis del lipidoma cardiaco se realizó mediante cromatografía líquida en tándem con espectrofotometría de masas.

Resultados

Las ratas HFD presentaron fibrosis cardiaca, estrés oxidativo y un aumento en el índice HOMA, adiposidad y los niveles circulantes de leptina así como una reducción en los niveles cardiacos del transportador de glucosa (GLUT 4) en comparación con las ratas controles. El análisis del lipidoma cardiaco mostró un aumento de los niveles de triglicéridos especialmente los que contenían ácido palmítico, esteárico o araquidónico, un incremento en los de diacilglicerol (DAG) aunque no cambios en los de diacilfosfocolina y una reducción en los de diacilfosfoetanolamina, diacilfosfoinositol o de esfingolípidos (SM) en las ratas HFD en comparación con las control. Después del

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ajuste por otras variables (estrés oxidativo, hipertrofia cardiaca, índice HOMA), los niveles de DAG fueron predictores independientes de fibrosis cardiaca mientras que los de SM fueron de los de niveles de GLUT4.

Conclusiones

La obesidad ejerce un impacto importante sobre el lipidoma cardiaco. Estos cambios parecen participar en el daño cardiaco en el contexto de la obesidad ya que los niveles de DAG podrían facilitar el desarrollo de fibrosis miocárdica y los de SM los de GLUT 4.

Palabras clave

Lipidomica, obesidad, remodelado cardiaco, fibrosis

Introduction

Obesity has become a relevant health problem that is reaching epidemic proportions worldwide¹. Obese individuals show a higher risk of cardiovascular morbidity and mortality, which has been explained through chronic low-grade inflammation, increased oxidative stress and the metabolic alterations associated with obesity which can affect cardiac function². These conditions are linked to excess lipid accumulation not only in adipose tissue but also in non-adipose tissues, including the heart, which occurs when the storage capacity of adipocytes is exceeded³.

Lipids are important regulators of cardiac function, not only as the main energy substrate for cardiac mitochondrial oxidative metabolism but also by their role in membrane phospholipid remodelling, their activity as signalling molecules and ligands for nuclear receptors. However, increased myocardial lipid accumulation elicits an imbalance between cardiomyocyte fatty acid uptake and fatty acid oxidation⁴, which can facilitate the accumulation of cardiotoxic metabolites that can exert deleterious effects on the myocardium⁵. Clinical studies with proton magnetic resonance spectroscopy have demonstrated that increased intramyocardial triglyceride (TG) accumulation occurs before cardiac dysfunction in patients with type 2 diabetes mellitus and correlates with body mass index^{6,7}. These data supporting a link between cardiac lipid accumulation and myocardial dysfunction. Experimental studies⁸⁻¹¹ have shown that the

1 accumulation of some lipid species, including diacylglycerol (DAG), lysophospholipids,
2 acyl carnitines, ceramides and TGs, can affect cardiomyocyte function and lead to
3 cardiac dysfunction. However, the potential mechanisms that link the lipid
4 accumulation with the functional alterations are not well established.
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8 Cardiac fibrosis is an important contributor to heart muscle dysfunction in obesity¹².
9 The excessive extracellular matrix (ECM) deposition arises from the imbalance between
10 ECM synthesis and degradation. An exacerbated deposition of ECM components can
11 cause an aberrant remodelling that favours functional alterations because a reduced
12 relaxing capability of the heart can increase its filling pressure and contribute to
13 diastolic dysfunction. However, whether or not changes in lipid profile associated with
14 obesity can affect cardiac fibrosis is still undetermined. Therefore, we explore the
15 impact of obesity on the heart lipid profile through a lipidomic analysis in rats with diet-
16 induced obesity as compared with controls. In addition, we evaluate whether the
17 specific changes in lipid species could be associated with cardiac fibrosis.
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29 **Methods**

30 **Animals**

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32 Male Wistar rats of 150 g (Harlan Ibérica, Barcelona, Spain) received either a standard
33 diet (3.5% fat; Harlan Teklad no. TD.2014; n=8) or a high-fat diet (HFD, 33.5% fat;
34 Harlan Teklad no. TD.03307, Madison, WI, USA; n=8) for 6 weeks. The Animal Care
35 and Use Committee of Universidad Complutense de Madrid approved all experimental
36 procedures according to the Spanish Policy for Animal Protection RD53/2013, which
37 meets the European Union Directive 2010/63/UE.
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51 Body weight was measured once a week. Food and water intake were determined
52 throughout the experimental period. Blood pressure (SBP) was estimated basally, at
53 mid-study and end-of-study through the use of a tail-cuff plethysmograph (Narco Bio-
54 Systems, Houston, TX, USA) in unrestrained animals. Serum and plasma were
55 collected, fat pads were weighed, and heart was dissected for further analysis at the end
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1 of the experimental period. Adiposity index was calculated as sum of fat pad
2 weight/(body weight-fat pad weight) x100).
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4 **Evaluation of cardiac structure and function**

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7 Cardiac structure and function were evaluated by transthoracic echocardiography with a
8 Philips CX50 (Philips, Netherlands) connected to a L12-3 MHz linear transducer in rats
9 anesthetized with isoflurane (2%; Esteve, Barcelona, Spain).
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13 Measurements of left ventricular (LV) end-diastolic diameter, end- systolic diameter,
14 interventricular septum (IVT) and posterior wall thickness (PWT) as well as
15 calculations of left ventricular ejection fraction (EF) and LV systolic chamber function
16 (pump function) were described elsewhere ¹³.
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22 **Morphological and histological evaluation**

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25 Cardiac tissue samples were dehydrated, embedded in paraffin and cut into 4µm-thick
26 sections. Sections were stained with picrosiriusred in in order to detect collagen fibers
27 and viewed with polarized light under dark-field optics to detect the birefringence of
28 collagen fibers. The area of cardiac interstitial fibrosis was identified as the ratio of
29 interstitial fibrosis or collagen deposition to the total tissue area after excluding the
30 vessel area from the region of interest. For each sample, 10 to 15 fields were analyzed
31 with a 40X objective under transmitted light microscopy (Leica DM 2000; Leica AG,
32 Germany). Myocytes (60–80 per animal) with visible nucleus and intact cellular
33 membranes were chosen for determination of cross-sectional area in cardiac sections
34 stained with hematoxylin and eosin.
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45 **Western blotting**

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48 Cardiac tissue lysates were separated by SDS-PAGE and transferred to 0.2µM
49 nitrocellulose membranes (Bio-Rad Laboratories, Germany) Blots were incubated with
50 antibodies against glucose transporter4 (GLUT4) (Santa Cruz Biotechnology Inc,
51 Heidelberg, Germany). Bound antibodies were detected after incubation with an HRP-
52 conjugated IgG and using the Super Signal West Dura Extended Duration Substrate
53 (Thermo Fisher Scientific Inc, Waltham, MA, USA). Equal loading of protein in each
54 lane was verified by β-actin (Sigma).
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Detection of superoxide anion production

The oxidative fluorescent dye dihydroethidium (DHE; Invitrogen, Grand Island, NY, USA) was used to evaluate superoxide anion ($O_2^{\cdot-}$) production. Cardiac and aorta tissue samples were embedded in tissue-freezing medium. 14- μ m thick sections were then cut with a cryostat, placed onto glass microscope slides and washed briefly in Krebs-HEPES buffer (in mmol/L: NaCl 130, KCl 5.6, $CaCl_2$ 2, $MgCl_2$ 0.24, HEPES 8.3, glucose 11, pH 7.4). Slides were then incubated with DHE (5×10^{-3} mmol/L) for 30 min at 37°C in a light-protected humidified chamber. Slides were subsequently washed with warm phosphate-buffered. Cardiac images were viewed by fluorescent laser scanning microscope (40X objective in a Leica DMI 3000 microscope) (Ex561 nm and Em610 nm) using the same imaging settings in each case. Three separate histological sections and four different fields in each section per animal were quantified and averaged for each experimental condition. The mean fluorescence densities in the target region were calculated. Results are expressed as an n-fold increase over the values of the control group.

Lipidomic analysis

Myocardial lipids were extracted and analyzed by ultrahigh performance liquid chromatography coupled to time-of-flight mass spectroscopy (UPLC-QToF-MS) using an Acquity UPLC System and a SYNAPT HDMS G2 (Waters, Manchester, UK) with electrospray ionization. Extraction of lipids was carried out from cardiac homogenates in methanol:chloroform mixture (1:2, v/v) and split into two aliquots. One aliquot was evaporated to dryness and the pellet re-suspended in acetone:2-propanol:ethanol (3:4:3, v/v/v) and used for TGs measurement. The other aliquot was evaporated to dryness and the pellet re-suspended in methanol:water (9:1, v/v/v) and used for phospholipids (PPLs) measurement. Extracts were kept at -80°C until analysis. Mass spectrometric analysis of TGs was performed in positive mode (ESI+) using the parameters that follow: capillary, 0.8 kV; sampling cone, 15 V; source temperature, 90 °C; desolvation temperature, 280 °C; cone gas, 40 L/h; and desolvation gas, 700 L/h. Data were acquired with the software MassLynx at a rate of 5 scans/s within the range 0-18 min, and m/z 100-1200 Da for the low-energy function and m/z 100-900 Da for the high-energy function (MS^E method, trap collision energy 30 V). LC and MS methods were optimized using the commercial standards TG (18:2/18:2/18:2) and TG

1 (16:0/16:0/16:0). These standards were also used to draw calibration curves for
2 quantification. Mass spectrometric analysis of PPLs was fitted as follows: capillary, 0.9
3 kV; sampling cone, 18 V; source temperature, 90 °C; desolvation temperature, 320 °C;
4 cone gas, 45 L/h; and desolvation gas, 900 L/h. Data were acquired with the software
5 MassLynx at a rate of 5 scans/s within the range 0-12 min and 100-1200 Da m/z for the
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7 low-energy function, and 50-900 Da m/z for the high-energy function (MS^E method,
8 trap collision energy 30 V), with ionization in positive mode (ESI+) for detection of
9 diacylphosphatidylcholines (PCs), ceramides (Cer) and sphingomyelins (SM), and with
10 ionization in negative mode (ESI-) for detection of other phospholipids, which were
11 diacylphosphatidylethanolamine (PE), diacylphosphatidylinositol (PI),
12 diacylphosphatidylglycerol (PG), and phosphatidic acids (PA). External commercial
13 standards, namely PI (8:0/8:0), PG (14:0/14:0), PE (12:0/12:0), PC (10:0/10:0) and PA
14 (14:0/14:0) were purchased from Cayman Chemical (Michigan, USA) and used for
15 method optimization and quantification.
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17 Up to three different chromatograms were manually checked for mass spectral peak
18 identification where possible. Within each chromatographic point, m/z values with an
19 intensity ≥ 700 were also checked for it in order to afford a defined chromatographic
20 peak (Extracted Ion Chromatogram, EIC); if positive, the elemental composition tool
21 was then used to determine all the possible chemical compositions ($C_nH_mO_pN_sP_rS_t$) that
22 were compatible with the isotopic distribution (M, M+1, M+2 and M+3 peaks) of a
23 given m/z value. Using LipidMaps, Metlin, CheBI, LipidBank and KEGG databases, a
24 certain elemental composition was examined for possible known compounds. Where
25 possible, acyl chains were identified by data from the high-energy function
26 (fragmentation). As well, specific fragments in the high energy function (MS^E) were
27 considered for identification, in particular m/z 184.07 for PCs and SMs in positive
28 ionization mode.
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30 **Statistical Analysis**

31 Data are expressed as mean \pm SEM. Normality of distributions was verified by means of
32 the Kolmogorov-Smirnov test. Data were analyzed using an unpaired Student's t-test to
33 assess specific differences among groups using GraphPad Software Inc. (San Diego,
34 CA, USA). Pearson correlation analysis was used to examine association among
35 different variables. To find the factors associated with cardiac fibrosis or protein levels
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1 of GLUT4, the β -correlation coefficients (slope or mean difference, along with their
2 95% CIs) were obtained using a linear regression model. The predetermined
3 significance level was $P < 0.05$.
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6 **Results**

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9 Experimental animals showed a progressive increase in body weight that was larger in
10 the animals fed a HFD than in those fed a control diet. A similar increase was observed
11 in adiposity index (Table 1). Our data demonstrated a significant increase in relative
12 heart weight in obese animals as compared with the control group (Table 1). The HFD
13 group showed also an increase in HOMA index (three-fold; Table 1), which suggests
14 insulin resistance. The echocardiographic values for both structural features and left
15 ventricular systolic function were similar in both groups (Table 1). No differences were
16 found in systolic blood pressure at the end of the experiment between both groups
17 (Table 1).
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27 Diet-induced obese animals showed an increase (2.5 fold) in cardiac interstitial fibrosis
28 in comparison to controls as demonstrated by the higher collagen volume fraction in the
29 HFD group (Figures 1A-1B). This increase was mainly due to the cross-linked collagen,
30 which increased 2.9 fold as compared with control group (Figures 1C-1D). No
31 differences were observed in cardiac myocyte cross-sectional area among any of the
32 groups (data not shown).
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39 The cardiovascular levels of O_2^- in obese animals were higher than those observed in
40 control animals as suggested by the higher fluorescence intensity in myocardial tissue
41 sections incubated with DHE (Figures 1E-1F). In agreement with previously reported
42 data in clinical and experimental studies, obese animals presented higher plasma leptin
43 levels as compared to normoweight rats (Table 1). In fact, the cardiac protein levels of
44 GLUT4 were reduced in HFD as compared to control rats (Figure 2).
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51 HFD rats showed an increase (16%) in the cardiac lipid content as compared with
52 control animals, although it did not reach statistical significance. The cardiac lipidomic
53 analysis detected overall 205 individual lipid subspecies over 12 different classes
54 encompassing, among others, TG, DAG, SM, CER and PPLs. The analysis showed a
55 relevant alteration in the cardiac lipid profile between HFD and control rats with
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1 changes identified in more than 50% of all species, although all lipid components are
2 not affected in the same manner. The main lipid component was the PPLs, with PC and
3 PE being the most abundant. Although no significant differences were observed
4 between total levels of PC between both groups (Figure 3A), a significant increase was
5 observed in those species with 20:4 acyl chain, ($p < 0.01$; Figure 3A). By contrast, total
6 cardiac PE levels were reduced (Figure 3; $p < 0.05$), a fact mainly due to the reduction of
7 the levels of PE containing palmitic acid ($p < 0.05$), PE/PC ratio was consequently lower
8 in HFD rats than in controls (2.3 ± 0.04 vs 2.7 ± 0.1 ; $p < 0.05$; respectively). In addition, an
9 increase was observed in lysoPE levels in HFD as compared with control animals. This
10 increase was also observed in total lysoPC (LPC) species in obese rats versus
11 normoweight animals ($p < 0.01$). This increase was a consequence of the rise ($p < 0.01$)
12 observed in those animals of the major component, the LPC (20:4) (Figure 3A). Both PI
13 and diacylphosphatidylserine (PS) species were also detected although at lower levels.
14 However, the impact of the HFD was different in PI and PS: no changes in total levels
15 of PS but a reduction ($p < 0.05$; Figure 3) in total PI levels, the reduction in PI being
16 mainly a consequence of the drop in PI (18:0/24:0), which was one of the most
17 abundant PI species detected in this study (Figure 3A).

18 Total TG levels were increased ($p < 0.01$; Figure 3A) in HFD rats as compared with
19 control animals by 2.7-fold. This increase was mainly due to those TG containing
20 palmitic or stearic acids (Figures 3B-3C). In fact, 78% and 100%, respectively, of the
21 detected TG species of each type were elevated, with the TG 50:1 (16:0/16:0/18:1), 52:1
22 (16:0/18:0/18:1) and 52:2 (16:0/18:1/18:1) being the most abundant species (data not
23 shown). The overall TG analysis showed that, as compared with control animals, the
24 levels of those containing only saturated fatty acids were higher (3.8-fold increase) in
25 HFD without significant changes in the levels of species containing only
26 polyunsaturated or monounsaturated fatty acids. HFD rats also exhibited an increase of
27 7.6 fold in cardiac TG arachidonic acid (20:4) as acyl chain ($p < 0.01$; Figure 3A).

28 Four species of DAG were detected in HFD group but only two of them were found in
29 the heart of the control group (DAG 38:6; DG 42:5), which were the most abundant
30 DAG species. Animals fed a HFD showed an increase in total DAG levels (0.85 folds;
31 $p < 0.05$; Figure 3A), since 3 (DAG 50:0; DAG 52:1; DAG 42:5) of the 4 species
32 detected were significantly elevated in HFD as compared with animals fed a control

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diet. In fact, the DAG containing C20:4 increased 0.6-fold ($p<0.05$; Figure 3A) in HFD group as compared with control group. SM represented only around 5% of the cardiac lipids in control animals, and this content dropped to 1.4% in HFD animals ($p<0.01$) because of the drastic decrease ($p<0.001$) observed in the main SM specie detected in this study(SM (d18:1/16:0). However, no reduction was observed in the levels of those SM with 20:4 (Figure 3A). No differences were observed in levels of either sphingosine-1-phosphate (data not shown), carnitine or in the two detected species of ceramide between both groups (Figure 3A). As shown in table 2, adiposity index was correlated with levels of leptin, HOMA index, total lipids, arachidonic acid containing TGs and LPC levels. A negative correlation was found between adiposity and total levels of SM and PI. HOMA index was positively correlated with leptin levels and C20:4-containing TG content but a negative correlation was found of HOMA index to levels of PI and PE (table 2).

In order to examine the relationship between cardiac lipid profile changes and cardiac fibrosis or cardiac levels of GLUT4, a linear regression analysis was performed. After adjustment for other variables which could affect cardiac fibrosis (HOMA index, ROS, and cardiac hypertrophy), it was found that total level of DAG could be considered an independent predictor of cardiac fibrosis (odds ratio 0.602; 95% CI, 0.012-0.683; $p=0.04$) whereas the level of total SM was shown to be an independent predictor of the cardiac level of GLUT 4 (odds ratio 28.18; 95% CI, 14.994-41.37; $p=0.001$)

Discussion

Cardiac lipotoxicity has been associated with cardiac functional alterations in the context of obesity, this fact suggesting that accumulation of lipids may exert a toxic effect on the myocardium^{4,14}. Even though cardiac lipotoxicity has been identified to accompany increases in TG levels, it is likely that the content and composition of other different lipid species are also altered and this imbalance can contribute to the cardiac damage associated with obesity, as well. In this study, we report a significant lipidomic remodelling in the heart of rats with diet-induced obesity with regard to normally fed rats, the remodelling involving more than 50% of the lipid species detected in the heart. These changes, although affecting cardiac TG to a significant degree, also modify other species, including DAGs, SMs, PIs and LPCs, and this feature suggests that obesity has

1 an effect on lipid content in the heart more than the one which had been foreseen. These
2 changes seem to be relevant because of the cardiac consequences of obesity since total
3 DAG content seems to be a determinant factor for cardiac fibrosis, as does SM levels
4 for the cardiac levels of GLUT 4; this suggests its potential role in the cardiac metabolic
5 alterations in the context of obesity.
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10 The data show that the increase in body weight observed in the model of diet-induced
11 obesity (HFD animals) was associated with an important impact on cardiac lipid
12 composition, with about 59% of the detected lipid species exhibiting a modified
13 content. However, the change trends were species-specific, which resulted in only a
14 slight increase in total lipids (16%) in HFD rats as compared with controls because of
15 the increase in some species counteracted the decrease in other species. TGs were the
16 main lipid class affected in regard to their content and profile, especially those
17 containing stearic and palmitic acid were shown to have levels increased up to 8- and
18 4.5-fold, respectively, in HFD as compared with controls; this fact closely reflects the
19 fatty acid composition of the diet. Indeed, significant correlations were found between
20 adiposity, it being measured by the index of obesity, and levels of either total TG or
21 those enriched with arachidonic and palmitic acids, such correlations supporting a close
22 link between excess caloric intake and TG accumulation in the myocardium. These data
23 are in agreement with those reported in previous studies, which demonstrated that
24 cardiac or circulating TG levels were elevated in both obese patients and models of diet-
25 induced obesity^{3,15-18}. More importantly, the changes observed in TG species in obese
26 animals indicate that it is due to a profile more prone to cardiovascular complications.
27 An imbalance due to either increased lipid uptake or decreased lipid oxidation has been
28 proposed as the main cause underlying lipid accumulation. This idea is supported by
29 studies in genetically modified mice, which affect a variety of components involved in
30 lipid transport, storage, and metabolism¹⁹⁻²³. An enhanced lipid uptake has been
31 suggested as a determinat factor involved in cardiac lipotoxicity in clinical studies in
32 obese patients²⁴. In addition, it has been suggested that other lipid species could be
33 facilitating heart TG accumulation. In this regard, Lim and Bodmer have shown that a
34 reduction in PE levels through the modulation of the activity of the sterol regulatory
35 element binding protein could facilitate lipogenesis in the heart of the model of easily-
36 shocked *Drosophila*²⁵. Supporting this concept is the fact that our data showed a
37 negative correlation between cardiac TG levels and those of PE (data not shown).
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2 Although different mechanisms have been bound to TG accumulation and insulin
3 resistance²⁶⁻²⁸, some studies have suggested that TG are metabolically inactive since
4 accumulation of TG has been observed in the muscle of insulin-sensitive women and
5 athletes²⁹. Similarly, plasma TG is not associated with insulin resistance in overweight
6 and obese patients³⁰. However, our data did not allow us to reach any conclusion
7 regarding this feature because it is difficult to consider that changes in cardiac lipid
8 species can allow for it to underlie the observed systemic insulin resistance. However,
9 our data show that total SM levels, and especially the levels of SM(18:1/16:0), were
10 independent predictors of GLUT4 cardiac levels, supporting previous data that suggest
11 a positive role for SMs in insulin sensitivity in patients³¹. Similarly, it has been shown
12 that dietary SM improves metabolic complications associated with diet-induced obesity
13 in mice³².

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25 Cardiac interstitial fibrosis is a common feature in the context of obesity, which
26 contributes to the pathogenesis of diastolic dysfunction^{12,33}. As previously
27 reported^{17,18,34}, HFD animals show an increase in interstitial fibrosis, although no
28 functional changes in cardiac function were observed, probably due to the relatively
29 short-time of evolution of obesity. The increase in interstitial fibrosis is mainly due to
30 crosslinking collagen that is less prone to degradation. We have recently reported that
31 the administration of an inhibitor of the activity of lysyl oxidase that catalyses the
32 covalent cross-link of collagen and elastin fibers³⁵ reduced cardiac fibrosis in rats with
33 diet-induced obesity³⁴. A variety of factors has been involved in the development of
34 cardiac fibrosis, including adipokines such as leptin. In fact, this role involved the
35 activation of oxidative stress, engaging downstream events, which mediate the
36 activation of PI3K/Akt pathway and, consequently, the production of end effectors.
37 Such effectors include TGF- β , CTGF and galectin-3, which are mainly responsible for
38 the final synthesis of ECM in cardiac myofibroblasts, the main factor responsible for
39 fibrosis^{36,37}. Cardiac fibrosis can cause an aberrant remodelling that favours functional
40 alterations, since a reduced relaxing capability of the heart can increase its filling
41 pressure and contribute to diastolic dysfunction. The general concept that lipotoxicity
42 can participate in cardiac fibrosis and functional alterations is widely accepted^{19,38-42}.
43 However, the potential mechanism involved is not well established. The present data
44 further extend this concept because it shows that the levels of DAG can predict those of

1 cardiac interstitial fibrosis in rats. In agreement with this concept, it has been reported
2 that the cardiac overexpression of cardiac-specific diacylglycerol kinase, an enzyme that
3 negatively controls the cellular levels of DAG, reduces cardiac fibrosis and improved
4 ventricular remodelling in mice with myocardial infarction, diabetes or aortic
5 constriction⁴³⁻⁴⁵.
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10 The potential underlying mechanisms are not well established but different data have
11 linked DAG to cardiac lipotoxicity^{10,46,47}. DAGs are intracellular second messengers
12 capable of increasing the activity of protein kinase C (PKC), which promotes cardiac
13 fibrosis and heart failure⁴⁸⁻⁵¹ through the activation of galectin-3 and oxidative
14 stress^{48,51}. Therefore, activation of these factor which we have previously reported can
15 participate in the cardiac fibrosis observed in rats fed a HFD¹⁸ could be a potential
16 mediator through which DAG can participate in the cardiac fibrosis in the context of
17 obesity. Interestingly, changes in the DAG fatty acid composition result in translocation
18 and activation of distinct PKC isoenzymes^{52,53}. Thus, it has been shown that 20:4 ω -6
19 enriched DAG are more efficient than those with 20:5 ω -3 or 22:6 ω -3 in activating
20 PKC δ , PKC ϵ and PKC α . On the contrary, activation of PKC β I by DAG-containing
21 arachidonic acid was significantly lower than that induced by the DAGs containing ω -3
22 PUFAs. Given that PKC isozymes play different roles, even opposites, in specific
23 aspects of cardiac remodelling in HFD, the differential activation of PKC isoforms
24 possibly leads to different pathophysiological effects. This phenomenon may be
25 implicated in the influence of ω -3 or ω -6 polyunsaturated fatty acids in health and
26 disease. Thus, our findings that DAG containing 20:4 ω -6 is increased in HFD group
27 might be linked to an activation of PKC isotypes such as PKC δ , PKC ϵ and PKC α . In
28 agreement, it has been reported that these isoforms can mediate cardiac fibroblast
29 proliferation and collagen production^{54,55}, and activation of PKC δ has been associated to
30 increased cardiac damage⁵⁶. Moreover, it has been reported that the presence of cardiac
31 DAG enriched with ω -3 is associated with a reduction of PKC ϵ and PKC α
32 translocation⁵³.
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54 Finally, it is worth mentioning that other observed changes could be relevant to
55 different aspects of the cardiac function that were not evaluated in the study, such as the
56 reduction in the ratio PE/PC. The levels of PC and PE are key regulators of the
57 membrane integrity as well as of its biophysical properties, such as curvature and
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1 rigidity, which influence protein functions. Thus, it has been recently reported that the
2 activity of GLUT4 is controlled by the membrane PPLs composition⁵⁷. The lipid
3 components of the membrane can impact not only cell function but also mitochondrial
4 function, since PE and PC are the predominant PPLS in mitochondrial membrane⁵⁸.
5 Reductions in PI levels, which play important roles in lipid signaling, cell signaling and
6 membrane trafficking, could also be relevant.
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12 In summary, these data suggest that obesity exerts an important impact on cardiac lipid
13 composition, although it does not modulate the different species in a similar manner.
14 While an increase in TG and DAG-species was observed, PPLs, the main lipid
15 component in the heart either does not change (PC, PS) or decrease (PE, PI). These
16 changes can participate in the cardiac damage in the context of obesity. Specifically,
17 DAG can facilitate the development of cardiac fibrosis and SM levels can modulate
18 cardiac GLUT4 levels and, in consequence, the decrease in SM observed in HFD rats
19 could facilitate the changes in the metabolic substrate use for myocardium that occurs in
20 obesity. Although further work is warranted to better understand the entire spectrum of
21 the cardiac functional consequences of the alterations on the lipid profile, the data
22 provide an understanding of these changes and an insight into their underlying
23 complexity.
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Table 1. Body weight, adiposity, relative heart weight, leptin levels, HOMA index, echocardiographic parameters and systolic blood pressure in control rats and rats fed a high fat diet (HFD).

	CT	HFD
Body Weight (g)	329.2±7.5	371.2±3.9***
Adiposity index	2.7±0.29	6.6±0.62***
HW/TL (mg/cm tibia)	205.1±7.6	243.2±8.4**
Leptin levels (ng/ml)	12.5±1.8	82.4±25.1*
HOMA Index	2.4±0.27	6.7±1.59*
IVT (mm)	1.73±0.14	1.79±0.2
PWT(mm)	1.81±0.2	1.74±0.2
EDD (mm)	5.2±0.43	4.9±0.4
ESD (mm)	2.6±0.25	2.8±0.24
EF (%)	85.3±3.5	84.8±3.2
FS (%)	46.1±0.4	46.9±2.9
SBP (mmHg)	122.9±3.4	124.8±3.3

HW: heart weight; TL: tibia length; HOMA index: the homeostasis model assessment; IVT: interventricular septum thickness; PWT: posterior wall thickness;

EDD: end-diastolic diameter; ESD: end-systolic diameter; EF: ejection fraction; FS:
fractional shortening; SBP: systolic blood pressure. Data values represent mean \pm S.E.M
of 6 animals. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. control group.

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Table 2. Associations found between adiposity index and HOMA Index and leptin plasma levels and cardiac lipid levels in control rats and rats fed a high fat diet (HFD).

	<i>r</i>	P value
Adiposity		
HOMA Index	0.706	0.023
Leptin	0.805	0.005
Total Lipids	0.808	0.005
TG 20:4	0.801	0.005
LPC	0.832	0.003
SM	-0.691	0.027
Total PI	-0.716	0.02
HOMA Index		
Leptin	0.891	0.001
TG 20:4	0.694	0.026
PE	-0.847	0.008
PI	-0.825	0.003

HOMA index: the homeostasis model assessment; TG: Triglycerides; SM: sphingolipid; PE: phosphoethanolamine; PI: phosphoinositol

Figure legends

1
2 **Figure 1. Consequences of a high fat diet in the heart of rats.** (A): Collagen volume
3 fraction (CVF) and (B) Representative microphotographs of myocardial sections
4 staining with picosirius red examined by light microscopy (magnification 40X) in heart
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6 from control rats (CT) and rats fed a high-fat diet (HFD). (C) Percentage of cross-linked
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8 collagen and (D) Representative microphotographs of myocardial sections staining with
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10 picosirius red examined by polarized light microscopy (magnification 40X) in heart
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12 from control rats (CT) and rats fed a high-fat diet (HFD). (E) Quantification of
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14 superoxide anions production in hearts from control rats (CT) and rats fed a high-fat
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16 diet (HFD) and (F) representative microphotographs of myocardial sections labeled
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18 with the oxidative dye hydroethidine by fluorescence microscopy (magnification 40X).
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20 Scale bar: 50 μ m. Values are mean \pm SEM of 8 animals. * p <0.05; *** p <0.001 vs control.
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29 **Figure 2. Consequences of a high fat diet in the cardiac levels of GLUT 4 in rats.**
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31 Protein levels of GLUT4. Quantification of band intensities was measured by
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33 densitometry and normalized to respective α -tubulin. Values are mean \pm SEM of 8
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35 animals. * p <0.05 vs control. * p <0.05; *** p <0.001 vs control.
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40 **Figure 3. Consequences of a high fat diet in the cardiac lipidome of rats.** (A)
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42 Changes in lipid levels in heart from control rats (CT) and rats fed a high-fat diet
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44 (HFD). (B) Levels of triglycerides enriched with palmitic acid (16:0). (C) Levels of
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46 triglycerides enriched with stearic acid (18:0); and (D) Levels of triglycerides enriched
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48 with arachidonic acid (20:4) in heart from control rats (CT) and rats fed a high-fat diet
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50 (HFD). Values are mean \pm SEM of 6 animals. ** p <0.01 vs control.
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Figure 1

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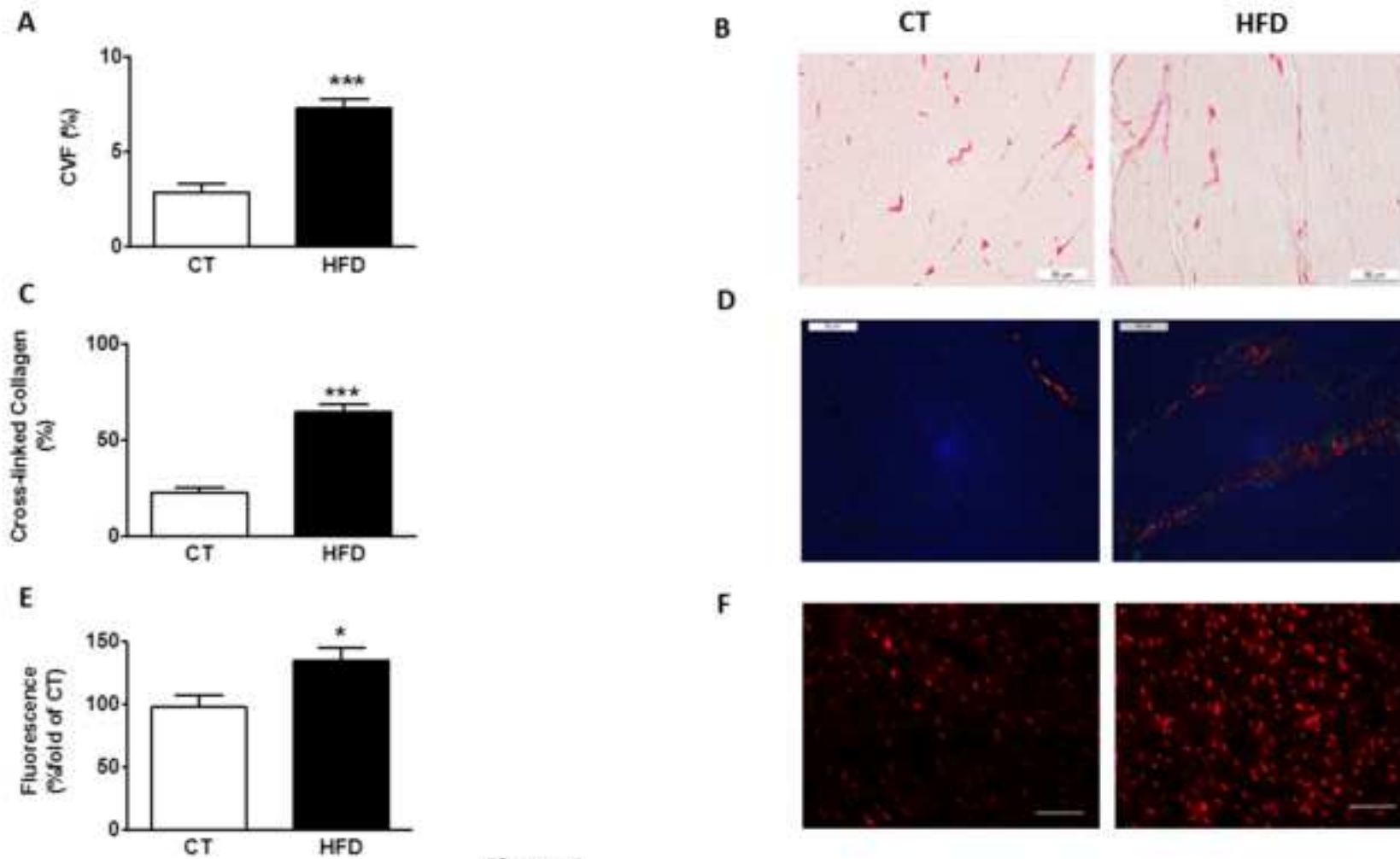


Figure 1

Figure2

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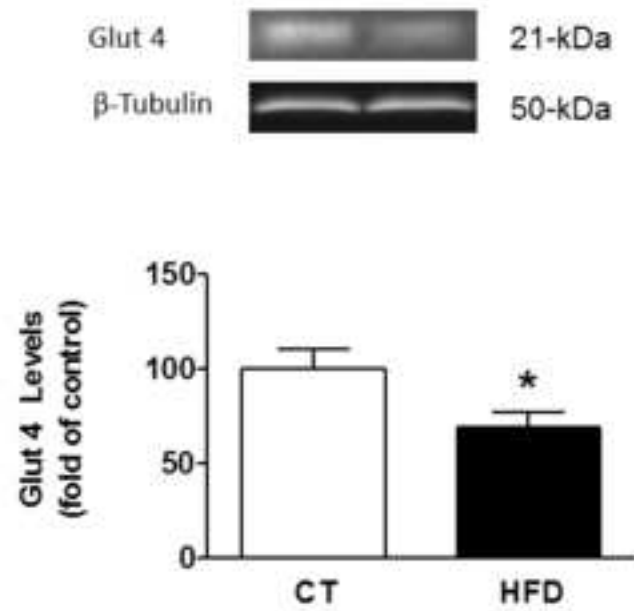


Figure 2

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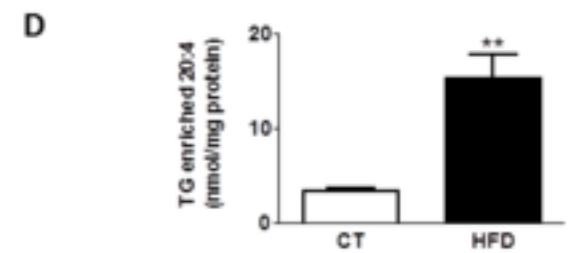
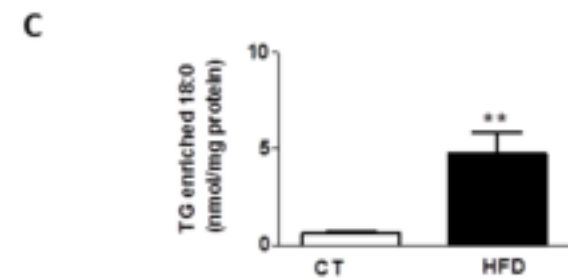
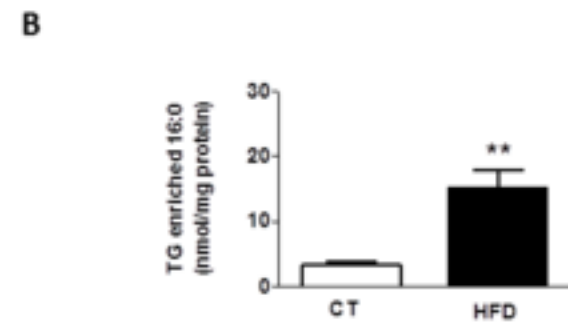
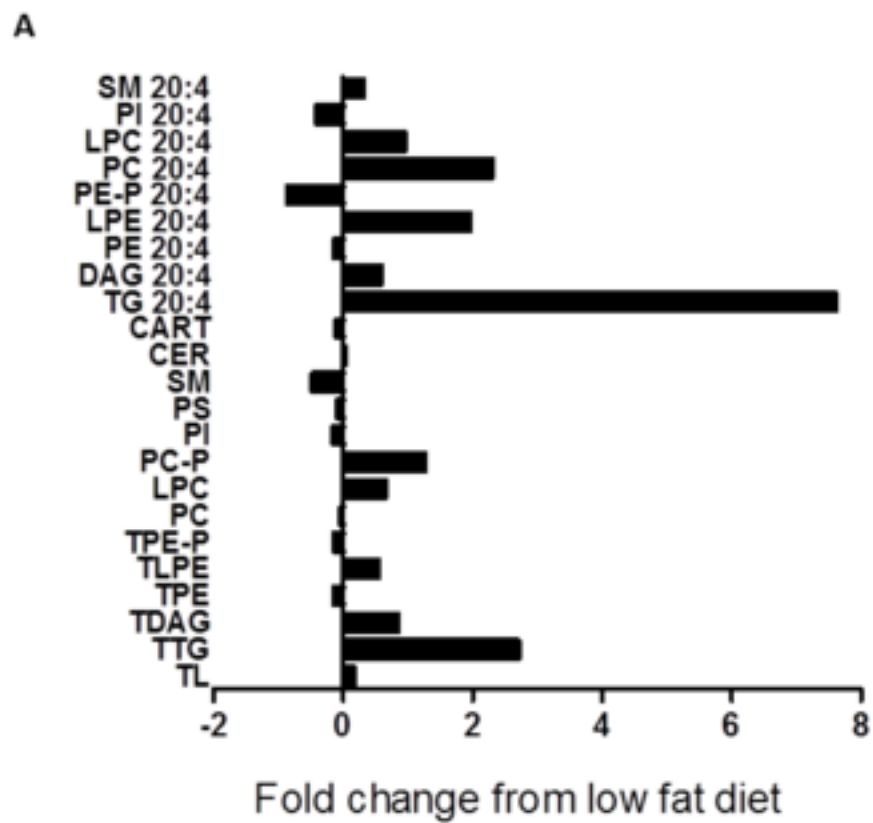


Figure 3

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The impact of obesity in the cardiac lipidomic and its consequences in the cardiac damage observed in obese rats

El impacto de la obesidad sobre el lipidoma cardiaco y sus consecuencias en el daño cardiaco en ratas obesas.

Gema Marín-Royo¹, Ernesto Martínez-Martínez¹, Beatriz Gutiérrez², Raquel Jurado-López¹, Isabel Gallardo², Olimpio Montero³, M^a Visitación Bartolomé^{4,7}, José Alberto San Román^{5,7}, Mercedes Salaices^{6,7}, María Luisa Nieto^{2,7*}, Victoria Cachofeiro^{1,7*}

¹Departamento de Fisiología, Facultad de Medicina, Universidad Complutense de Madrid and Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Spain.

² Instituto de Biología y Genética Molecular, CSIC-Universidad de Valladolid, Spain.

³Centro de Desarrollo Biotecnológico, CSIC, Valladolid, Spain

⁴ Departamento de Oftalmología y Otorrinolaringología, Facultad de Psicología, Universidad Complutense, Madrid, Spain.

⁵Instituto de Ciencias del Corazón (ICICOR), Hospital Clínico Universitario de Valladolid, Valladolid, Spain

⁶Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid and Instituto de Investigación Hospital Universitario La Paz (IdiPAZ), Spain.

⁷Ciber de Enfermedades Cardiovasculares (CIBERCV). Instituto de Salud Carlos III. Madrid, Spain

* : These authors contributed equally to this work.

Corresponding author: V. Cachofeiro, Departamento de Fisiología, Facultad de Medicina, Universidad Complutense, Madrid, 28040, Spain. Phone: 34-913941489; FAX: 34-913941628. E-mail: vcara@uclm.es

Author contributions

GMR performed data analysis and contributed to discussion and the writing of the manuscript. EMM and OM performed experiments and data analysis and contributed to discussion and the writing of the manuscript. BG, RJL, IG performed experiments and data analysis. MVB, JASR and MS contributed to discussion and the writing of the manuscript. MLN and VC designed the study, performed experiments and data analysis and wrote the manuscript.

Conflict of interest

None

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