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**BENEFICIAL EFFECTS OF LEPTIN TREATMENT IN A SETTING OF CARDIAC
DYSFUNCTION INDUCED BY TRANSVERSE AORTIC CONSTRICTION IN
MOUSE**

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Key Points Summary

- Leptin, is a 16kDa pleiotropic peptide, primary secreted by adipocytes, but also produced by other tissues including the heart.
- Controversy exists regarding the adverse and beneficial effects of Leptin on the heart
- We analyzed the effect of a non-hypertensive dose of leptin on cardiac function, $[Ca^{2+}]_i$ handling and cellular electrophysiology, which participate in the genesis of pump failure and related arrhythmias both in control mice and in mice subjected to chronic pressure-overload by transverse aorta constriction
- We find that Leptin activates mechanisms that contribute to cardiac dysfunction in physiological conditions. However after the establishment of pressure overload, an increase in Leptin levels has protective cardiac effects rescuing the cellular HF phenotype.
- These beneficial Leptin effects involve restoration of APD via normalization of I_{to} and SR Ca^{2+} content via rescue of control SERCA levels and RyR2 function modulation, leading to normalization of Ca^{2+} handling parameters.

ABSTRACT

Leptin, is a 16kDa pleiotropic peptide, primary secreted by adipocytes, but also produced by other tissues including the heart. Evidence support that Leptin may have either adverse or beneficial effects on the heart. In order to have further insights, in the present study we analyzed the effect of Leptin treatment on cardiac function, $[Ca^{2+}]_i$ handling and cellular electrophysiology, which participate in the genesis of pump failure and related arrhythmias both in control mice and in mice subjected to chronic pressure-overload by transverse aorta constriction (TAC). Three weeks after surgery, animals received either Leptin (0.36 mg/kg/day) or Vehicle via osmotic minipumps for 3 weeks. Echocardiographic measurements showed that whereas Leptin treatment was deleterious on cardiac function in Sham, Leptin had a cardioprotective effect following TAC. $[Ca^{2+}]_i$ transient in cardiomyocytes followed similar pattern. Patch-clamp experiments showed prolongation of action potential duration (APD) in TAC and Leptin-treated Sham animals, whereas following TAC Leptin reduced the APD towards control values. APD variations were associated with decreased transient outward potassium current and KV4.2 and KChIP2 protein expression. TAC myocytes presented higher incidence of triggered activities and spontaneous Ca^{2+} waves. These proarrhythmic manifestations, related to CaMKII and RyR2 phosphorylation, were reduced by Leptin.

Our results demonstrate that whereas Leptin treatment was deleterious on cardiac function in control animals, Leptin had a cardioprotective effect following TAC, normalizing cardiac function and reducing arrhythmogeneity at the cellular level.

Abbreviations list: Cardiovascular Diseases , CVD ; Ca^{2+} /Calmodulin-dependent protein Kinase II, CaMKII; Heart Failure, HF ; Transverse aorta constriction, TAC; Transient outward potassium current, I_{to} ; Action Potential Duration ,APD ; Sarcoplasmic Reticulum,

SR ; Ryanodine Receptors, (RyR2) ; Early Afterdepolarizations, EADs ; delayed after depolarizations, DADs ; Fractional Shortening, FS ; Ejection Fraction , EF.

INTRODUCTION

Leptin is a 16 kD adipokine that regulates food intake and energy metabolism at a central level. It is produced and secreted by adipose tissue, but also by other tissues including the heart. Leptin acts via the Leptin-receptor (ObR), which is present on cells of kidney, pancreas, liver, muscle, heart, and the vasculature (Abel *et al.*, 2008; Sweeney, 2010).

Obese patients usually present high circulating Leptin levels that are generally consequence of a status of Leptin resistance and a down-regulation of ObRs in the hypothalamus. However, there is experimental evidence supporting that Leptin resistance does not occur in cardiac tissue of obese individuals. Several studies have reported that ObR are not down-regulated in cardiomyocytes and that they preserve full responsiveness to Leptin (Abe *et al.*, 2007; Guzmán-Ruiz *et al.*, 2010; Stucchi *et al.*, 2011; Leifheit-Nestler *et al.*, 2013).

Obesity is associated with the prevalence of many cardiovascular diseases (CVD), including hypertension, coronary disease and heart failure (HF) (Lavie *et al.*, 2014). However, the link among obesity, hyperleptinemia, and development of CVD is not completely understood. Several clinical studies (Sierra-Johnson *et al.*, 2007; Liu *et al.*, 2010; Christakos *et al.*, 2016) but not all (Thøgersen *et al.*, 2004; Brennan *et al.*, 2007), have supported a role of hyperleptinemia as a risk marker for CVDs. On the other hand, disruption of the Leptin signaling pathway within the mouse heart has been reported to cause left ventricular hypertrophy (LVH) (Baartscheer *et al.*, 2003) and it has been proposed that hyperleptinemia can play a protective role in HF through activation of ObR signaling in cardiomyocytes (McGaffin *et al.*, 2008; Fernandez-Velasco *et al.*, 2012). Moreover, several studies have reported a better prognosis and lower mortality in overweight and obese patients with HF than in those patients with HF but normal body mass index (Horwich *et al.*, 2001; Oreopoulos *et al.*, 2008; Lavie *et al.*, 2013).

It is well known that almost half of HF patients die from sudden cardiac death, most probably due to ventricular arrhythmias. Electrophysiological remodeling, including reduced expression of potassium channels and prolonged action potential duration are typical features of HF that make the heart more prone to early afterdepolarizations (EADs) and fatal arrhythmias. In addition, failing hearts show enhanced diastolic Ca^{2+} leak via cardiac ryanodine receptor (RyR2) that can generate delayed after depolarizations (DADs) and ventricular arrhythmias. Alteration in intracellular Ca^{2+} handling and reduced expression of potassium channels have been proposed as mechanisms involved in the genesis of HF related arrhythmias (Nattel *et al.*, 2007; Lehnart *et al.*, 2009).

The aim of the present study was to assess the effect of a non-hypertensive dose of Leptin on cardiac electrical remodeling and sarcoplasmic reticulum Ca^{2+} leak in a mouse model with pre-existing pathological hypertrophy and cardiac dysfunction induced by transverse aortic constriction (TAC).

Our results demonstrate that in the presence of a cardiac pathology, Leptin treatment has a beneficial effect by normalizing cardiac function, and reducing arrhythmogenesis. The mechanisms involved included a significant increase of Kv4.2 and KChIP expression that normalized I_{to} density and APD reduction and a significant reduction of Ca^{2+} waves frequency as a consequence of normalization of Ser-2815 RYR2 phosphorylation levels. Although the effect of Leptin on control animals cardiac function was deleterious it was not accompanied with increased arrhythmogenicity.

METHODS

Ethical Approval

All experiments on mice were performed with the approval of the Bioethical Committee of the Complutense University of Madrid, following recommendations of the Spanish (Proex 035-15) and European guidelines (2010/63/EU) and according to the ethical principles laid down by the French (Ministry of Agriculture) Directives for the care of laboratory animals (B9201901).

Animals

Mice were housed in cages of 5 and fed *ad libitum*. Ten weeks C57Bl/6 male mice (Jackson laboratories), under anesthesia (50 mg/kg ketamine plus 8 mg/kg xylazine i.p) during the whole procedure, underwent transversal aortic constriction (TAC). Depth of anesthesia was confirmed by toe pinch. After thoracotomy, the aortic arch was constricted with a thread (6-0 silk suture) together with a 27-gauge needle (\varnothing 0.42 mm). Then the needle was removed, leaving a region of stenosis which partially reduced the vascular light. Sham group underwent the same procedure without placement of the suture. Three weeks after surgery, before the establishment of cardiovascular dysfunction (Ejection fraction Sham $76.41\% \pm 2.53$ vs. TAC $77.00\% \pm 1.84$ and Shortening fraction Sham $39.99\% \pm 2.24$ vs. TAC $40.29\% \pm 1.74$) animals were randomly divided into 4 groups (Sham vehicle, Sham Leptin, TAC vehicle and TAC Leptin). A micro-osmotic pump containing either Leptin (0.36 mg/kg/day) or Vehicle (20 mmol/L Tris, 150 nmol/L NaCl) was subcutaneously implanted in every mouse (Sham and TAC groups). Mice were finally sacrificed for experiments 3 weeks after osmotic pump implantation.

Drugs and Devices:

Leptin was purchased from Sigma Aldrich Co (St Louis, MO) and the stock solution was prepared according to manufacturer instructions. Fluo-3 AM was purchased from Molecular Probes-Life Technologies ® (Eugene, OR) and Collagenase type II from (Worthington Biochemical Corporation, Lakewood, NJ). Caffeine and other products are from Sigma Aldrich Co (St Louis, MO).

Osmotic minipumps were obtained from Alzet® (Cupertino, CA). The 5 MHz transducer Vivid 9 for M-mode echocardiography was obtained from General Electric Healthcare (Piscataway, NJ).

We used a Leica SP5 confocal microscopy (Leica Microsystems, Germany) for Ca²⁺ imaging and Ca²⁺ image analyses were performed using IDL software (Research Systems, Inc., Boulder, CO, USA) and homemade routines. For the whole-cell patch-clamp method, we used Axopatch 1D amplifier with pClamp6 software (Axon Instruments, Sunnyvale, CA).

Primary antibodies: SERCA [1:1000] (Thermo Fisher, Waltham, MA), Phospholamban [1:5000] (Ser16 and Thr17), Phospholamban [1:1000] and Phospho-RyR [1:1000] (Ser2815; Badrilla Ltd. Leeds, UK), RyR [1:1000] (Affinity Bioreagents. Golden, CO), pCaMKII (Thr286) [1:1000] and CaMKII [1:1000] (Cell signaling antibodies. Danvers, MA), Kv4.2 [1:500] (EMD Millipore. Darmstadt, Germany), the voltage-gated K⁺ channel-interacting proteins 2 [1:1000] (KCHIP2; Affinity Bioreagents. Golden, CO) and GAPDH [1:5000] (Ambion. Carlsbad, CA). Specific bands were detected using the Amersham™ ECL™ protein detection system (General Electric Healthcare. Piscataway, NJ) and were analyzed using ImageJ software ([NIH](#)).

Echocardiography

Transthoracic echocardiography was performed the day previous to the experiment using a 5MHz transducer under 3% isoflurane gas anesthesia. Two-dimensional-guided (2D) M-mode echocardiography was used to determine contractile parameters such as fractional shortening (FS) or ejection fraction (EF).

Cardiomyocytes isolation

Mice were heparinized (4 IU/g, i.p.) and anesthetized (sodium pentobarbital, 50 mg/kg, i.p.). The depth of anesthesia was determined by assessing loss of the pedal reflex by toe pinch. Ventricular myocytes were isolated using a collagenase perfusion method as previously described (Gómez-Hurtado *et al.*, 2014; Delgado *et al.*, 2015).

Confocal imaging

Ca²⁺ tolerant rod shaped myocytes were loaded with the fluorescence Ca²⁺ dye Fluo-3 AM (6 μmol/L) for 30 min. Images were obtained after at least 30 supplementary minutes, to allow for complete dye deesterification, with confocal microscopy (objective w.i. 63x) fitted with a white light laser tuned to 500 nm. Excitation was collected at >510 nm. All experiments were performed at room temperature (23°C).

To record Ca²⁺ transients, Fluo-3 loaded cells were field stimulated by two Pt electrodes at 2 Hz until steady state before recording. Spontaneous Ca²⁺ sparks and waves were obtained in quiescent cells after [Ca²⁺]_i transients recordings. For Sarcoplasmic Reticulum (SR) Ca²⁺ load estimation, cardiac myocytes were rapidly perfused with 10 mmol/L caffeine right after field-stimulation to empty SR. Because [Ca²⁺]_i reached during caffeine experiments may fall in the non-linear part of the fluorescence-Ca²⁺ curve of Fluo-3, significant changes in [Ca²⁺]_i may provide small differences in fluorescence. Thus we calculated [Ca²⁺]_i levels according to the

formula and diastolic $[Ca^{2+}]_i$ previously published (Gómez *et al.*, 1996) and the Fluo-3 AM Kd values experimentally measured in cardiomyocytes (Loughrey *et al.*, 2003).

Electrophysiological measurements

Action potentials (AP) and K^+ currents were recorded using whole cell patch clamp method with solutions and protocols as previously described (Ouvrard-Pascaud *et al.*, 2005). Trigger Activity was defined as the number of spontaneous AP recorded as a result of delayed post-depolarizations (DAD) large enough to reach the threshold potential for activation of a regenerative inward current.

Western blot

Cardiac tissue was homogenized and centrifuged at 500 rpm for 1 min at 4°C to eliminate cellular debris. The supernatants were used for immunoblotting. The extracted proteins (15-30 μ g) were separated on SDS-PAGE gels and transferred to polyvinylidenedifluoride membranes. The membranes were blocked with 5% milk and incubated overnight with primary antibodies.

ELISA

To analyze Leptin concentration in heart homogenates we use Leptin mouse ELISA KIT KMC2281 Novex® (Life Technologies) according to manufacturer instructions.

STATISTICS

Data are presented as means \pm SEM. Statistical analysis was performed with Graphpad Prism 5.0. Gaussian distribution of the samples was assessed by D'Agostino-Pearson test. Then, statistical significance was evaluated by one-way ANOVA followed by Bonferroni post-test in those samples that followed a normal distribution or by

Kruskal-Wallis followed by Dunn's post-test in those samples that did not follow a normal distribution. For statistical comparison of abnormal diastolic Ca^{2+} release and trigger activity occurrence, the χ^2 test was used as previously described (Ruiz-Hurtado *et al.*, 2012) and then data are presented as percentage. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Chronic Leptin treatment slightly impairs cardiac function in healthy mice, but prevents cardiac dysfunction in mice subjected to pressure overload.

Analysis of cardiac Leptin levels did not show significant differences between Sham and TAC mice (Table 1). After 3 weeks treatment, we observed a 25% increase in cardiac Leptin levels either in Sham or TAC animals. Neither TAC nor chronic Leptin treatment modified mice body weight (BW) or tibia length (TL), however, TAC induced an increase in heart weight normalized by BW or by TL. This cardiac hypertrophy induced by TAC was reduced by 3 weeks of Leptin treatment, at the whole organ level, but also at the cardiomyocyte level, as attested by membrane capacitance measurements (Table 1). Whereas Leptin treatment in Sham mice impaired cardiac contractility, it improved cardiac function in TAC animals, measured by M-mode Echocardiography (Figure 1A). As shown in Figure 1 B-C, on average, 3 weeks treatment with Leptin (Sham Leptin) slightly but significant reduced EF and FS, although by less amplitude than TAC. When treated for 3 weeks with Leptin, TAC mice showed improved EF and FS compared to those mice TAC treated only with Vehicle.

Leptin treatment after TAC rescued $[\text{Ca}^{2+}]_i$ transients and contractile function in cardiomyocytes.

Cardiac pump function is dependent on the cardiomyocytes contraction, which is activated by Ca^{2+} . To get an insight into the cellular mechanism responsible for the modification of cardiac function, we investigated whether Leptin modulated $[\text{Ca}^{2+}]_i$ transients in isolated cardiomyocytes. Figure 2A shows representative line-scan images recorded from ventricular myocytes isolated from mice from all 4 experimental groups. Consistent with the reduced cardiac function observed by Echocardiography, 3 weeks Leptin treatment reduced $[\text{Ca}^{2+}]_i$ transient amplitude (Figure 2B) and cell shortening (Figure 2C). In contrast, in mice subjected to TAC, where $[\text{Ca}^{2+}]_i$ transient amplitude and contraction were reduced compared to Sham-operated animal (consistent with failing function), Leptin was able to restore $[\text{Ca}^{2+}]_i$ transient amplitude and cell shortening to levels similar to those observed in control group. The SR Ca^{2+} content could be responsible for the $[\text{Ca}^{2+}]_i$ transient amplitude reduction. Indeed, the SR Ca^{2+} load was reduced in cells isolated from TAC compared to Sham animals, as it is characteristic of HF. Three weeks Leptin treatment also depressed SR Ca^{2+} load, although the reduction was smaller than that induced by TAC. The SR Ca^{2+} load in cells isolated from TAC animals having received Leptin was partially restored (Figure 2 D-E). As shown in Figure 2F, Leptin treatment after TAC prevented SERCA2 down-regulation induced by TAC hearts. This can contribute to restore SR Ca^{2+} content and $[\text{Ca}^{2+}]_i$ transient amplitude. Of note, we did not observed changes either in total expression or phosphorylation (in either residue Ser16 or Thr17) of PLB (Figure 2G-H).

Leptin rescued electrophysiological alterations in mice that underwent TAC.

Ca^{2+} release from the SR, which induces contraction, is activated during action potential (AP). Cardiac hypertrophy is accompanied by a prolongation of AP duration, mainly due to a reduction in the transient outward K^+ current (I_{to}) (Beuckelmann *et al.*, 1993; Ravens & Cerbai, 2008). In order to test whether Leptin had modulator effect in these parameters, we carried out patch-clamp experiments to measure APs and K^+ currents. Figure 3A shows representative

traces of APs recorded in different cardiomyocytes isolated from Sham and TAC mice treated either with Vehicle or with Leptin. Leptin treatment by itself increased AP duration (APD) measured at 20% (APD₂₀) (Sham vehicle 1.62 ± 0.13 ms vs. Sham Leptin 2.28 ± 0.27 ms), 50% (APD₅₀) (Sham vehicle 5.12 ± 0.51 ms vs. Sham Leptin 7.98 ± 1.12 ms) and 90% (APD₉₀) of repolarization (Sham vehicle 73.18 ± 8.76 ms vs. Sham Leptin 107.3 ± 14.58 ms) (Figure 3B). Following TAC, cells isolated from Vehicle treated mice showed the classical hypertrophic induced prolongation of APD measured at 20% (TAC vehicle 3.44 ± 0.85 ms), 50% (TAC vehicle 11.85 ± 1.99 ms) and 90% of repolarization (TAC vehicle 189.12 ± 41.72 ms) (Figure 3B). In contrast, Leptin treatment shortened the APD in TAC cardiomyocytes (Figure 3B) at 20% (TAC Leptin 1.69 ± 0.14 ms), 50% (TAC Leptin 7.23 ± 0.85 ms) and 90% of repolarization (TAC Leptin 148.28 ± 30.03 ms).

To further analyze the mechanisms involved in APD alteration by Leptin and/or TAC, we measured the main K⁺ currents responsible for the AP repolarization phase. In adult mouse ventricle, outward K⁺ currents involved in cardiac repolarization consist of 3 components distinguishable by their specific time and voltage dependency and sensitivity to pharmacological agents: a transient current (I_{to}), a low-concentration 4-aminopyridine-sensitive current (I_{Kur}), and a noninactivating steady-state component (I_{ss}) (Brouillette *et al.*, 2004). Three weeks Leptin treatment of Sham-operated mice reduced I_{to} densities (Figure 4A left panel) without modifying I_{Kur} (Figure B left panel) or I_{ss} (Figure 4C left panel) densities. In mice subjected to TAC and treated with Vehicle (Figures 4 A-C right panel), densities of I_{to} and I_{Kur} , but not I_{ss} , were reduced compared to Sham cells. Leptin treatment reversed I_{to} density downregulation to levels similar to those observed in control mice (Figures 4A right panel) but did not modify the reduction of I_{Kur} densities (Figure 4B right panel). These results are consistent with the AP waveforms (Figure 3). The decrease in I_{to} density could be the result of a depression in channel expression. We analyzed protein expression of the main α subunit that

encode I_{to} channel in mice (Kv4.2) and also the auxiliary subunit KChIP2 (Patel & Campbell, 2005). Figures 4D and E show that Kv4.2 and KChIP2 protein expression were reduced in those groups that presented cardiac dysfunction (Sham Leptin and TAC Vehicle), compared to Sham Vehicle. In TAC hearts, Leptin treatment normalized Kv4.2 and KChIP2 protein expression.

Leptin treatment prevents arrhythmic manifestations in cardiac myocytes isolated from TAC mice by reverting CaMKII RyR2 phosphorylation

During AP recordings, we observed oscillations in membrane potential or delayed post-depolarizations (DAD) following completion of the driven AP, which eventually, triggered spontaneous AP (Figure 5A). The occurrence of these triggered activities (TA) was greatly enhanced by TAC (Figure 5B), and reduced by Leptin treatment after TAC. DADs are commonly initiated by non-electrically driven, propagating spontaneous Ca^{2+} release from the SR via the RyR2s, named Ca^{2+} waves (Pogwizd & Bers, 2004). Consistently, spontaneous Ca^{2+} wave occurrence (Figure 5C-D) were prominent in the TAC group. Although Leptin did not promote any effect on Ca^{2+} waves occurrence in healthy cells, it was able to reduce the occurrence of Ca^{2+} waves in cardiomyocytes isolated from TAC animals to control levels (Figure 5D). In addition, we found that NCX expression was increased in all experimental groups in comparison with sham-vehicle group (Figure 5E). It is widely reported that NCX expression is elevated in hypertrophy and HF both in humans and animal models (Despa *et al.*, 2002; Pieske *et al.*, 2002; Baartscheer *et al.*, 2003; Louch *et al.*, 2010). Thus, the fact that leptin treatment of TAC animals did not prevent this increase tend to rule out a direct NCX involvement in leptin antiarrhythmogenic effects.

Enhanced Ca^{2+} waves occurrence, suggests leaky RyR2 channels (Zima *et al.*, 2010). Several studies (Bers, 2002; Ruiz-Hurtado *et al.*, 2012) previously found that an increase in CaMKII

activation (measurable by CaMKII auto-phosphorylation at Thr286) and subsequent RyR2 phosphorylation at the CaMKII site (Ser2815) are involved in this effect. To test the involvement of this mechanism in the observed effects, we first analyzed the CaMKII auto-phosphorylation in our four experimental groups. Figure 6A shows that CaMKII was more activated in TAC mice treated with Vehicle than in Sham mice, and that Leptin treatment was able to reduce this effect on TAC mice, without significantly altering CaMKII activation in Sham operated animals. Consequently, when we analyzed RyR2 phosphorylation, we observed an increase of Ser2815 phosphorylation levels in TAC mice, which was significantly prevented by Leptin treatment (Figure 6B). In addition, we confirmed that phospho-Ser2809 RyR2, was increased in the failing groups (provide data here). We then examined RyR2s activity in situ, by visualizing spontaneous Ca^{2+} sparks. Figure 6C shows examples of Ca^{2+} sparks recorded in isolated cardiomyocytes obtained from Sham and TAC mice treated either with Vehicle or with Leptin. Neither TAC nor Leptin treatment significantly modified Ca^{2+} sparks frequency (Figure 6D). However, Ca^{2+} sparks amplitude was significantly higher in cells isolated from TAC animals and this effect was blunted prevented by Leptin treatment (Figure 6E), consistent with RyR phosphorylation levels (Figure 6B). Because RyR2 activity, and hence Ca^{2+} sparks occurrence, also depends on SR Ca^{2+} load, we normalized Ca^{2+} spark frequency by the total amount of Ca^{2+} stored in the SR. Figure 6F shows that cardiac myocytes isolated from TAC animals presented significantly higher frequency of Ca^{2+} sparks for a given SR Ca^{2+} load, consistent with RyR2 phosphorylation at the S2815 site. This increase was attenuated by Leptin treatment (Figure 6F). Ca^{2+} waves are originated by spontaneous openings of RyR2s as Ca^{2+} sparks, which then propagate to neighboring RyR2s clusters. Thus, Ca^{2+} sparks spread in time and space are important in the probability of a Ca^{2+} spark to initiate a Ca^{2+} wave. Figure 7 shows that Leptin treatment diminished Ca^{2+} spark duration and width in both experimental groups, thus limiting Ca^{2+} spread and probability to activate an arrhythmogenic Ca^{2+} wave.

DISCUSSION

In the last two decades several studies have determined that plasma Leptin levels oscillate among 5-15 ng/ml in normoweight individuals (Diez *et al.*, 1984; Tejerina *et al.*, 1984) whereas in obese patients these levels are significantly increased, reaching values among 15-500 ng/ml (Considine *et al.*, 1996; Soliman *et al.*, 2002).

Moreover, obesity ($BMI \geq 30$) is associated with altered cardiac function. In a follow-up study over 14 years with 5881 patients from Framingham study, it has been reported a gradual increase in HF risk related to an increase in BMI (Lehnart *et al.*, 2009). However, it is important to note that within HF patients, elevated BMI is not a risk factor for worse outcomes (Lavie *et al.*, 2013). This discrepancy between increased CVD risk associated with obesity and better outcomes of CVD found in obese patients has led to the obesity paradox theory. In our study we tested the hypothesis that leptin could have deleterious or protective effect in cardiac function depending on the previous existence of a cardiac pathology.

To accomplish this aim we produced transverse aortic constriction (TAC) in mice, that is characterized for LV hypertrophy development 3 weeks after intervention, whereas after 6 weeks a systolic dysfunction also occurs (Nakamura *et al.*, 2001). Echocardiographic results confirmed reduced cardiac EF and FS 6 weeks after surgery, validating the model. Leptin treatment in Sham-operated animals induced a smaller but significant depression in cardiac function, consistent with *in vitro* studies showing impaired cardiomyocyte contractility by

Leptin (Dong *et al.*, 2006). It is important to note that after 3 weeks Leptin treatment (0.36 mg/kg/day) we did not observe neither left ventricular hypertrophy in our mice nor an increase in the membrane capacitance of the cardiomyocytes isolated from those mice. It is important to note here that although Leptin effects on cardiac hypertrophy remains controversial, there are previous studies supporting Leptin pro-hypertrophic effects on isolated neonatal cardiomyocytes (Fernandez-Velasco *et al.*, 2003; Tajmir *et al.*, 2004; Fernandez-Velasco *et al.*, 2006; Smani *et al.*, 2010). However, our data is consistent with a previous study published by Abe Y. et al in 2007 (Abe *et al.*, 2007) where they did not find any differences in left ventricular mass after 4 weeks of chronic Leptin infusion with a non-hypertensive dose (0.32 mg/Kg/day). Moreover, it has been previously shown that electrical remodeling precedes cellular hypertrophy after myocardial damage (Perrier *et al.*, 2004). Strikingly, contractile function evaluated by M-mode echocardiography was improved by Leptin treatment after TAC, revealing a beneficial effect of Leptin in the stressed heart. $[Ca^{2+}]_i$ transient analysis of isolated cardiomyocytes obtained from these mice was consistent with the echocardiographic outcomes. Taken together, these results support that a rise in Leptin levels induces deleterious effects in physiological conditions but may be cardioprotective in pathological situations. Leptin signaling has been shown to have an important role in maintaining ATP formation, as KO of Leptin in stressed mice is lethal (Hall *et al.*, 2012). Thus in the setting of HF, Leptin may help, among other plausible mechanisms, to refill the SR with Ca^{2+} , providing ATP to the SERCA pump or by increasing SERCA expression levels which are known to be reduced in HF (Hobai & O'Rourke, 2001). As a matter of fact, HF is characterized by metabolic alterations that contribute to contractile abnormalities. ATP content is decreased and the main energy source is shifted from fatty acid utilization towards glucose in hypertrophy and HF (Sorokina *et al.*, 2007; Ingwall, 2009). There is experimental evidence that supports the idea that Leptin shifts myocardial metabolism towards fatty acid utilization (Witte *et al.*, 2016), which could

be beneficial in the setting of HF. In fact, 3 weeks Leptin treatment after TAC helped preserving the intracellular Ca^{2+} stores and restored SERCA levels that were decreased in TAC mice.

Increased arrhythmia susceptibility is a hallmark of HF (Tomaselli & Marbán, 1999). It is well known that alterations in APD due to reduced repolarizing K^+ currents lead to longer cardiac AP or long-QT interval and predisposes patients to ventricular arrhythmias (Ravens & Cerbai, 2008). Therefore, patients with impaired K^+ channels function are at increased risk for sudden cardiac death. In this regard, it is also well documented that changes in I_{to} function is one of the most important electrophysiological abnormalities related to the left ventricular hypertrophy and HF (Benitah *et al.*, 1993; Beuckelmann *et al.*, 1993; Gómez *et al.*, 1997). Thus, we analyzed the electrophysiological remodeling that occurs in our model. We found that Leptin in physiological conditions moderately increases APD. Interestingly, in TAC mice where we observed a marked increase of APDs Leptin treatment completely rescued the APD20 prolongation induced by TAC, and significantly shortened the prolongation at APD50 and APD90. In rodents, I_{to} current has a major contribution to phase 1 of ventricular repolarization and APD. Therefore, we hypothesize that the effects of Leptin in APD could be mediated by Leptin ability to modulate K^+ currents. We found that Leptin itself reduced I_{to} densities without modifying I_{Kur} and I_{ss} , which is consistent with the APD increases, whereas TAC reduced both I_{to} and I_{Kur} . The action potential "plateau phase" is mainly Ca^{2+} -mediated but in rodent due to highly prevalence of multiple K^+ currents there is no clear plateau phase, and repolarization is rapid (Nerbonne & Kass, 2005). When K^+ currents are decreased (not only I_{to} but also I_{Kur}), this plateau phase become apparent, as observed after TAC. Even if leptin in TAC model blunted I_{to} reduction, this is not associated with prevention of I_{Kur} reduction and then the plateau phase is still apparent. This is emphasized by the fact that in sham, leptin treatment induced a reduction of I_{to} without alteration in I_{Kur} and no obvious appearance of plateau phase. However, the most important finding was that I_{to} density was restored by Leptin

in TAC mice to levels similar to those observed in control mice through a mechanism that involve normalization of protein expression levels of Kv4.2 and KChIP2. Our results are consistent with a previous *in vitro* study in which Leptin induced an increase of I_{to} current and protein expression on 48 h cultured ventricular cardiomyocytes (Gómez-Hurtado *et al.*, 2014), supporting the effect of Leptin in modulating K^+ channel expression.

It is also well established that spontaneous Ca^{2+} release during diastole can trigger ventricular arrhythmias. When spontaneous SR Ca^{2+} release occurs and Ca^{2+} signaling propagates along the myocyte as Ca^{2+} waves, the Na^+/Ca^{2+} exchanger is activated and able to generate transient inward currents that result in DADs. If DADs are large enough, they may turn into spontaneous APs and initiate an arrhythmia (Sedej *et al.*, 2010; Galimberti & Knollmann, 2011). In our model, we found higher occurrence of Ca^{2+} sparks (Figure 6F) and arrhythmogenic Ca^{2+} waves only in TAC group, related to CaMKII activation and RyR2 hyperphosphorylation by CaMKII. Leptin by itself did not activate CaMKII and decreased Ca^{2+} sparks amplitude without altering their frequency. Thus although Leptin treated Sham animals showed cardiac dysfunction, Leptin did not enhance arrhythmogenic Ca^{2+} waves. In TAC animals, however, RyR2 hyperphosphorylation at the CaMKII site, as previously reported (Ruiz-Hurtado *et al.*, 2012; Fischer *et al.*, 2013; Bers, 2014; Fischer *et al.*, 2014), increased RyR2 open probability, as manifested by the enhanced Ca^{2+} spark frequency relative to the SR Ca^{2+} load, which contributes to higher propensity to present Ca^{2+} waves. This was also normalized by Leptin treatment, which prevented CaMKII hyperactivation. CaMKII regulation involves a number of different processes and a number of phosphatases regulated both directly and indirectly by Ca^{2+} . Among these multiple mechanisms it has been reported that CaMKII autophosphorylation is sensitive to the duration, magnitude and frequency of the calcium transients (De Koninck & Schulman, 1998) and there is evidence that increased action potential duration enhanced CaMKII activity (Grueter *et al.*, 2007). In this regard, Leptin-treatment

prevention of increased APD might contribute to the normalization of CaMKII phosphorylation. On the other hand, CaMKII can be activated by ROS as well (Erickson *et al.*, 2008; Curran *et al.*, 2014). In this regard, Leptin activation of AMPK (Hardie, 2003) and subsequent increased expression of the metabolic regulator PGC-1 α (Jäger *et al.*, 2007) might result in increased mitochondrial biogenesis and improved mitochondrial function and oxygen consumption, and therefore reduced ROS production (Lehman *et al.*, 2000).

Altogether our results provide evidence that Leptin activates mechanisms that contribute to cardiac dysfunction in physiological conditions. However after the establishment of pressure overload, an increase in Leptin levels may have protective cardiac effects rescuing the cellular HF phenotype. These beneficial Leptin effects involve restoration of APD via Ito upregulation and SR Ca²⁺ content via rescue of control SERCA levels and RyR2 function modulation, leading to normalization of Ca²⁺ handling parameters. We acknowledge that more in depth studies are needed in order to fully understand Leptin effects in the human heart, especially those regarding I_{to} currents regulation since the main alpha subunit for Ito differs in the rodent (Kv4.2) and the human heart (Kv4.3), but our data point to Leptin as a good candidate involved in the pathophysiological mechanisms related to obesity (Romero-Corral *et al.*, 2006; Oreopoulos *et al.*, 2008; Rider *et al.*, 2011; Littnerova *et al.*, 2015; Zamora *et al.*, 2016). In this regard, recent reports (Wannamethee *et al.*, 2014; Mishra *et al.*, 2015) have shown that HF patients with high circulating Leptin levels were at lower risk of mortality from cardiovascular disease supporting a protecting role of Leptin once the pathology is established. This phenomenon, while not quite well understood has been related to adipose tissue function, and low Leptin levels in HF patients have been proposed to reflect loss of adipose tissue due to cachexia which is associated with reduced survival (Filippatos *et al.*, 2000; Wannamethee *et al.*, 2014). However, our data show that direct effects of Leptin on ion channels and Ca²⁺

handling related proteins could be also involved in the more favorable clinical outcomes observed in overweight and obese HF patients.

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Author contributions

N.G-H., AM.G., JP.B. and C.D. conceived and designed the work; N.G-H., A.D-R.,P.M., M.F-V., A.V-B., R.A., J.S., acquired the data, N.G-H., A.D-R.,P.M., M.F-V., A.V-B., R.A., J.S., AM.G., JP.B. and C.D. analyzed and interpreted the data. N.G-H., AM.G., JP.B. and C.D. wrote the manuscript. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Disclosures

None to declare

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AUTHOR'S TRANSLATIONAL PERSPECTIVE

Our data supports the idea that high Leptin levels contribute to increase CVD risk under physiological conditions but may be cardioprotective in pathological situations such as heart failure. In the failing TAC mouse model Leptin prevents SR Ca^{2+} load decline maintaining physiological protein levels of SERCA2. Leptin treatment also prevents the electrical TAC-remodeling via potassium current normalization and prevention of ventricular arrhythmia occurrence due to RyR2 activity normalization. These opposite effects of Leptin depend on the patho-physiological state of the heart. In conclusion our study contributes to better understand the “obesity paradox” phenomenon at the cardiac level.

REFERENCES

- Abe Y, Ono K, Kawamura T, Wada H, Kita T, Shimatsu A & Hasegawa K. (2007). Leptin induces elongation of cardiac myocytes and causes eccentric left ventricular dilatation with compensation. *Am J Physiol Heart Circ Physiol* **292**, H2387-2396.
- Abel ED, Litwin SE & Sweeney G. (2008). Cardiac remodeling in obesity. *Physiol Rev* **88**, 389-419.
- Baartscheer A, Schumacher CA, Belterman CN, Coronel R & Fiolet JW. (2003). $[Na^+]_i$ and the driving force of the Na^+/Ca^{2+} -exchanger in heart failure. *Cardiovasc Res* **57**, 986-995.
- Benitah JP, Gomez AM, Bailly P, Da Ponte JP, Berson G, Delgado C & Lorente P. (1993). Heterogeneity of the early outward current in ventricular cells isolated from normal and hypertrophied rat hearts. *J Physiol* **469**, 111-138.
- Bers DM. (2002). Cardiac excitation-contraction coupling. *Nature* **415**, 198-205.
- Bers DM. (2014). Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu Rev Physiol* **76**, 107-127.
- Beuckelmann DJ, Näbauer M & Erdmann E. (1993). Alterations of K^+ currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res* **73**, 379-385.
- Brennan AM, Li TY, Kelesidis I, Gavrilu A, Hu FB & Mantzoros CS. (2007). Circulating leptin levels are not associated with cardiovascular morbidity and mortality in women with diabetes: a prospective cohort study. *Diabetologia* **50**, 1178-1185.
- Brouillette J, Clark RB, Giles WR & Fiset C. (2004). Functional properties of K^+ currents in adult mouse ventricular myocytes. *J Physiol* **559**, 777-798.
- Christakos S, Dhawan P, Verstuyf A, Verlinden L & Carmeliet G. (2016). Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiol Rev* **96**, 365-408.

- Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ & Bauer TL. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* **334**, 292-295.
- Curran J, Tang L, Roof SR, Velmurugan S, Millard A, Shonts S, Wang H, Santiago D, Ahmad U, Perryman M, Bers DM, Mohler PJ, Ziolo MT & Shannon TR. (2014). Nitric oxide-dependent activation of CaMKII increases diastolic sarcoplasmic reticulum calcium release in cardiac myocytes in response to adrenergic stimulation. *PLoS One* **9**, e87495.
- De Koninck P & Schulman H. (1998). Sensitivity of CaM kinase II to the frequency of Ca²⁺ oscillations. *Science* **279**, 227-230.
- Delgado C, Ruiz-Hurtado G, Gómez-Hurtado N, González-Ramos S, Rueda A, Benito G, Prieto P, Zaragoza C, Delicado EG, Pérez-Sen R, Miras-Portugal MT, Núñez G, Bosca L & Fernández-Velasco M. (2015). NOD1, a new player in cardiac function and calcium handling. *Cardiovasc Res* **106**, 375-386.
- Despa S, Islam MA, Weber CR, Pogwizd SM & Bers DM. (2002). Intracellular Na⁽⁺⁾ concentration is elevated in heart failure but Na/K pump function is unchanged. *Circulation* **105**, 2543-2548.
- Diez J, Delgado C & Tamargo J. (1984). An analysis of the positive inotropic effect of AR-L 115 BS on isolated rat atria. *Arch Int Pharmacodyn Ther* **269**, 70-82.
- Dong F, Zhang X & Ren J. (2006). Leptin regulates cardiomyocyte contractile function through endothelin-1 receptor-NADPH oxidase pathway. *Hypertension* **47**, 222-229.
- Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, Bartlett RK, Lowe JS, O'Donnell SE, Aykin-Burns N, Zimmerman MC, Zimmerman K, Ham AJ, Weiss RM, Spitz DR, Shea MA, Colbran RJ, Mohler PJ & Anderson ME. (2008). A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. *Cell* **133**, 462-474.
- Fernandez-Velasco M, Goren N, Benito G, Blanco-Rivero J, Bosca L & Delgado C. (2003). Regional distribution of hyperpolarization-activated current (I_f) and hyperpolarization-activated cyclic nucleotide-gated channel mRNA expression in ventricular cells from control and hypertrophied rat hearts. *Journal of Physiology-London* **553**, 395-405.
- Fernandez-Velasco M, Prieto P, Terron V, Benito G, Flores JM, Delgado C, Zaragoza C, Lavin B, Gomez-Parrizas M, Lopez-Collazo E, Martin-Sanz P & Bosca L. (2012). NOD1 Activation Induces Cardiac Dysfunction and Modulates Cardiac Fibrosis and Cardiomyocyte Apoptosis. *Plos One* **7**.
- Fernandez-Velasco M, Ruiz-Hurtado G & Delgado C. (2006). I-K1 and I-f in ventricular myocytes isolated from control and hypertrophied rat hearts. *Pflugers Archiv-European Journal of Physiology* **452**, 146-154.

- Filippatos GS, Tsilias K, Venetsanou K, Karambinos E, Manolatos D, Kranidis A, Antonellis J, Kardaras F, Anthopoulos L & Baltopoulos G. (2000). Leptin serum levels in cachectic heart failure patients. Relationship with tumor necrosis factor-alpha system. *Int J Cardiol* **76**, 117-122.
- Fischer TH, Eiringhaus J, Dybkova N, Förster A, Herting J, Kleinwächter A, Ljubojevic S, Schmitto JD, Streckfuß-Bömeke K, Renner A, Gummert J, Hasenfuss G, Maier LS & Sossalla S. (2014). Ca²⁺/calmodulin-dependent protein kinase II equally induces sarcoplasmic reticulum Ca²⁺ leak in human ischaemic and dilated cardiomyopathy. *Eur J Heart Fail*.
- Fischer TH, Herting J, Tirilomis T, Renner A, Neef S, Toischer K, Ellenberger D, Förster A, Schmitto JD, Gummert J, Schöndube FA, Hasenfuss G, Maier LS & Sossalla S. (2013). Ca²⁺/calmodulin-dependent protein kinase II and protein kinase A differentially regulate sarcoplasmic reticulum Ca²⁺ leak in human cardiac pathology. *Circulation* **128**, 970-981.
- Galimberti ES & Knollmann BC. (2011). Efficacy and potency of class I antiarrhythmic drugs for suppression of Ca²⁺ waves in permeabilized myocytes lacking calsequestrin. *J Mol Cell Cardiol* **51**, 760-768.
- Grueter CE, Colbran RJ & Anderson ME. (2007). CaMKII, an emerging molecular driver for calcium homeostasis, arrhythmias, and cardiac dysfunction. *J Mol Med (Berl)* **85**, 5-14.
- Guzmán-Ruiz R, Somoza B, Gil-Ortega M, Merino B, Cano V, Attané C, Castan-Laurell I, Valet P, Fernández-Alfonso MS & Ruiz-Gayo M. (2010). Sensitivity of cardiac carnitine palmitoyltransferase to malonyl-CoA is regulated by leptin: similarities with a model of endogenous hyperleptinemia. *Endocrinology* **151**, 1010-1018.
- Gómez AM, Bénitah JP, Henzel D, Vinet A, Lorente P & Delgado C. (1997). Modulation of electrical heterogeneity by compensated hypertrophy in rat left ventricle. *Am J Physiol* **272**, H1078-1086.
- Gómez AM, Cheng H, Lederer WJ & Bers DM. (1996). Ca²⁺ diffusion and sarcoplasmic reticulum transport both contribute to [Ca²⁺]_i decline during Ca²⁺ sparks in rat ventricular myocytes. *J Physiol* **496 (Pt 2)**, 575-581.
- Gómez-Hurtado N, Fernández-Velasco M, Fernández-Alfonso MS, Boscá L & Delgado C. (2014). Prolonged leptin treatment increases transient outward K⁺ current via upregulation of Kv4.2 and Kv4.3 channel subunits in adult rat ventricular myocytes. *Pflugers Arch* **466**, 903-914.
- Hall ME, Smith G, Hall JE & Stec DE. (2012). Cardiomyocyte-specific deletion of leptin receptors causes lethal heart failure in Cre-recombinase-mediated cardiotoxicity. *Am J Physiol Regul Integr Comp Physiol* **303**, R1241-1250.

- Hardie DG. (2003). Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology* **144**, 5179-5183.
- Hobai IA & O'Rourke B. (2001). Decreased sarcoplasmic reticulum calcium content is responsible for defective excitation-contraction coupling in canine heart failure. *Circulation* **103**, 1577-1584.
- Horwich TB, Fonarow GC, Hamilton MA, MacLellan WR, Woo MA & Tillisch JH. (2001). The relationship between obesity and mortality in patients with heart failure. *J Am Coll Cardiol* **38**, 789-795.
- Ingwall JS. (2009). Energy metabolism in heart failure and remodelling. *Cardiovasc Res* **81**, 412-419.
- Jäger S, Handschin C, St-Pierre J & Spiegelman BM. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A* **104**, 12017-12022.
- Lavie CJ, Alpert MA, Arena R, Mehra MR, Milani RV & Ventura HO. (2013). Impact of obesity and the obesity paradox on prevalence and prognosis in heart failure. *JACC Heart Fail* **1**, 93-102.
- Lavie CJ, McAuley PA, Church TS, Milani RV & Blair SN. (2014). Obesity and cardiovascular diseases: implications regarding fitness, fatness, and severity in the obesity paradox. *J Am Coll Cardiol* **63**, 1345-1354.
- Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM & Kelly DP. (2000). Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* **106**, 847-856.
- Lehnart SE, Maier LS & Hasenfuss G. (2009). Abnormalities of calcium metabolism and myocardial contractility depression in the failing heart. *Heart Fail Rev* **14**, 213-224.
- Leifheit-Nestler M, Wagner NM, Gogiraju R, Didié M, Konstantinides S, Hasenfuss G & Schäfer K. (2013). Importance of leptin signaling and signal transducer and activator of transcription-3 activation in mediating the cardiac hypertrophy associated with obesity. *J Transl Med* **11**, 170.
- Littnerova S, Parenica J, Spinar J, Vitovec J, Linhart A, Widimsky P, Jarkovsky J, Miklik R, Spinarova L, Zeman K, Belohlavek J, Malek F, Felsoci M, Kettner J, Ostadal P, Cihalik C, Spac J, Al-Hiti H, Fedorco M, Fojt R, Kruger A, Malek J, Mikusová T, Monhart Z, Bohacova S, Pohludkova L, Rohac F, Vaclavik J, Vondrakova D, Vyskocilova K, Bambuch M & Dusek L. (2015). Positive influence of being overweight/obese on long term survival in patients hospitalised due to acute heart failure. *PLoS One* **10**, e0117142.
- Liu J, Butler KR, Buxbaum SG, Sung JH, Campbell BW & Taylor HA. (2010). Leptinemia and its association with stroke and coronary heart disease in the Jackson Heart Study. *Clin Endocrinol (Oxf)* **72**, 32-37.

- Louch WE, Hougen K, Mørk HK, Swift F, Aronsen JM, Sjaastad I, Reims HM, Roald B, Andersson KB, Christensen G & Sejersted OM. (2010). Sodium accumulation promotes diastolic dysfunction in end-stage heart failure following Serca2 knockout. *J Physiol* **588**, 465-478.
- Loughrey CM, MacEachern KE, Cooper J & Smith GL. (2003). Measurement of the dissociation constant of Fluo-3 for Ca²⁺ in isolated rabbit cardiomyocytes using Ca²⁺ wave characteristics. *Cell Calcium* **34**, 1-9.
- McGaffin KR, Sun CK, Rager JJ, Romano LC, Zou B, Mathier MA, O'Doherty RM, McTiernan CF & O'Donnell CP. (2008). Leptin signalling reduces the severity of cardiac dysfunction and remodelling after chronic ischaemic injury. *Cardiovasc Res* **77**, 54-63.
- Mishra S, Harris TB, Hsueh WC, Hue T, Leak TS, Li R, Mehta M, Vaisse C & Sahyoun NR. (2015). The Association of Serum Leptin with Mortality in Older Adults. *PLoS One* **10**, e0140763.
- Nakamura A, Rokosh DG, Pacanaro M, Yee RR, Simpson PC, Grossman W & Foster E. (2001). LV systolic performance improves with development of hypertrophy after transverse aortic constriction in mice. *Am J Physiol Heart Circ Physiol* **281**, H1104-1112.
- Nattel S, Maguy A, Le Bouter S & Yeh YH. (2007). Arrhythmogenic ion-channel remodeling in the heart: heart failure, myocardial infarction, and atrial fibrillation. *Physiol Rev* **87**, 425-456.
- Nerbonne JM & Kass RS. (2005). Molecular physiology of cardiac repolarization. *Physiol Rev* **85**, 1205-1253.
- Oreopoulos A, Padwal R, Kalantar-Zadeh K, Fonarow GC, Norris CM & McAlister FA. (2008). Body mass index and mortality in heart failure: a meta-analysis. *Am Heart J* **156**, 13-22.
- Ouvrard-Pascaud A, Sainte-Marie Y, Bénitah JP, Perrier R, Soukaseum C, Nguyen Dinh Cat A, Royer A, Le Quang K, Charpentier F, Demolombe S, Mechta-Grigoriou F, Beggah AT, Maison-Blanche P, Oblin ME, Delcayre C, Fishman GI, Farman N, Escoubet B & Jaisser F. (2005). Conditional mineralocorticoid receptor expression in the heart leads to life-threatening arrhythmias. *Circulation* **111**, 3025-3033.
- Patel SP & Campbell DL. (2005). Transient outward potassium current, 'Ito', phenotypes in the mammalian left ventricle: underlying molecular, cellular and biophysical mechanisms. *J Physiol* **569**, 7-39.
- Perrier E, Kerfant BG, Lalevee N, Bideaux P, Rossier MF, Richard S, Gómez AM & Benitah JP. (2004). Mineralocorticoid receptor antagonism prevents the electrical remodeling that precedes cellular hypertrophy after myocardial infarction. *Circulation* **110**, 776-783.

- Pieske B, Maier LS, Piacentino V, Weisser J, Hasenfuss G & Houser S. (2002). Rate dependence of $[Na^+]_i$ and contractility in nonfailing and failing human myocardium. *Circulation* **106**, 447-453.
- Pogwizd SM & Bers DM. (2004). Cellular basis of triggered arrhythmias in heart failure. *Trends Cardiovasc Med* **14**, 61-66.
- Ravens U & Cerbai E. (2008). Role of potassium currents in cardiac arrhythmias. *Europace* **10**, 1133-1137.
- Rider OJ, Petersen SE, Francis JM, Ali MK, Hudsmith LE, Robinson MR, Clarke K & Neubauer S. (2011). Ventricular hypertrophy and cavity dilatation in relation to body mass index in women with uncomplicated obesity. *Heart* **97**, 203-208.
- Romero-Corral A, Montori VM, Somers VK, Korinek J, Thomas RJ, Allison TG, Mookadam F & Lopez-Jimenez F. (2006). Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. *Lancet* **368**, 666-678.
- Ruiz-Hurtado G, Gomez-Hurtado N, Fernandez-Velasco M, Calderon E, Smani T, Ordonez A, Cachofeiro V, Bosca L, Diez J, Gomez AM & Delgado C. (2012). Cardiotrophin-1 induces sarcoplasmic reticulum Ca^{2+} leak and arrhythmogenesis in adult rat ventricular myocytes. *Cardiovascular Research* **96**, 81-89.
- Sedej S, Heinzel FR, Walther S, Dybkova N, Wakula P, Groborz J, Gronau P, Maier LS, Vos MA, Lai FA, Napolitano C, Priori SG, Kockskemper J & Pieske B. (2010). Na^+ -dependent SR Ca^{2+} overload induces arrhythmogenic events in mouse cardiomyocytes with a human CPVT mutation. *Cardiovasc Res* **87**, 50-59.
- Sierra-Johnson J, Romero-Corral A, Lopez-Jimenez F, Gami AS, Sert Kuniyoshi FH, Wolk R & Somers VK. (2007). Relation of increased leptin concentrations to history of myocardial infarction and stroke in the United States population. *Am J Cardiol* **100**, 234-239.
- Smani T, Calderón-Sánchez E, Gómez-Hurtado N, Fernández-Velasco M, Cachofeiro V, Lahera V, Ordoñez A & Delgado C. (2010). Mechanisms underlying the activation of L-type calcium channels by urocortin in rat ventricular myocytes. *Cardiovasc Res* **87**, 459-466.
- Soliman AT, Omar M, Assem HM, Nasr IS, Rizk MM, El Matary W & El Alaily RK. (2002). Serum leptin concentrations in children with type 1 diabetes mellitus: relationship to body mass index, insulin dose, and glycemic control. *Metabolism* **51**, 292-296.
- Sorokina N, O'Donnell JM, McKinney RD, Pound KM, Woldegiorgis G, LaNoue KF, Ballal K, Taegtmeyer H, Buttrick PM & Lewandowski ED. (2007). Recruitment of compensatory pathways to sustain oxidative flux with reduced carnitine palmitoyltransferase I activity characterizes inefficiency in energy metabolism in hypertrophied hearts. *Circulation* **115**, 2033-2041.

- Stucchi P, Guzmán-Ruiz R, Gil-Ortega M, Merino B, Somoza B, Cano V, de Castro J, Sevillano J, Ramos MP, Fernández-Alfonso MS & Ruiz-Gayo M. (2011). Leptin resistance develops spontaneously in mice during adult life in a tissue-specific manner. Consequences for hepatic steatosis. *Biochimie* **93**, 1779-1785.
- Sweeney G. (2010). Cardiovascular effects of leptin. *Nat Rev Cardiol* **7**, 22-29.
- Tajmir P, Ceddia RB, Li RK, Coe IR & Sweeney G. (2004). Leptin increases cardiomyocyte hyperplasia via extracellular signal-regulated kinase- and phosphatidylinositol 3-kinase-dependent signaling pathways. *Endocrinology* **145**, 1550-1555.
- Tejerina MT, Delgado C, Diez J & Tamargo J. (1984). Inotropic and electrophysiological effects of PY 108-068 on isolated cardiac preparations. *Arch Int Pharmacodyn Ther* **271**, 64-80.
- Thøgersen AM, Söderberg S, Jansson JH, Dahlén G, Boman K, Nilsson TK, Lindahl B, Weinehall L, Stenlund H, Lundberg V, Johnson O, Åhrén B & Hallmans G. (2004). Interactions between fibrinolysis, lipoproteins and leptin related to a first myocardial infarction. *Eur J Cardiovasc Prev Rehabil* **11**, 33-40.
- Tomaselli GF & Marbán E. (1999). Electrophysiological remodeling in hypertrophy and heart failure. *Cardiovasc Res* **42**, 270-283.
- Wannamethee SG, Shaper AG, Whincup PH, Lennon L, Papacosta O & Sattar N. (2014). The obesity paradox in men with coronary heart disease and heart failure: the role of muscle mass and leptin. *Int J Cardiol* **171**, 49-55.
- Witte KK, Byrom R, Gierula J, Paton MF, Jamil HA, Lowry JE, Gillott RG, Barnes SA, Chumun H, Kearney LC, Greenwood JP, Plein S, Law GR, Pavitt S, Barth JH, Cubbon RM & Kearney MT. (2016). Effects of Vitamin D on Cardiac Function in Patients With Chronic HF: The VINDICATE Study. *J Am Coll Cardiol* **67**, 2593-2603.
- Zamora E, Díez-López C, Lupón J, de Antonio M, Domingo M, Santesmases J, Troya MI, Díez-Quevedo C, Altimir S & Bayes-Genis A. (2016). Weight Loss in Obese Patients With Heart Failure. *J Am Heart Assoc* **4**, e002468.
- Zima AV, Bovo E, Bers DM & Blatter LA. (2010). Ca²⁺ spark-dependent and -independent sarcoplasmic reticulum Ca²⁺ leak in normal and failing rabbit ventricular myocytes. *J Physiol* **588**, 4743-4757.

FIGURE LEGENDS

Figure 1. Chronic Leptin treatment impairs cardiac function in control but improves cardiac outcomes after TAC mice. **(A)** Representative M-mode echocardiographic images of Sham and TAC mice treated either with Vehicle or with Leptin. **(B-C)**. Average ejection fraction **(B)** and shortening fraction **(C)** measured by M-mode echocardiography. Data are mean±SEM. (N=6-8 mice * p <0.05, *** p <0.001 vs. Sham Vehicle, † p <0.05, ††† p <0.001 vs. TAC Vehicle, # p <0.05, ## p <0.01 vs. Sham Leptin)

Figure 2. Leptin treatment depresses cellular $[Ca^{2+}]_i$ transients and contraction in Sham but restores it in TAC cardiomyocytes. **(A)** Representative line-scan confocal images of $[Ca^{2+}]_i$ transients obtained in Fluo-3 loaded cardiomyocytes from Sham and TAC mice treated either with Vehicle or with Leptin. **(B)** Average $[Ca^{2+}]_i$ transient amplitude measured as peak F/F_0 , where F is the fluorescence trace and F_0 the fluorescence in the diastolic period in each experimental group. **(C)** Cellular shortening expressed in percentage of cell length in each experimental group. **(D)** Representative line-scan images of cardiac myocytes from each experimental group during field stimulation at 2 Hz and rapid 10 mmol/L caffeine application. **(E)** Average SR content. **(F)** Representative immunoblots (top) and average ratio of protein

levels (bottom) expressed as % of SERCA normalized by GAPDH. (G) Representative immunoblots (top) and average ratio of protein levels (bottom) expressed as % of p-PLB (Ser16) of the total PLB and (H) p-PLB (Thr16) normalized by the total PLB. Data are mean±SEM. (N=4-6 mice; n=50-70 cells in B & C and n=20-40 cells in D. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. Sham Vehicle, † $p < 0.05$, †† $p < 0.001$ vs. TAC Vehicle, # $p < 0.05$, ### $p < 0.001$ vs. Sham Leptin)

Figure 3. Leptin treatment prolonged Action Potential Duration in control mice but reverted it in mice that underwent TAC. (A) Representative action potential recorded in cardiomyocytes from Sham (left) and TAC (right) mice treated either with Vehicle or with Leptin. (B) Average action potential duration (APD) measured at 20% (left), 50% (middle) and 90% (right) of the repolarization. Data are mean±SEM. (N=4-6 mice; n=18-25 cells *** $p < 0.001$ vs. Sham Vehicle, ††† $p < 0.001$ vs. TAC Vehicle, ### $p < 0.001$ vs. Sham Leptin)

Figure 4. TAC-induced alterations of K^+ currents and Kv4.2 and KChIP2 expression are rescued by Leptin. Representative current traces (left) and I-V relationships (right) of I_{to} (A), I_{Kur} (B) and I_{ss} (C) recorded in isolated cardiomyocytes from Sham (left panels) and TAC (right panels) mice treated either with Vehicle or with Leptin. The current density and times scales are the same for Sham and TAC groups. Data, normalized to cell capacitance, are presented as mean±SEM. (N=4-6 mice; n=10-24 cells * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs. Vehicle treated group). See the text for statistical comparisons between Sham and TAC. Representative immunoblots (top) and average protein levels normalized by GAPDH and expressed as % of Sham vehicle (bottom) for Kv4.2 (D) and KChIP2 (E) in cardiac tissue from Sham and TAC mice treated either with Vehicle or with Leptin. Data are mean±SEM. (N=3-4 mice ** $p < 0.01$, *** $p < 0.001$ vs. Sham Vehicle, †† $p < 0.01$, vs. TAC Vehicle, # $p < 0.05$, ## $p < 0.01$ vs. Sham Leptin)

Figure 5. Leptin treatment reduced the occurrence of trigger activities and spontaneous Ca^{2+} waves induced by TAC. (A) Representative example of delayed post-depolarization (DAD) and spontaneous action potential (TA, triggered activity) recorded in ventricular cardiomyocytes isolated from TAC mouse. (B) Percentage of cells eliciting TA (N=4-6 mice; n=19-28 cells). The number of cells with TA/total analyzed cells is noted above each bar. (C) Representative example of spontaneous Ca^{2+} wave recorded in TAC cardiomyocyte at rest. (D) Percentage of cardiomyocytes presenting Ca^{2+} waves (N=4-6 mice; n=46-67 cells). The number of cells with Ca^{2+} waves/total analyzed cells is noted above each bar. (E). Representative immunoblots (top) and average ratio of protein levels (bottom) expressed as % of NCX normalized by GAPDH. Data are presented as percentage. *** $p < 0.001$, ** $p < 0.01$ vs. Sham Vehicle, ††† $p < 0.001$ vs. TAC Vehicle, ### $p < 0.001$ vs. Sham Leptin).

Figure 6. Chronic Leptin treatment reduces the TAC-induced CaMKII activation and RyR2 phosphorylation. (A-B) Representative immunoblots (top) and average ratio of protein levels (bottom, expressed as % of p-CaMKII (Thr286) of the total CaMKII in (A) and p-RyR2 (Ser2815) normalized by the total RyR2 (B) in hearts (N=3-4 mice) from Sham and TAC mice treated either with Vehicle or with Leptin. (C) Representative line-scan images presenting Ca^{2+} sparks recorded in cardiomyocytes from Sham (left 2 panels) and TAC (right 2 panels) mice treated either with Vehicle or with Leptin, as indicated in the labels below each image. Average Ca^{2+} sparks frequency per second and per 100 μm (D), their amplitude as peak F/F_0 , (E), and Ca^{2+} sparks frequency per second and per 100 μm normalized to SR load measured as F/F_0 (F) in each studied group (N=4-6 mice; n=50-70 cells). Data are mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. Sham Vehicle, † $p < 0.05$, ††† $p < 0.001$ vs. TAC Vehicle, # $p < 0.05$, ### $p < 0.001$ vs. Sham Leptin

Figure 7. Ca^{2+} spark duration and width. Average Ca^{2+} sparks duration and width in each studied group (N=4-6 mice; n=50-70 cells). Data are presented as mean \pm SEM. *** $p < 0.001$

vs. Sham Vehicle, †p<0.05, †††p<0.001 vs. TAC Vehicle, ## p<0.01, ###p<0.001 vs. Sham Leptin).

Table 1. Macroscopic parameters of mice, cell capacitances and cardiac Leptin levels.

	Sham Vehicle	Sham Leptin	TAC Vehicle	TAC Leptin
<u>Body weight (g)</u>	28.72±2.75	27.65±2.44	27.99±0.58	26.69±1.70
<u>Tibia length (mm)</u>	22.19±0.12	22.0±0.11	21.86±0.04	21.63±0.09
<u>Heart weight / body weight (mg/g)</u>	7.84±0.45	7.91±0.39	10.23±0.69*	8.78±0.60†
<u>Heart weight/tibia length (mg/mm)</u>	10.10±2.35	9.89±2.16	13.11±2.85*	11.06±1.98†
<u>Leptin concentration in heart</u>				
<u>homogenates (pg/μg protein)</u>	376.3±15.39	477.8±4.95*	412.5±37.55	463.4±29.53*
<u>Membrane capacitance (pF)</u>	182.4±7.5	187.2±12.6	220.4±9.9***	183.4±9.2†††

Data are expressed as mean ± SEM. (*p<0.05, ***p<0.001 vs. Sham Vehicle; †p<0.05, †††p<0.001 vs. TAC Vehicle. N=15, n=33 Sham Vehicle; N=14, n=23 Sham Leptin; N= 7, n=34 TAC Vehicle; N=8, n=34 TAC Leptin). N is number of animals, n is number of cells.