Murine model of left ventricular diastolic dysfunction and electromechanical uncoupling following high fat diet

Serena L'Abbate, Nicole Di Lascio, Giuseppina Nicolini, Francesca Forini, Francesco Faita, Claudia Kusmic

CNR Institute of Clinical Physiology, Pisa, Italy

Running title: Cardiac electromechanical impairment in obese mice

Corresponding author:
Claudia Kusmic
Institute of Clinical Physiology
Italian National Research Council
56124 Pisa, Italy
Phone: +39-050-3152669 Fax: +39-050-3152166
Email: kusmic@ifc.cnr.it

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Abstract

Background/Objectives. It is well established that obesity is an independent risk factor for cardiac death. In particular various cardiac alterations have been described in obese patients such as long QT on ECG, impaired diastolic filling of the left ventricle (LV) and all-type arrhythmias. In the present study the above alterations were all reproduced in a mouse model of fat diet-induced obesity.

Animals/Methods. In C57BL6 mice fed on a high fat (n=20, HF–Group) or standard diet (n= 20, C–Group) for 13 weeks, balanced by sex and age, we examined heart morphology and function by high frequency-ultrasounds and electric activity by surface ECG. Besides, the autonomic sympathovagal balance (heart rate variability) and the arrhythmogenic susceptibility to adrenergic challenge (i.p. isoproterenol) were compared in the two groups as well as glucose tolerance (i.p. glucose test) and liver steatosis (ultrasounds).

Results. Body weight in HF–Group exceeded C–Group at the end of the experiment (+ 28% $p<0.01$). An abnormal ventricular repolarization (long QTc on ECG) associated with impaired LV filling rate and increased LV mass was found in HF–Group as compared to C. Moreover, HF–Group showed higher heart rate, unbalanced autonomic control with adrenergic prevalence and a greater susceptibility to develop rhythm disturbances under adrenergic challenge (i.p. isoprenaline). Impaired glucose tolerance and higher liver fat accumulation were also found in HF mice compared to C.

Conclusions. The described murine model of 13 weeks on HF diet well reproduced the cardiovascular and metabolic disorders reported in clinical obesity suggesting its potential utility as translational mean suitable for testing new pharmacotherapeutic approaches to the treatment of obesity and its comorbidity.

Introduction

Obesity with its associated metabolic disorders is an epidemic condition seriously undermining public health. More than one-third of the world population is overweight or obese, and the threat that obesity places on health care in terms of enhanced risk of cardiovascular (CV) diseases and mortality has now become a global concern[1,2] .

The impact of obesity on the CV system includes a wide range of disorders ranging from impaired cardiac output to increased left ventricle (LV) mass, wall thickness and function[3–5]. Moreover, lengthening of QT interval on ECG has been described in obese patients[6,7]. There is a growing clinical interest in the relationship between adipose accumulation and cardiac electrical dysfunction. The latter contributes to the general picture of electrical instability associated with greater susceptibility to arrhythmias and higher incidence of sudden cardiac death[8–12]. Likewise, numerous studies have shown an association between atrial fibrillation and the increased size of the left atrium as well as LV diastolic dysfunction[8,13]. Despite the clear epidemiological association, the underlying mechanisms linking obesity to the CV alterations are still object of investigation. As matter of fact, several conditions such as arterial hypertension, diabetes and obstructive sleep apnea are frequently associated with obesity, making the specific pathophysiological mechanisms difficult to be identified.

In order to investigate in a more controlled setting the CV alteration associated with obesity and the relative mechanisms, a few different validated animal models have been proposed. Among these, the wild type mouse in which obesity is induced by over nutrition (when energy intake exceeds expenditure) has proved effective in recapitulating most of the cardiac alterations found in clinical obesity in the absence of confounding factors such as atherosclerosis or overt hyperglycemia. Unfortunately, studies using this model have been focused on only one of the many alterations associated to high fat (HF) and/or hypercaloric diet, making it difficult to overview the entire picture and to investigate the relationships between subnormal features. Indeed, some studies have

investigated the effect of short- and long-term HF diet on LV structure or function by echocardiography or hemodynamic measurements[14–18] while other have focused on the HF diet effect of the electrical properties of cardiomyocytes, manifested by the lengthening of the QT period and the ventricular arrhythmogenesis[19]. Alternatively, the interest has been directed towards the specific effect of HF diet-induced obesity on atrial electrical activity[20] or the impairment of autonomic heart rate (HR) control[21].

Therefore, to the best of our knowledge, a broad non-invasive and *in vivo* characterization of the wild type mouse model of HF–induced obesity, describing the structural and functional cardiac alterations in conjunction with common metabolic disorders associated with obesity such as reduced glucose tolerance and liver fat accumulation, is still lacking. In view of these considerations, the aim of this study is a multimodal investigation of electrical, structural and mechanical cardiac functions together with glucose tolerance and liver fat content in the C57BL6 mouse fed with HF diet at three time points (basal, 9 and 13 weeks). In particular, animals are characterized in terms of cardiac morphology and function (ultrasounds), electrical activity, arrhythmogenic susceptibility, and autonomic control of HR (ECG), in the metabolic context of glucose tolerance (glucose test) and liver fat content (ultrasounds).

Material and Methods

Animals and experimental protocol

The study was approved by the Local Ethical Panel (Prot. n° 397/2018-PR) and conforms to principles of laboratory animal care demanded by European Directive and Italian laws.

Forty C57BL/6 mice (sex ratio 1:1, 7–9 week–old) were purchased from Charles Rivers Laboratories (Milano, Italy). See Supplementary Information for full details on animal housing conditions. At the beginning of the 13–week protocol, mice were divided into 2 groups (n=20 each, balanced by sex and age): mice fed with high fat diet (PF4051/D, 60% of energy from fat;

Mucedola srl, Settimo Milanese, Italy) (HF–Group) and mice fed with standard chow (C–Group). Mice were examined for cardiac and metabolic characterization basally (T_0), after 9 weeks of diet (T_1) and again after 13 weeks of diet (T_2) with non–invasive techniques: high-frequency ultrasound (hf–US) imaging, surface ECG recordings, glucose tolerance test. Body weight was assessed prior to each investigation.

At endpoint T_2, mice were challenged with i.p. isoprenaline, a β_1-adrenoreceptor agonist, to test the susceptibility to arrhythmias.

High frequency–ultrasound examinations

All the animals underwent examination with a high-resolution US imaging system (Vevo 3100, FUJIFILM VisualSonics Inc, Toronto, Canada). See Supplementary Information for more details on examination conditions.

Regarding cardiac assessment, images were acquired using B-mode modality with the 40 MHz probe in parasternal long axis and short axis views (Figure 1a–b), and transmitral LV inflow velocities by means of pulse–waved Doppler (Figure 1c). See Supplementary Information for full details on parameters evaluated.

Concerning liver fat content assessment, images from a sagittal projection showing the liver and the right kidney simultaneously were acquired using B-mode modality. Two regions-of-interest were placed in the liver and in the kidney parenchyma respectively, and the ratio between the correspondent mean grey-level intensities was calculated and used as a surrogate index of steatosis degree[22] (that we called steatoscore) (Figure 1d).

Electrocardiographic recordings and analysis

Surface heart's electrical activity was recorded through standard lead configuration (i.e. type I, II, III, aVR, aVL, aVF) using needle electrodes inserted subcutaneously into the limbs of sedated mice

(1% isoflurane). See Supplementary Information for full details on acquisition conditions and measurements assessed.

Measurements included heart rate, P and QRS morphology, PR interval, QRS duration, rate corrected QT interval (QTc) and the rate corrected JT interval (JTc), index of ventricular repolarization. For QTc and JTc calculation the correction equation recommended by Mitchell[23] was used, which is based on the Bazett formula and adapted for mice: $QTc = QT/(RR/100)^{1/2}$

To investigate the sympathovagal balance we assessed heart rate variability (HRV)[24,25]: the beat-to-beat interval variation in 2–min segment of ECG recording was analyzed by the HRV LabChart module (ADInstruments Ltd.). See Supplementary Information for more details.

Arrhythmogenic response to β-adrenergic challenge

To compare the arrhythmic response to β-adrenergic stimulation sedated mice (1% isofluorane in pure oxygen) were challenged with isoproterenol (Isoprenaline Chlohydrate, Monico S.p.A., Venezia, Italy) administered intraperitoneally in bolus at a dose of 2 mg/kg of body weight.

ECG was continuously recorded in a time frame of 15 min, with baseline ECG recordings initiated at 5 min before the isoproterenol injection. See Supplementary Information for more details on arrhythmias' classification.

Glucose tolerance test

To assess systemic responsiveness to glucose loading, intraperitoneal glucose tolerance test (IPGTT) were performed on awake mice fasted for 6 h. See Supplementary Information for more details.

Statistical analysis

Data were analyzed with SPSS Version 23 (IBM, New York, NY, USA). All the parameters fulfilled the test of normality. Data are presented as mean ± standard deviation (SD). Inter-group differences were examined by one-way ANOVA and intra-group longitudinal variations by means of Student's t-test for paired samples. The effect of time per treatment was evaluated by General Linearized Model ANOVA for repeated measurements. Correlation analysis was performed using Pearson's test. Tests were considered statistically significant when $p < 0.05$.

Results

Effect of HF diet on body weight and metabolic parameters

Compared with controls, mice on HF diet gained more body weight over time reaching statistical difference at T_1, which continued through the end of the experiment at 13 weeks (Figure 2a). The ANOVA analysis indicated a significant interaction between groups and time ($p<0.001$). The significant 22% increase in the body weight of control animals over the 13-week period of standard diet ($p<0.001$, T_2 vs T_0) fitted with the standard body weight growth curves for mice matched for strain and age. High fat diet feeding induced a significant extra body weight of 15% and 28% with respect to standard diet at T_1 and T_2 respectively ($p=0.003$ and $p<0.001$ vs standard diet).

In 6–hour fasting animals, the blood glucose was similar in the two groups over the experimental period (137±17 mg/dl in C–Group vs 135±31 mg/dl in HF–Group, determined at T_2, p=ns), with measured levels compatible with those reported as the reference range for mice matched for strain and fasting conditions (100-160 mg/dl). On the other hand, the comparison of the glucose tolerance curves showed a significant impairment of the glycemic homeostasis in HF–Group compared to C-Group as expressed by the areas under the glycemic curves reaching statistical difference from T_1 onwards ($p<0.001$ vs control group at both T_1 and T_2) (Figure 2b).

Steatoscore values were higher for the HF–Group than those in control animals at both T_1 ($p=0.05$ vs C–Group) and T_2 ($p<0.001$ vs C–Group) (Figure 2c). The longitudinal comparison for the HF–

Group showed a progressive significant increase in the hepatic fat content according to the time of diet exposure (0.62 ± 0.04 vs 0.72 ± 0.07, $p<0.005$ T_0 vs T_1 and 0.72 ± 0.07 vs 0.87 ± 0.1, $p<0.001$ T_1 vs T_2).

Effect of HF diet on cardiac hf–US parameters

The results of the hf–US analysis for C– and HF–Groups at T_0, T_1 and T_2 are reported in Table 1. The comparison of all the measurements analyzed at T_0 revealed no differences between the two groups, ruling out any bias in animal allocation into the groups. The same comparison performed at T_1 showed a limited number of significant differences between HF– and C–Groups. In particular, the E/A ratio and the deceleration time (Dt), parameters reflecting the diastolic function, were higher in HF– with respect to C–Group ($p<0.05$ and $p<0.005$ vs C–Group, respectively). However, the ANOVA run at T_2 evidenced higher values of LV mass ($p<0.005$), diastolic thickness (LVPWT and IVST, $p<0.05$ and $p<0.005$, respectively), HR ($p<0.05$) and a marked impairment in LV diastolic filling in HF compared to C–group. In general, all the parameters featuring the entire diastolic filling phase of LV resulted altered: the time of early filling (Dt, $p<0.005$), the total time of mitral flow (MVet, $p<0.05$), and the IVRT ($p<0.05$) were longer and the E/A ratio ($p<0.005$) was higher in HF mice than in controls.

The longitudinal evaluation of the C–Group failed to display any differences with age. Conversely, the same longitudinal analysis of HF–Group pointed out a significant increase according to the time of diet exposure of HR ($p<0.05$ T_2 vs T_0), LVPWT and IVST ($p<0.05$ T_2 vs both T_0 and T_1) and LV mass ($p<0.05$ T_1 vs T_0 and $p<0.05$ T_2 vs both T_0 and T_1). Concerning diastolic function, on the one hand E/A ratio and Dt significantly increased from T_1 onwards ($p<0.001$ and $p<0.05$ T_1 and T_2 vs T_0, respectively), on the other hand, MVet and IVRT resulted significantly prolonged only at the end point (respectively $p<0.05$ T_2 vs both T_0 and T_1 for MVet and $p<0.05$ T_2 vs T_0 for IVRT).

197 *Effect of HF diet on surface ECG parameters*

198 The main ECG characteristics are reported in Table 2 for the two groups at each time point. The
199 comparison of all the parameters analyzed at T_0 revealed agreeable similarity between the two
200 groups. The ANOVA analysis run at T_1 revealed that 9 weeks of HF diet induced a significant
201 decrease of the RR interval with respect to standard diet ($p<0.001$), whose values corresponded to
202 HR of 495 ± 39 bpm for the HF–group and 455 ± 21 bpm for the C–group ($p<0.02$). Moreover, HF–
203 group showed longer QTc and JTc intervals than C–group with total time of ventricle electrical
204 activity (QTc) prolonged by about 15% and time of ventricular repolarization (JTc) lengthened by
205 about 30% with respect to C–group ($p<0.001$ for both). The same comparison repeated at T_2
206 showed a similar pattern of results for RR interval, QTc and JTc ($p<0.001$ for all). No differences
207 were found for the other parameters measured.

208 The longitudinal comparison performed for C–Group failed to reveal any significant difference
209 among the indices analyzed. Conversely, the same evaluation carried on HF–Group indicated that
210 the alterations of RR interval, QTc and JTc arisen from T_1 onwards, without any significant
211 progression at T_2.

212 The FFT spectral analysis of heart rate revealed that at T_0 the normalized power within LFr and HFr
213 was similar between the two groups, as well as the relative strength of the sympathovagal balance
214 indexed by LFr/HFr ratio (Table 2). The ANOVA analysis performed at both T_1 and T_2 resulted in
215 significant difference between mice fed HF or standard diet. In particular, HF diet consumption
216 decreased HRV by increasing normalized LFr power and reducing normalized HFr with respect to
217 control diet ($p<0.001$ for both T_1 and T_2). As a consequence, the balanced properties of the two
218 arms of the autonomic nervous system expressed by LFr/HFr ratio resulted significantly affected by
219 HF diet ($p<0.001$ for both T_1 and T_2), suggesting an unbalanced autonomic control with
220 sympathetic overdrive in the HF–Group.

The longitudinal comparison failed to evidence any difference in the spectra parameters of C–Group over time, while pointed out that spectral alterations observed in HF–Group arisen from T_1 onwards, without any significant progression at T_2 ($p<0.001$ for T_1 and T_2 vs T_0 for all parameters).

Relationship between measured variables affected by HF diet

Correlation matrix of ECG diagnostic signs, morpho–functional LV indices and metabolic parameters significantly affected by HF diet at T_2 is shown in Table 3. All the significant relationships found between variables were positive linear correlations. The metabolic indicators – namely steatoscore and IPGTT– altered by high fat diet correlated well with each other and were associated with changes in cardiac electrical activity (QTc/JTc), impaired autonomic control and altered mitral flow. The sympathovagal unbalance correlated with the LV diastolic filling, but not with LV hypertrophy. Furthermore, in addition to the expected correlation with heart rate, the altered HRV was associated with the lengthening of the ventricular repolarization, despite the positive correction of HR with repolarization markers. The overall alterations of LV diastolic filling were associated with altered ECG indices of repolarization. Out of the analyzed parameters, Dt was the weakest in association with all the other indices, while the MVet emerged as the most reliable mark of diastolic function. The significant correlation between IVRT, QTc/JTc and LV hypertrophy underscored the specific relationship of diastolic relaxation time with both the lengthening of ventricular repolarization and the increase of LV mass. Hypertrophic markers were correlated with diet–induced alterations in cardiac electrical activity, but not with the overall LV diastolic filling impairment, except for IVRT.

Effect of HF diet on the arrhythmogenic response to β-adrenergic stimulation

Serial ECG recordings to test the arrhythmogenic response to adrenergic stimulation were completed at T_2 in control (n=10) and HF mice (n=10). All animals showed regular sinus rhythm at

baseline. Following bolus injection of isoproterenol, a selective β_1-agonist, HR increased in all mice. Despite the significant difference of the basal HR between C– and HF–Groups (466 ± 37 bpm and 504 ± 45 bpm respectively, $p<0.05$), the chronotropic response to isoproterenol was similar in the two groups at both 1 min (711 ± 25 bpm and 709 ± 66 bpm) and 15 min (660 ± 13 bpm and 664 ± 26 bpm in C and HF respectively) post drug challenge, with maximal heart rate reached within the first min. Delayed up to 1–2 min after adrenergic stimulation 40% of mice in the HF–Group developed events of paroxysmal atrial fibrillation and/or flutter (Figure 3). In addition, between 5 and 10 min post isoprenaline, HF–group manifested rhythm and conduction disorders including sinus dysrhythmia (30%), premature ventricular beats, atrio-ventricular dissociation, and Mobitz 2 atrioventricular block. In contrast, no atrial fibrillation or flutter was documented in the control mice, while the only abnormalities recorded were sinus dysrhythmia in one case and Mobits 2 atrioventricular block in another.

Discussion

In the present study we provide a broad description of the cardiac structural, electrical and mechanical alterations induced by high fat diet at 9 and 13 weeks in the wild type mouse. We also describe the autonomic unbalance favoring the sympathetic arm, the enhanced arrhythmogenic susceptibility to β_1-adrenergic stimulation, the reduced glucose tolerance and the increased liver fat accumulation associated with HF diet.

Several studies have used the mouse model of diet-induced obesity to investigate cardiac dysfunction and the underlying mechanisms. However, they were mainly addressed toward either electrical alterations[19,20] or structural and functional remodeling[16–18,26], underscoring the possible relationships between the two aspects.

The technical approach we used, namely electrocardiography and hf–ultrasonography, represents the main strength of this work that provides novel insight into the relation between diastolic

function and electrical repolarization. On the one hand, the ultrasonic approach allows an *in vivo* multiorgan analysis through a safe and nonionizing imaging, particularly useful in longitudinal studies aimed to identify alterations related to obesity progression. On the other hand, surface ECG is the simplest and less expensive technology to provide reliable information on heart rate, heart rate variability, and arrythmogenicity.

QTc and JTc elongation

One of the main results derived from the analysis of the cardiac electrical profile in response to the high-fat dietary regimen was the significant lengthening of the QTc and the JT intervals. These results indicate that, within the ventricular action potential (depolarization and repolarization) the lone repolarization phase was significantly impaired, as indicated by the prolonged JT interval. This alteration was longitudinally evident as early as a 9–week period of HF diet. Although multiple controlled studies demonstrated that QTc and QT were significantly longer in overweight and obese human subjects[27], to our knowledge only one study has previously shown prolonged QT at 12–14 weeks by implanted telemeters, as well as more frequent ventricular ectopic beats in the same animal strain fed with HF diet[19]. The authors attributed the impairment of cardiac repolarization to the decreased expression of voltage-gated potassium channels, suggesting a pro-arrhythmic electrophysiological remodeling in *obese* heart. Unfortunately, the above study did not address the relationship among ECG abnormalities and LV structural and functional changes. Indeed, due to the electro-mechanical coupling of cardiomyocytes, such an evident electric disorder on the ECG tracing should imply mechanical dispersion in the ventricular relaxation phase and should therefore be studied well beyond the outline of electrophysiology. In this context, different clinical evidences of contractile function alterations have been accumulated in patients with genetic or congenital long QT interval syndrome[28–31]. In a retrospective study of patients with clinical suspicion of heart

failure with preserved EF, Wilcox et al[29], found a linear association between a prolonged QTc duration and a tissue Doppler marker of abnormal ventricular relaxation.

Altered LV diastolic function

In our study, by integrating ECG and ultrasound imaging evidences, all the outstanding parameters of altered diastolic function (E/A, MVet and IVRT) positively correlated with prolonged ventricular repolarization (QTc/JTc), confirming in our diet–induced obese mouse model the association observed by Wilcox in long QT syndrome patients. Indeed, in our study concurrently with the QTc and JTc lengthening, mild signs of diastolic dysfunction were evident at 9 weeks of HF diet (E/A ratio and early filling deceleration time) with a progressive worsening at 13 weeks. Besides, the development of hypertrophy, underscored by increased LV mass and wall thickness, may have progressively reduced the ventricular compliance thus contributing to the gradual worsening of diastolic relaxation time.

Thus, the original finding of a positive correlation between elongated QTc/JTc and impaired diastolic filling rate in our study supports the hypothesis that electromechanical coupling might represent the pathophysiologic link between altered myocyte repolarization and abnormal LV relaxation.

Systolic function preserved

Interestingly, we did not find any effect of HF diet on LV systolic performance. This observation suggests that cardiac dysfunction secondary to diet-induced obesity follows, in the first instance, the traits of heart failure with preserved ejection fraction. These results are in line with a previous study reporting the impairment of diastolic, but not systolic function in the same mouse strain after 12 weeks of HF diet[32]. However, conflicting data are present in the literature on this issue. Some studies report no adverse effect of HF diet on LV systolic function over few weeks or even several months of diet[14,15], while others report significant LV systolic dysfunction over a wide range of

duration of HF feeding[17,26,33]. Recently, Ternacle and colleagues[18] in a longitudinal study using echocardiographic radial strain imaging have shown, as early as 5 weeks of HF diet feeding, a significant diastolic dysfunction associated with only subclinical systolic dysfunction and preserved ejection fraction, followed by an overt systolic failure only after 20 weeks.

These heterogeneous and controversial results may be ascribed to different animal age, the use of different imaging techniques and/or to the number and kind of parameters considered. In the present work, state of the art preclinical ultrasound technology was adopted to evaluate cardiac function, using post-processing techniques that allow the calculation of different parameters to maximize the reliability and reproducibility of the results.

Sympathovagal balance (heart rate variability)

In our study, the relationship between HF diet and HRV was explored and, according to the results, a clear sympathovagal unbalance with a prevalence of the sympathetic arm was evident from 9 weeks of fat diet onwards. The linear correlation analysis performed at T_2 indicated that altered autonomic control was associated with the development of both glucose intolerance and hepatic fat accumulation. This finding is in line with the cardiac autonomic dysfunction reported in non-diabetic obese subjects[34–38] and long term HF fed mice[21]. Our analysis revealed also an association between impaired HRV and the disorders of ventricular repolarization (QTc/JTc), independently of HR values. Several studies reported that diabetic patients with sympathetic dysfunction have prolonged QT interval[39–43], as well as obese subjects and patients with essential hypertension[43–46]. These observations suggest the autonomic nervous system as a possible determinant of the duration of the cardiac action potential, although the underlying mechanisms remain elusive.

Arrhythmic response to adrenergic challenge

Both the reduced HRV and lengthening of QTc interval can be associated with higher ectopic ventricular and atrial events in humans[47–49] and in non–obese animal models[50,51]. In our study, despite the lengthening of QTc/JTc and the reduced HRV, no spontaneous atrial and/or ventricular arrhythmic events were recorded under anesthesia in mice fed a HF diet for 13 weeks. A similar observation has been reported in sedated diet–induced or genetic obese mice[20,52]. Conversely, other studies have shown that HF diet in the same mouse strain favored the appearance of ventricular arrhythmias both in conscious and anesthetized animals[19,53].

In our study, according to a previous report[52], arrhythmias were differentially triggered by adrenergic stress in obese or control mice. Obese group was characterized by atrial fibrillation/flutter, sinus node rhythm dysfunction, ventricular ectopic beats and atrio-ventricular conduction defects.

Study limitations

The study has some limitations deserving to be acknowledged. Functional determinations were performed in anesthetized mice. Several studies in the literature reported that the concentration of isoflurane used in this study (i.e., 1.5%) preserved HR and LV function and morphology[54–5657]. Nevertheless anesthesia partly reduces the total power of HRV[57], thus conclusions from the frequency domain analysis should be drawn with caution if used outside of relative comparisons. Moreover, there are significant differences between human and murine electrophysiology, especially in terms of the different contribution of ionic currents of repolarization and the chamber specificity of subtypes of potassium channels[19,58,59]. Nevertheless, mouse models have provided important insights into the genetic and molecular control of human electrophysiology, and notably in long QT syndrome[60].

Conclusion

The present study proves that cardiac dysfunction developed by diet-induced obesity in mice is characterized by diastolic electro–mechanical impairment and greater susceptibility to develop arrhythmias under adrenergic stimulation. The correlations highlighted by our analysis between the different affected parameters related to cardiac, autonomic and metabolic functions, provide new and potentially important information for further mechanistic investigations.

This work clearly shows the potentials of our HF murine model as translational mean suitable for testing new pharmaco–therapeutic approaches to the treatment of obesity and its comorbidity.

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Conflict of interest

None

Figure legends

Figure 1. Ultrasound scans (a) Parasternal long axis view of the left ventricle. LV: left ventricle; RA: right atrium; LA: left atrium; Ao: aortic root. White arrow points to the semilunar valve leaf. (b) Parasternal short axis view of the left ventricle. (**c**) PW-Doppler of transmitral flow velocity obtained in parasternal 4-chamber view. E: peak of early LV filling wave; A: peak of late filling wave; Dt: deceleration time of early filling; IVRT: isovolumic relaxation time; IVCT: isovolumic contraction time; Aet: aortic ejection time. (d) Sagittal scan of liver and right kidney. Mean gray levels within the ROIs (red circles) in the liver and kidney parenchyma were compared.

Figure 2. Histograms show body weight (a), glucose tolerance expressed by the area under the curve (AUC) (b) and hepatic fat accumulation expressed by steatoscore (c) in the control group (white bars) and in HF fed mice (black bars) at the three time experimental time points: basal (T_0), 9 weeks (T_1) and 13 weeks (T_2) of diet. Data are presented as mean ± SD (n=20 for each group).

Figure 3. Representative electrophysiological disorders documented by ECG monitoring under adrenergic challenge. Normal sinus rhythm is shown in (a). Irregular heartbeat manifestations include sinus dysrhythmia (b), flutter (c), atrial fibrillation (d), atrio-ventricular dissociation (e), premature ventricular beat (f), and Mobitz 2 atrio-ventricular block (g). Incidence of arrhythmias following adrenergic challenge was higher in mice fed with HF diet relative to controls.

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