High levels of myocardial antioxidant defense in aging non-diabetic normotensive Zucker obese rats

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Running head: Heart in obese rats

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ABSTRACT

Chronic renal failure often induces left ventricular hypertrophy. We assessed whether the heart is affected in Zucker obese rat, a model of chronic renal failure associated with obesity, glucose intolerance, and insulin resistance without hypertension or hyperglycemia. After systemic blood pressure measurement, the heart, the aorta, and the kidneys were removed from anesthetized 9 and 13 month-old Zucker obese and lean control male rats (n = 33, n = 24, n = 25, and n = 21, respectively). Determination of left ventricular geometry, quantification of myocardium collagen density and measurement of heart antioxidant enzyme activity were made, as well as aorta and kidney parameters. Mean blood pressure remained at a normal range whatever the age and group considered. Whereas kidney structure and function were severely impaired, no sign of myocardial infarction or inflammatory process was noticed. A moderate left ventricular hypertrophy was observed in 13 months-old obese rats. While heart malondialdehyde was stable with age and among groups, antioxidant enzyme activity was higher in obese rats. In conclusion, in absence of hypertensive or hyperglycemic disorders, the heart seems to display a sufficient line of defense against oxidative stress during the development of cardiac hypertrophy.
**Keywords**: Left ventricular hypertrophy; renal failure; oxidative stress; Zucker rats; hyperlipidemia.

**INTRODUCTION**

Zucker obese rats develop, at 3-months and onwards, severe structural alterations of the kidneys and renal impairment leading to renal failure in 9-month-old animals (1-6). We have recently shown that these alterations are initiated in absence of hypertension, diabetes and inflammatory cell infiltration (6), but might derive from an oxidative stress in kidney tissue (5, 7). Oxidative stress is associated with an increase in the production of reactive oxygen species (ROS), such as superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$), two molecules playing a key-role in the generation of toxic ROS during mitochondrial respiration. Cells contain both enzymatic and non-enzymatic anti-oxidants that form the first line of defense against ROS (8). Briefly, superoxide dismutase isoforms (SOD), present in the cytosol (Cu/Zn-SOD) and mitochondria (Mn-SOD), convert superoxide anion to hydrogen peroxide, which is detoxified to H$_2$O by glutathione peroxidase (GPx) and catalase. The activity of GPx requires a supply of reduced glutathione, which is the main intracellular antioxidant.

Left ventricular hypertrophy (LVH) is an independent predictor of cardiovascular morbidity and mortality (9, 10). Chronic renal failure induces LVH
in approximately 40% of the patients, a figure that rises up to 75% by the onset of end-stage renal disease \((11, 12)\). Mild-to-moderate obesity, another predictor of coronary heart disease, congestive heart failure, stroke and cardiovascular death \((13)\), are associated with increased LVH \((14-19)\), even in absence of hypertension or other confounding factors \((20)\).

Due to these relationships between renal failure, obesity, and LVH, we might hypothesize that 9-month-old and older Zucker obese rats develop severe LVH and cardiovascular morbidity. The aim of this study was to investigate the consequences of such severe renal insufficiency and massive obesity on the age-dependent morphological alteration of the heart, with regards to the myocardial oxidant/antioxidant balance, in absence of other confounding factors such as hypertension and diabetes. Using automated image analysis, we measured the development of LVH and the expansion of subendocardial and subepicardial fibrosis in obese and in control lean age-matched littermates, aged 9 and 13 months. In heart homogenates, we determined the amounts of tissue malondialdehyde (MDA), the major product of lipid peroxidation, and of reduced glutathione, and the activity of the Cu/Zn- and Mn-SOD, GPx and catalase. We showed that despite dramatic renal alteration and massive obesity, in the absence of hypertension and hyperglycemia, neither significant structural alteration occurred, nor lipid
peroxidation products increased with age in hearts of Zucker obese rats in which antioxidant defenses were high.

**MATERIAL AND METHODS**

*Animals*

Male Zucker rats were obtained from our local husbandry (INSERM U465, Paris, France). Our obese Zucker rat strain differs from commercial Zucker strains by the absence diabetes\(^{3, 4}\). Lean *Fa/fa* (ZL) and obese *fa/fa* (ZO) rats were identified and selected at 4 weeks of age by visual examination of inguinal fat deposits. They were raised in standard husbandry conditions, fed regular laboratory chow *ad libitum* (M25, Extralabo, Provins, France) and had free access to water. At the time of sacrifice at 9 and 13 months, animals were anesthetized with pentobarbital (i.p., 0.1 mL/100 g body weight). The heart and the kidneys were rapidly removed and weighed, as well as the descending thoracic aorta. Samples of heart tissue were directly frozen in liquid nitrogen for enzyme activity determination. Transversal median sections of heart, aorta, and kidney were fixed in 10% formol, dehydrated at room temperature through ethanol series, and embedded in paraffin. Sections (4-µm thick) were cut and stained with hematoxylin-eosin for routine histology, or with Sirius red or with elastic stain for morphometric study of heart and aorta, respectively. Animal care complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of
Laboratory Animals (National Institutes of Health publication 86-23, revised 1989, authorization 00577, 1989, Paris, France) and followed the newest guiding principles for research (21).

Biological parameters

For determination of renal parameters, one week before sacrifice fasting animals were housed individually in metabolic cages with free access to water. Twenty-four hour urine samples were collected and a blood sample was obtained by orbital sinus puncture into tubes containing heparin.

At time of sacrifice, mean blood pressure was calculated as

\[ MBP = \text{diastolic pressure} + \frac{1}{3} (\text{systolic} - \text{diastolic pressures}) \]

that have been determined using a captor inserted through a catheter into the abdominal aorta. Blood samples, obtained through this catheter, were collected into ethylene-diamine-tetraacetic-acid (EDTA) containing Vacutainers (Becton-Dickinson, Meylan, France) and were centrifuged at 2,500 rpm for 10 min at 4°C.

Plasma and urine creatinine and plasma glucose concentrations were measured on a Synchron CX7 Beckman analyser (Beckman, Fullerton, CA, USA). Proteinuria was determined using the Coomassie Protein Assay Reagent (Pierce, Rockford, IL, USA) with bovine serum albumin as standard.

Histological and morphometric studies
For qualitative assessment of histological alterations, a single investigator who was unaware of the nature of the experimental groups, studied standardized transversal median sections of the heart, stained with hematoxylin-eosin. The geometry of the left ventricle was assessed by computer-assisted morphometry on Sirius Red stained sections, as previously described in our laboratory (22) (Fig. 1). Briefly, each field sent to the image analyzer was transmitted by a video camera connected to a microscope and transformed into a digital binary image. The entire left ventricular surface area was included in one field (final magnification x 15, giving a final resolution of 30 µm per pixel). Thus, left ventricle lumen perimeter (Fig. 1A) and left ventricular surface area were measured (Fig. 1B), using the same sequence of mathematical and morphological analysis operations for each field. This low magnification field was also used for quantification of the large collagen domains such as perivascular and any eventual microscar collagens (Fig. 1C). Morphometric analysis of isolated aortas was performed according to the same procedure. Myocardial interstitial collagen was determined at a magnification of x 250 (final resolution, 0.48 µm per pixel). Collagen surface area was measured by analyzing 20 fields in the subendocardium and 20 fields in the subepicardium for each rat, excluding fields in which there were microscars or coronary arteries (Fig. 1D). Interstitial collagen density was obtained from the ratio of the collagen surface area and the corresponding left ventricular surface area.
Semiquantitative assessment of renal tubulo-interstitial injury in the cortex (including tubular dilatation, atrophic tubular cells, proteinaceous tubular casts, interstitial fibrosis and inflammatory cell infiltrates) was graded on a scale of 0 to 4 (0 = normal; 0.5 = small focal areas of damage; 1 = involvement of less than 10% of the cortex; 2 = involvement of 10 to 25% of the cortex; 3 = involvement of 25 to 75% of the cortex; 4 = extensive damage involving more than 75% of the cortex), in 10 random microscopic fields at X10 magnification to form a semi-quantitative index of tubulo-interstitial damage.

**Immunohistochemistry**

The differentiation and activation of interstitial mononuclear cells were assessed by incubating deparaffinized heart sections with a mouse monoclonal antibody specific for a monocyte/macrophage cytoplasmic marker (ED1 antibody, Serotec, Oxford, UK), diluted 1:1000 in Tris-buffered saline pH 7.4 containing 0.1% bovine serum albumin (BSA, Sigma Chemical, St Louis, MO, USA), for 60 min at room temperature (RT). Then, the sections were washed in Tris-buffered saline and incubated with rabbit anti-mouse immunoglobulin antibody (Dako Corporation, Carpinteria, CA, USA) and alkaline-antiphosphatase alkaline complexes (diluted 1:75) (Dako). The enzyme was revealed with freshly prepared Fast Red Substrate System (Dako) containing 0.33 mg/mL levamisole (Sigma) to reduce the staining background. Sections
were counterstained with hematoxylin. The number of positive cells in squares of 1.105 mm² distributed over the myocardium was counted, with a minimum of 13 squares surveyed per heart section as defined by a preliminary analysis of convergence.

**Oxidative stress parameters**

Small fragments of heart tissue (about 250 mg) were rinsed with 0.15 M NaCl containing 1mM EDTA, frozen and stored in liquid nitrogen for less than three weeks. Tissues were homogenized in ice-bath for 30 sec with 0.3 M perchloric acid HClO₄ using an Ultra Turax homogenizer (Janken Kunkel Ika-Werk, Staufen, Germany). The homogenate was centrifuged at 2,300 g for 15 min at 4°C. Following neutralization with trioctylamine (0.2 v) and trichlorofluoromethane (0.8 v), the supernatant was used for determination of MDA after its conjugation to thiobarbituric acid (TBA) and antioxidant enzyme activities. TBA-MDA was measured by fluorometry according to the technique of Conti et al. (23). In order to minimize within-run variations, each sample was performed in triplicate. Samples from each category were assayed at the same time. Reduced glutathione concentration was determined from frozen heart samples homogenized in 3 volumes of 5% trichloroacetic acid (Merck), according to the technique of Ellman (24) with 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) (Sigma) as reagent. The activities of Cu/Zn- and Mn-SOD, catalase and GPx were measured from frozen heart tissues homogenized in 10 mM
phosphate buffer pH = 7.8, EDTA 1 mM, as described by Thérond et al. (25). Tissue protein content was determined using the Coomassie protein assay.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was carried out using a two-way ANOVA analysis of variance with age and group (genotype) as factors, followed by Bonferroni-Dunn tests (Statview 5.0 software, Abaccus Concept Inc, Berkeley, CA, USA). The overall age effect (A), group effect (G) and interaction (I) reached statistical significance if $P < 0.05$. In cases of interaction between the factors, one-factor analysis of variance was used at one level of the other factor.

RESULTS

As previously observed (4), ZL and ZO rat body weight (BW) rapidly increased in the first months of life, then plateaued at 9-month-onwards in ZO rats, while ZL rat BW still increased between 9 and 13 months. At 13 months, ZO BW was 1.26-fold ZL BW (Table 1). Heart weight, constant between 9 and 13 months, was 10% higher in ZO than in ZL rats (Table 1). Blood pressure remained at a normal range, whatever the age and the genotype of the animals (Table 1). However, systolic blood pressure was slightly higher in ZO than in ZL rats at 9 and 13 months (overall group effect, $P = 0.051$). Plasma glucose
concentration, close to 8 mmol/L in both groups, did not change with age (Table 1).

Histological aspect of heart sections appeared normal and well preserved, with no sign of patent inflammation, scars or necrosis (Fig. 2). With age, cardiac myocytes showed a mild enlargement in ZO rats at 13 months (Fig. 2C-D). Automated image analysis evidenced a 16% increased hypertrophy of the left ventricle in 13-month-old obese rats (ZO vs. ZL, $P < 0.0001$) as compared to the 9-month-old ZL or ZO rats. Collagen density detectable at low magnification, representing only pericoronary collagen domain, microscar collagen being absent in this model, was less than 0.2% of the ventricle surface area, whatever the age or the genotype of the animals (overall genotype effect, $P = 0.37$) (Table 2). Interstitial collagen, evidenced by the increase in microscopic collagen density visualized at a higher magnification, was at 9 months already more developed in the subendocardial domain in ZO than in ZL rats, did not increase afterwards, and occupied 1.5% and 1% of the ventricle surface area, respectively (Table 2). In the subepicardial domain, this interstitial fibrosis remained faint ($\approx 1\%$ of the left ventricle surface area) and identical in both ZL and ZO rats (subendocardial vs. subepicardial domains, overall effect, $P = 0.02$) (Table 2). Thus, the 16% increased LVH was essentially due to a mild enlargement of the cardiomyocytes (Figure 2) and not to fibrosis. No sign of inflammatory cell infiltrates was detectable: less than three macrophages (ED1+)
12 cells) per mm² of heart section were observed, whatever the group or the age of the animals (group effect, \( P = 0.76 \); age effect, \( P = 0.90 \); interaction, \( P = 0.76 \)). LVH was not linked to any modification of the aorta, which lumen surface area to vascular wall surface area ratio remained at constant value, whatever the age or the genotype of the animals (ZL vs. ZO, 9 months: \( 3.24 ± 0.80, n = 10 \), vs. \( 3.11 ± 0.05, n = 9 \); 13 months: \( 3.29 ± 0.13, n = 7 \), vs. \( 3.13 ± 0.18, n = 4 \); age effect, \( P = 0.73 \); genotype effect, \( P = 0.17 \); interaction, \( P = 0.88 \)).

ZO rats showed functional and structural renal impairment as compared to ZL rats with marked increase in proteinuria and tubulo-interstitial lesions and decrease in creatinine clearance (Table 3). That latter parameter worsened with age (p<0.05).

As shown in Figure 3, malondialdehyde, a major product of oxidative stress, and glutathione levels did not change in heart samples, whatever the age or the genotype of the animals. All antioxidant enzyme activities, GPx, catalase, Cu/Zn- and Mn-SOD, were higher in ZO than in ZL samples at 9 and 13 months. Moreover, the Mn-dependent form of SOD was 1.5-fold more active than the Cu/Zn-dependent form in ZO group.
**DISCUSSION**

Between 9 and 13 months of age, Zucker obese rats displayed a moderate LVH, due to a mild enlargement of cardiomyocytes. A faint and diffuse subendocardial fibrosis, with no sign of myocardial infarction or inflammatory process, was observed at 9 months and did not progress afterwards, while subepicardial collagen density was similar in ZL and ZO rat groups and did not vary with age. This contrasts with marked structural and functional renal impairment. Heart tissue malondialdehyde, the major product of lipid peroxidation, did not increase with age and remained at the same level in obese and in control lean littermates. Meanwhile, all antioxidant enzyme activities were high, with a peculiar amplification of the Mn-SOD activity. This indicates that the line of defense against an oxidative stress was increased in Zucker obese rats.

Chronic renal insufficiency is associated with elevated cardiovascular morbidity and mortality (26) in which progressive left ventricular dilation seems to be the first characteristic morphological pattern of dialysis patients (27-32). Cardiac alteration appears to progress rapidly as renal function declines (31, 33-37). It is therefore surprising that, despite drastic renal failure, heart of ZO rat did not present significant structural alteration, besides a moderate LVH. The known, or strongly suspected, causes of the cardiac abnormalities in patients with chronic renal failure are highly diverse and include hypertension and
metabolic disturbances, such as diabetes mellitus, hyperinsulinemia or dyslipidemia. Our Zucker obese rats displayed normal mean systemic blood pressure values, although systolic pressure was slightly, but at the limit of significance ($P = 0.051$), higher in ZO than in ZL rats. This slight increase in systolic blood pressure could explain the LVH we have observed: increasing systolic blood pressure levels, even within ranges close to conventional normotension, were independently associated with progressive LVH in patients with progressive renal disease under dialysis over a 1-yr period (38). Diabetes, another disorder involved in cardiovascular diseases, must be excluded as a primary factor implicated in the LVH development in ZO rats. Actually, the strain developed in our laboratory, despite presenting glucose intolerance and insulin resistance, still controls its glycemia at a normal level at the cost of severe hyperinsulinemia (4-6) and does not display glycated hemoglobin and advanced glycation products (5). Indeed, insulin resistance and hyperinsulinemia might be implicated in LVH of Zucker rats. The angiotensin-converting enzyme inhibitors captopril and enalapril reversed insulin resistance and the associated cardiovascular complications such as cardiac hypertrophy, hypertension and proteinuria in the Zucker obese rats (39) and hyperinsulinemia lowers endothelial-dependent vasodilatation in response to 5HT in chronic hypertriglyceridemic patients (40). The mechanisms by which insulin resistance intervenes on cardiac hypertrophy remain to be clarified. Cardiac inflammation was not observed in our model contrary to what is observed in the kidney (41).
This could partly explain the ventricle hypertrophy and the absence of severe heart alteration that we have observed in Zucker obese rats. Experimental studies have shown that uremic toxins are capable of depressing myocardial function (42, 43). In addition, parathyroid hormone (PTH) may be a potent uremic toxin, which in experimental conditions has several adverse effects on myocardium cell functions and metabolism (44, 45). Furthermore, in dialysis patients, secondary hyperparathyroidism has been implicated in the pathogenesis of left ventricular dysfunction (46). Again, these factors might intervene in a low manner in ZO rats, in which myocardial alteration remained faint. Finally, obesity has been considered to be a type of volume-overload state with increased plasma and circulating volume associated with increased cardiac output and stroke volume (47, 48). This has not been measured in our study. However, it is noteworthy that LVH noticed in ZO rats was not associated to an increased aorta size which reflects the amount of blood in aortic column and which is a parameter of heart workload.

Cardiac output and oxygen consumption are all increased in direct proportion to the gained weight. Studies have demonstrated that subjects with LVH have compromised myocardial oxygen supply because of endothelial dysfunction and diminished coronary vasodilator reserve, even in the absence of angiographic coronary artery disease (16, 49, 50). Animal model studies have also suggested that myocardial ischemia may induce LVH (51), and, conversely, that
LVH may result in more extensive myocardial infarct size (52). The detrimental effect of hypertriglyceridemia in obesity seems to be due to its enhancing effect on lipoprotein oxidizability (53). A direct association between LVH, oxygen consumption and ROS production probably occurs in ZO heart tissue. Oxidative stress has been demonstrated in ZO kidney, and was one of the key factors leading to the dramatic renal alterations that characterize this strain of rats (5, 7). In the kidney, the activity of Cu/Zn-SOD was increased while the activity of GPx and catalase were decreased, leading to an accumulation of toxic superoxide anion $O_2^{-}$ (5). By contrast, we showed in this study that the heart activities of all antioxidant enzymes were higher, producing a stronger line of defense of the myocardium against oxidative stress, commonly observed during heart failure (54, 55). Moreover, malondialdehyde, the major product of lipid peroxidation, was not increased in ZO heart tissue. On the one hand, our results are in discrepancy with the findings of Vincent et al. (56) who showed an association of obesity and increased myocardial oxidative stress in Zucker obese rats. However, no information was available about the diabetic and/or hypertensive state of the animals, which were used by these authors. As the Zucker strain from commercial suppliers often displays hyperglycemia and mild hypertension, it is therefore likely that oxidative stress observed by Vincent et al. was the consequence of such disorders. On the other hand, our results are in agreement with the observation of Gupta et al. (57) who reported that a higher antioxidative capacity of the ventricle occurs during the stable
phase of hypertrophy with no clinical sign of heart failure. When hearts were challenged with ROS, the hypertrophied hearts showed less functional depression and lipid peroxidation compared to the non-hypertrophied groups (58, 59). During the transition phase between hypertrophy to heart failure, an increase in oxidative stress was evident from the delayed development of heart failure after antioxidant treatment with vitamin E (54). Rats with chronic heart hypertrophy, pretreated with vitamin E, showed a reduction in the rate of mortality, decreased content of MDA and improved myocardial function (60), with a reduced incidence of pathologic dysrhythmias (60, 61). Low concentrations of ROS or exposure for a transient period may stimulate the signal transduction mechanisms both for myocytes function and gene expression. On the other hand, high concentrations of ROS or exposure for a prolonged period seem to produce deleterious effects in both cardiac and vascular myocytes (55).

In conclusion, our results strengthen that in the absence of diabetes or hypertension, the heart in obese and hyperlipidemic rats seems to be able to display a sufficient line of defense against oxidative stress during the development of cardiac hypertrophy.
ACKNOWLEDGEMENTS

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**LEGENDS OF FIGURES**

**Figure 1:** Automated morphometric analysis of left ventricular geometry and collagen deposits. At low magnification (x 15), surface areas of ventricular lumen (A) and myocardium (B) and density of perivascular collagen (C) were automatically determined. At higher magnification (x 250, D), interstitial microscopic collagen density was measured.

**Figure 2:** Histological aspect of left ventricular parenchyma of 9-month-old ZL (A) and ZO (C) rats and of 13-month-old ZL (B) and ZO (D) rats. With age, myocardial cells hypertrophied, more in ZO than in ZL
myocardium, while interstitial fibrosis developed. No sign of cardiac ischemia or scars has been detected. (Sirius red stained sections, x150).

**Figure 3: Malondialdehyde, glutathione, and antioxidant enzymes in heart samples of 9- and 13-month-old ZL and ZO rats.** MDA, measured as thiobarbituric reactive substances (TBARS, see Methods), and glutathione (G-SH) levels did not change (MDA: age effect, $P = 0.06$; genotype effect, $P = 0.10$; interaction, $P = 0.35$. G-SH: age effect, $P = 0.51$; genotype effect, $P = 0.68$; interaction, $P = 0.67$).

By contrast, all antioxidant enzyme activities were higher in ZO rat hearts:

- GPx: age effect, $P = 0.009$; genotype effect, $P = 0.02$; interaction, $P = 0.07$;
- Catalase: age effect, $P = 0.32$; genotype effect, $P = 0.0001$; interaction, $P = 0.083$;
- Cu/Zn-SOD: age effect, $P = 0.04$; genotype effect, $P = 0.003$; interaction, $P = 0.17$;
- Mn-SOD: age effect, $P = 0.37$; genotype effect, $P < 0.0001$; interaction, $P = 0.21$.

$n = 12$ and $12$ ZL rats and $8$ and $11$ ZO rats at 9 and 13 months, respectively.

*: in absence of genotype/age interaction, different from the ZL value at both ages ($P < 0.05$).
Table 1: Physiological and Biological parameters (mean ± SEM)

<table>
<thead>
<tr>
<th>AGE</th>
<th>9 months</th>
<th></th>
<th>13 months</th>
<th></th>
<th>Statistical effect</th>
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<td>LEAN</td>
<td>OBSE</td>
<td>LEAN</td>
<td>OBSE</td>
<td></td>
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<tr>
<td>Body weight (BW)</td>
<td>436 ± 7</td>
<td>621 ± 11$</td>
<td>479 ± 6†</td>
<td>604 ± 21$</td>
<td>Genotype: P &lt; 0.0001</td>
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<td>(g)</td>
<td>n = 24</td>
<td>n = 33</td>
<td>n = 21</td>
<td>n = 25</td>
<td>Age: P = 0.33</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Interaction: P = 0.03</td>
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<td>Heart weight (HW)</td>
<td>1.33 ± 0.06</td>
<td>1.47 ± 0.04 *</td>
<td>1.37 ± 0.04</td>
<td>1.52 ± 0.07 *</td>
<td>Genotype: P = 0.006</td>
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<td>(g)</td>
<td>n = 24</td>
<td>n = 33</td>
<td>n = 21</td>
<td>n = 25</td>
<td>Age: P = 0.36</td>
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<td>Interaction: P = 0.86</td>
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<td>Systolic Blood Pressure</td>
<td>94 ± 2</td>
<td>102 ± 4</td>
<td>99 ± 3</td>
<td>106 ± 5</td>
<td>Genotype: P = 0.051</td>
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<td>(mm Hg)</td>
<td>n = 22</td>
<td>n = 15</td>
<td>n = 20</td>
<td>n = 17</td>
<td>Age: P = 0.23</td>
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<td>Interaction: P = 0.89</td>
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<tr>
<td>Diastolic Blood Pressure</td>
<td>66 ± 2</td>
<td>70 ± 3</td>
<td>71 ± 3</td>
<td>69 ± 5</td>
<td>Genotype: P = 0.69</td>
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<td>(mm Hg)</td>
<td>n = 22</td>
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<td>Interaction: P = 0.38</td>
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<tr>
<td>Mean Blood Pressure 1</td>
<td>75 ± 2</td>
<td>81 ± 3</td>
<td>80 ± 3</td>
<td>81 ± 4</td>
<td>Genotype: P = 0.30</td>
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<tr>
<td>(mm Hg)</td>
<td>n = 24</td>
<td>n = 15</td>
<td>n = 20</td>
<td>n = 17</td>
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<td>Interaction: P = 0.53</td>
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<td>Glycemia</td>
<td>7.85 ± 0.36</td>
<td>7.53 ± 0.34</td>
<td>8.34 ± 0.44</td>
<td>8.03 ± 0.04</td>
<td>Genotype: P = 0.17</td>
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<td>(mmol/L)</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 6</td>
<td>n = 5</td>
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<td>Interaction: P = 0.51</td>
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1 Mean blood pressure = diastolic pressure + 1/3(systolic pressure - diastolic pressure)

*: in absence of genotype/age interaction, significant genotype effect at both ages;

$: different from the age-matched lean value : P < 0.05 ; †: different from 9 months lean values : P < 0.0001.
Table 2: Geometry of the left ventricle and density of pericoronary and interstitial collagen domains (mean ± SEM)

<table>
<thead>
<tr>
<th>AGE</th>
<th>9 months</th>
<th>13 months</th>
<th>Statistical effect</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>LEAN</td>
<td>OBESE</td>
<td>GENOTYPE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P = 0.89</td>
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<td>Lumen surface area (mm²)</td>
<td>36.90 ± 3.21</td>
<td>32.20 ± 2.02</td>
<td>34.89 ± 1.94</td>
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<td>Left ventricle myocardium surface area (mm²)</td>
<td>71.30 ± 2.19</td>
<td>72.52 ± 2.48</td>
<td>68.15 ± 1.83</td>
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<td>Pericoronary collagen density (%)</td>
<td>0.118 ± 0.012</td>
<td>0.096 ± 0.019</td>
<td>0.070 ± 0.009</td>
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<td>Microscopic collagen density Sub-endocardium (%)</td>
<td>0.991 ± 0.123</td>
<td>1.524 ± 0.121</td>
<td>1.041 ± 0.121</td>
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<td>Microscopic collagen density Sub-epicardium (%)</td>
<td>1.057 ± 0.131</td>
<td>1.051 ± 0.142</td>
<td>1.165 ± 0.149</td>
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*: in absence of genotype/age interaction, significant genotype effect at both ages;  
†: different from the lean value : P < 0.0001; #: different from 9 months obese value : P = 0.006 ;  
$: different from 9 months lean value : P = 0.006.
Table 3: Renal parameters (mean ± SEM)

<table>
<thead>
<tr>
<th>AGE</th>
<th>9 months</th>
<th>13 months</th>
<th>Statistical effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LEAN</td>
<td>OBESE</td>
<td>LEAN</td>
</tr>
</tbody>
</table>
| Proteinuria (mg/24h) | 11.6 ± 6.4 | 310 ± 49 * | 16.5 ± 11.8 | 302 ± 51 * | Genotype: \( P < 0.0001 \)  
Age: \( P = 0.97 \)  
Interaction: \( P = 0.86 \) |
|     | (n = 12) | (n = 12) | (n = 13) | (n = 10) |
| Creatininemia (mmol/l) | 58.5 ± 3.1 | 73.0 ± 25.8 $ | 60 ± 5 | 176 ± 0.52 $† | Genotype: \( P = 0.0003 \)  
Age: \( P = 0.013 \)  
Interaction: \( P = 0.008 \) |
|     | (n = 12) | (n = 12) | (n = 13) | (n = 10) |
| Creatinine clearance / Kidney weight (ml/min/g) | 0.527 ± 0.008 | 0.275 ± 0.095 * | 0.337 ± 0.069 | 0.115 ± 0.065 * | Genotype: \( P = 0.0026 \)  
Age: \( P = 0.028 \)  
Interaction: \( P = 0.94 \) |
|     | (n = 12) | (n = 12) | (n = 13) | (n = 10) |
| Scores of tubulo-interstitial lesions | 0.583 ± 0.327 | 3.83 ± 0.17 * | 0.583 ± 0.201 | 3.98 ± 0.020 * | Genotype: \( P < 0.0001 \)  
Age: \( P = 0.71 \)  
Interaction: \( P = 0.71 \) |
|     | (n = 12) | (n = 12) | (n = 13) | (n = 10) |

*: in absence of genotype/age interaction, significant genotype effect at both ages;  
$: $ different from the age-matched lean value: \( P < 0.05 \); †: different from 9 months obese values: \( P < 0.0001 \).
Conti et al. Figure 2
Conti et al., Figure 3.