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Cardiac remodeling associated with chronic kidney disease is enhanced in a rat model of metabolic syndrome: Preparation of mesenchymal transition

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Keywords

Chronic kidney disease · Insulin resistance · Obesity · Cardiac fibrosis · Mesenchymal transition

Abbreviations

CKD Chronic kidney disease; EndMT Endothelial to mesenchymal transition

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Abstract

Cardiac alteration due to chronic kidney disease is described by tissue fibrosis. This remodeling involves myofibroblasts of various origins, including epithelial or endothelial to mesenchymal transitions. In addition, obesity and insulin resistance together or separately seem to exacerbate cardiovascular risk in chronic kidney disease (CKD). The main objective of this study was to assess if pre-existing metabolic disease exacerbates CKD-induced cardiac alterations. In addition, we hypothesised that endothelial to mesenchymal transition participates in this enhancement of cardiac fibrosis. Rats fed cafeteria type diet for 6 months underwent a subtotal nephrectomy at 4 months. Cardiac fibrosis was evaluated by histology and qRT-PCR. Collagens and macrophages were quantified by immunohistochemistry. Endothelial to mesenchymal transitions were assessed by qRT-PCR (CD31, VE-cadherin, α -SMA, nestin) and also by CD31 immunofluorescence staining. Rats fed with cafeteria type regimen were obese, hypertensive and insulin resistant. Cardiac fibrosis was predominant in CKD rats and was highly majored by cafeteria regimen. Collagen-1 and nestin expressions were higher in CKD rats, independently of regimen. Interestingly, in rats with CKD and cafeteria diet we found an increase of CD31 and α -SMA co-staining with suggest an implication of endothelial to mesenchymal transition during heart fibrosis. We showed that rats already obese and insulin resistant had an enhanced cardiac alteration to a subsequent renal injury. Cardiac fibrosis process could be supported by a involvement of the endothelial to mesenchymal transition phenomenon.

Introduction

Diabetes and hypertension are the most common etiologies of chronic kidney diseases [1]. Cardiovascular disease is the leading cause of morbidity and mortality in CKD patient [2] and their prevalence increases with decreasing glomerular filtration rate [3]. Heart failure secondary to CKD define the type 4 cardiorenal syndrome [4]. Cardiomyocytes hypertrophy is a hallmark of cardiac remodeling in the early stages of cardiac alteration. This process, which is a major element involved in type 4 cardiorenal syndrome, induces the activation of cellular signals of metabolic pathways capable of increasing the production of extracellular matrix leading to fibrosis [5, 6]. Interstitial or perivascular cardiac fibrosis is a deleterious phenomenon resulting from the predominance of collagen synthesis compared to its degradation. Accumulation of this fibrosis progressively leads to a loss of compliance of the myocardial wall and a lack of contraction. These phenomena conduce to the appearance of dysfunction and thus promote heart failure progression.

Excessive synthesis of extracellular matrix (ECM) is essentially caused by activated fibroblasts, named myofibroblasts [7]. These specialized fibroblasts are mesenchymal cell presenting a hybrid phenotype, between fibroblast and smooth muscle cell. There are characterized by a granular endoplasmic reticulum and a highly developed golgi apparatus related to a collagen turnover deregulation. Indeed, collagen precursors excess as well as signaling molecules that regulate interaction of the ECM with the parenchyma are observed [8]. On the other hand, these cells exhibit peripheral α -SMA myofilaments conferring them

contractile properties [9], super adhesion type junctions allowing them to communicate, to synchronize their contraction and to form bonds with ECM. These characteristics lead to the tissue remodeling initiation [10]. Myofibroblasts origins are various and among them is the endothelio-mesenchymal transition (EndMT) [11]. Mainly induced by TGF- β , this transition process is associated with a phenotypic change of endothelial or epithelial cells and a cytoskeleton restructuration. Increased expression of mesenchymal markers such as α -SMA, COLL1, fibronectin, vimentin and FSP-1 (fibroblast specific protein-1) is observed. At the same time, these cells lose their specific markers like CD31/PECAM-1 or VE-cadherin. These variations allow detecting the presence of endothelial or epithelial to mesenchymal transitions. Moreover, an immune and inflammatory response accompanied with macrophage infiltration is observed in the cardiac tissue [12]. This may lead to sustained production of various growth factors, including TGF- β [13], thus maintaining the profibrotic mechanisms.

Among the risk factors for cardiovascular diseases such as hypertension and diabetes, insulin resistance and obesity are also known to be major determinants of cardiovascular risk in CKD [14]. The incidence of cardiovascular risk factors and cardiovascular diseases also are higher in diabetic patients [15]. Obesity or occidental rich diet model increase cardiovascular risk factors and progression of CKD [16–18]. CKD is associated with a significant burden of cardiovascular disease, and several studies evaluated the association between metabolic disease, renal impairment and cardiovascular events. These results do not allow to establish or not if cardiovascular risk is enhanced with an insulin resistance phenotype in the CKD population [19]. Heart failure is one of the cardiovascular risk factors consecutive to CKD and involves several mechanisms such as left ventricular hypertrophy, cardiac fibrosis and inflammation [20]. Only few studies reported that insulin-resistance is positively correlated to cardiac hypertrophy in a population of CKD patients [19]. To date, no study evaluated if cardiac remodeling is enhanced in patients presenting several chronic diseases whose prevalence is high in Western countries.

To the best of our knowledge, there is no study evaluating if cardiac fibrosis associated to CKD is worsened in a pre-existing insulin-resistant profile. Therefore, the main objective of the present study was to assess tissue and cell damages in the cardiac muscle resulting from the development of CKD in rats with established obesity and insulin resistance. In addition, we hypothesized that endothelial to mesenchymal transitions (EndMT) processes contribute to cardiac fibrosis formation.

Materials and methods

Ethics

The present experiment complied with the European and French laws (Agreement D34-172-25 and 1562–18,348) and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institute of health (National academies Press US, 8th edition, 2011). Rats were housed in a climate controlled conditions with a 12 h light/ dark cycle in a room temperature.

Animal model

Two groups of 34 male Wistar rats (Charles River France) were fed standard diet (SAFE A04) or a so-called “cafeteria” diet for 6 months. The cafeteria diet consists of a range of appetizing foods intended for human consumption, selected for their high energy, fat, sugar and salt

content (e.g. soda, lard, cake, potato crisps, sweets, cheese). It is very representative of the food excesses observed in the western diet. After 4 months, half of the animals in each diet group (standard or cafeteria) were subjected to a reduction in renal mass by right nephrectomy and ligation of two left renal artery branches, the inferior and superior branches leaving only the posterior branch intact (5/6th Renal ablation and infarction (a/I) surgery).

At the end of the experiment (6 months), rats were anaesthetized (isoflurane 2%) and a catheter was inserted into the right carotid artery for arterial pressure measurement and blood sampling. Then, the heart, left kidney and epididymal adipose tissue were removed, weighed, rinsed and stored at -80°C . The left ventricle (LV) including septum was weighted separately and cut into several pieces. Arterial blood was collected in heparinized sample, centrifuged at 4°C and plasma was stored at -80°C .

Biochemicals analysis

Three days before the end of the protocol, rats were fasted for 5 h and blood was taken from the tail artery for glucose and insulin measurement. Glucose concentration was measured by blood glucose meter (Freestyle Optium). Insulin concentration was determined by ELISA (ultrasensitive rat insulin, Mercodia). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula: $\text{HOMA-IR} = (\text{fasting blood glucose in mg/dl} * \text{fasting plasma insulin in mU/ml}) / 20$ [49].

Plasma creatinine level was determined by colorimetric assay (Cobas 8000 analyser, Roche).

Histochemical and immunohistochemical or fluorescence staining

For cardiac collagen staining, one piece of the LV was formalin- fixed, paraffin-embedded, and 3–5 μm slices were cut for histological analysis. The collagen volume fraction was determined by Sirius red staining (Sigma-Aldrich), and for quantification we divide the Sirius red staining by the total surface of the heart slice [21].

CD68 immunohistochemical staining was used as a marker of rat macrophages and determined in LV tissues, as previously described [22]. For quantification, the same ratio as for Sirius Red was performed. Briefly, anti-CD68 (1:100, Biorad), was incubated overnight at 4°C , revelation was performed with Universal vectastain ABC kit and ImmPACT AEC (Vector Laboratories) following the instructions of suppliers. Then, the sections were mounted in an aqueous mounting medium, VectaMount™ AQ (Vector Laboratories) and examined under a light microscope (Nikon Eclipse TE300). Quantification was performed with Image J software on pictures taken from 10 fields of LV tissue at 200- fold magnification. The area of Sirius red and CD68 staining was measured on LV tissue fields.

Immunofluorescence staining: On formalin-fixed slices, primary antibody anti-CD31 (1:100, ab28364, Abcam) was used for immunofluorescence staining coupled with primary antibody anti- α -SMA (1:200, ab7817, Abcam) and with appropriate secondary antibodies. The slices were mounted with vectashield vibrance mounting medium (Vector H-1800, Vector Laboratories). Fluorescence was observed with Axio-imager microscope (Zeiss). CD31/ α -SMA positive cells were counted for all blood vessels for each slice. We used Image J software for analysis.

Table 1 Primer pairs used for qPCR

Name	Forward primer	Reverse primer
CD31 (PECAM)	5'-CCTTCTGCTCTGTTC AAGCCT-3'	5'-GAGAGGTGGTGGCTGACATCC-3'
COL1A1	5'-GACTGTCCCAAACCCCAAAA-3'	5'-TGGGTCCTCGACTCCTATG-3'
Elastin	5'-GGAAAGTTCC TGGTTCGGT-3'	5'-AACCTTGGCCTTGACTCCTG-3'
FSP1	5'-TCAGCACTTCTCTCTCTGG-3'	5'-TCTGTCTTCTCC CCAGGAA-3'
Gal3	5'-AGCCCAACGC AAACAGTATC-3'	5'-GGCTTCAA CCAGGACTGTA-3'
GDF-15	5'-TGTTCCTGCTGCTCTGTG-3'	5'-TCGCACCTCTGGACTGAGTATC-3'
IL33	5'-CCCTGAGCACATA CAACGACC-3'	5'-CACCATCAGCTTCTTCCCATC-3'
Nestin	5'-TTGGCTTCTTGGACCCCAAG-3'	5'-GGGCTCTGACCTCTGCATTT-3'
RPLP0	5'-CACTGGCTGAAAAGGTCAAAG-3'	5'-GACTTGGTGTGAGGGGCTTA-3'
sST2	5'-CGTTA CCTTCCTGTGCCATT-3'	5'-CTCCATTTC CAATCATGTG-3'
ST2L	5'-ATGATGGCAAATGGAGAAT-3'	5'-TTCTAGACCCCAAGGATGTTT-3'
TGF- β 1	5'-GACCGCAACAACGCAATCT-3'	5'-GACAGCCACTCAGGGTATC-3'
VE-Cadherin (CADH5)	5'-TAGGCAAGTCAAGTCAA GCGT-3'	5'-AGTCTCAGGTTTTCGCCAG-3'
Vimentin	5'-TGGGGCTGCGAGAAAATTG-3'	5'-GGTCAAGACGTGCCAGAGAA-3'
α -SMA	5'-CCGGACTAAGACGGGAATC-3'	5'-TTGTCA CACACCAAGGCAGT-3'

Analysis of mRNA expression by real-time PCR

Immediately after removal and weighing, another piece of the left ventricle was frozen in «RNA later» solution (Life Technologies). Tissues were then dissociated in RLT buffer using a FastPrep Homogenizer (MP Biomedicals). Reverse transcription reactions were performed on 500 ng total RNA using a reverse transcription Takara Kit (Takara Bio Europe) and real-time quantitative PCR was done with the use of the Lightcycler 480 II real time PCR instrument (Roche). Primer pairs were generated using NCBI primer design application and were designed to span an exon-exon junction (Integrated DNA Technologies, Leuven, Belgium). Forward and reverse primers sequences (Table 1) were used at a 60 °C annealing temperature. Data on gene expression were normalized according to RPLP0 housekeeping gene expression.

Statistics

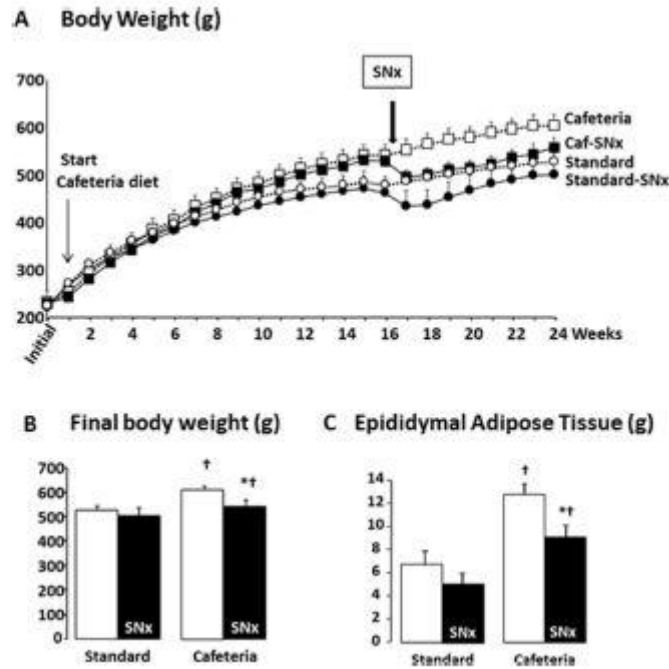
Quantitative data were expressed as mean \pm SEM. Statistical analysis were performed using two factor analysis of variance (ANOVA) and post hoc comparisons were performed by means of Fisher's Protected Least Significant Differences (PLSD) test using STATVIEW software. A *P* value of < 0.05 was considered significant.

Results

Influence of cafeteria regimen and renal mass reduction on metabolic parameters

The increase in body weight was more marked in the cafeteria group from week 6. The difference was maintained after renal mass reduction despite the loss of weight induced by the surgery (Fig. 1A). Compared to their respective standard group, final body weight was significantly higher in rats fed cafeteria diet, irrespective of the renal mass reduction (Fig. 1B). This increase in body mass was associated with elevated adiposity, also independently of the renal mass reduction (Fig. 1C).

Fig. 1 Effect of cafeteria diet on animals weight and adipose tissue. **A** Body weight evolution of rats during the diet. **B** Final body weight. **C** Final epididymal adipose tissue weight. *S/6th* (a/l) subtotal nephrectomy; *Caf* cafeteria diet. * indicates $P < 0.05$ vs intact group in the same diet (*S/6th* (a/l) effect); † indicates $P < 0.05$ vs Standard group with the same renal surgery (diet effect)



As reported in Table 2, elevated creatinine levels were observed in rats with renal mass reduction. As expected, insulin level and HOMA-IR increased in rats fed cafeteria diet, indicating a diet-related insulin resistance phenomenon. Subtotal nephrectomy tended to lower, and to the same extent, plasma insulin and the HOMA-IR, whatever the diet. No modification of blood calcium and phosphorus levels following renal mass reduction was observed. Cafeteria diet was associated with an increase in left kidney weight and more clearly when indexed to tibia length in rats which have not undergone surgery (intact rats). The usual compensatory hypertrophy of the remaining left kidney was observed in the *S/6th* nephrectomy group, and as observed in intact rats, it was more marked in cafeteria fed rats.

Table 2 Metabolic parameters, organs weight and blood pressure after 6 months of Standard or Cafeteria feeding without (Intact) or with later (at 4 months) subtotal nephrectomy (*S/6th* (a/l))

Diet Surgery	Standard intact	Standard <i>S/6th</i> (a/l)	Cafeteria intact	Cafeteria <i>S/6th</i> (a/l)
Plasma creatinine $\mu\text{mol/L}$	34.2 ± 5.1	$54.0 \pm 7.0^*$	31.7 ± 1.2	$70.5 \pm 10.3^*$
Plasma calcium	2.40 ± 0.05	2.57 ± 0.04	2.38 ± 0.05	2.56 ± 0.10
Plasma phosphorus	1.60 ± 0.06	1.58 ± 0.09	1.57 ± 0.04	1.80 ± 0.25
Glycaemia mg/dL	90 ± 2	94 ± 2	95 ± 2	91 ± 3
Insulin mU/L	92 ± 17	66 ± 22	$177 \pm 13^\dagger$	$112 \pm 19^{\dagger*}$
HOMA-IR	3.3 ± 0.8	2.6 ± 1.0	$6.9 \pm 0.6^\dagger$	$4.4 \pm 0.7^{\dagger*}$
Final body weight g	527 ± 17	500 ± 35	$610 \pm 13^\dagger$	$539 \pm 23^{\dagger*}$
Left kidney Wt mg	1290 ± 34	1477 ± 82	1332 ± 34	$1833 \pm 160^{\dagger*}$
Left kidney Wt index $\text{mg}\cdot\text{cm}^{-1}$	290 ± 7	341 ± 20	$321 \pm 10^\dagger$	$444 \pm 43^{\dagger*}$
Heart Wt mg	1093 ± 45	1285 ± 49	1188 ± 30	$1450 \pm 61^*$
Heart Wt index $\text{mg}\cdot\text{cm}^{-1}$	254 ± 10	297 ± 12	$286 \pm 8^\dagger$	$333 \pm 17^{\dagger*}$
Left ventricular Wt mg	793 ± 37	$972 \pm 34^*$	868 ± 22	$1067 \pm 48^*$
LV Wt index $\text{mg}\cdot\text{cm}^{-1}$	184 ± 9	$224 \pm 8^*$	209 ± 6	$260 \pm 13^*$
RV Wt index $\text{mg}\cdot\text{cm}^{-1}$	43 ± 1	47 ± 2	49 ± 2	$54 \pm 2^*$
Tibia length cm	4.30 ± 0.04	4.33 ± 0.03	4.16 ± 0.04	$4.12 \pm 0.04^\dagger$
Mean arterial pressure mmHg	106 ± 10	$139 \pm 24^\dagger$	105 ± 6	114 ± 9
Pulse pressure mmHg	33 ± 2	$54 \pm 4^\dagger$	37 ± 3	$42 \pm 3^\dagger$

HOMA-IR: homeostatic model assessment for insulin resistance; Wt: weight; LV: left ventricle; RV: right ventricle; MAP: Mean arterial pressure. * indicates $P < 0.05$ vs intact group in the same diet (*S/6th* (a/l) effect); † indicates $P < 0.05$ vs Standard group with the same renal surgery (diet effect)

Cardiac morphological and histological alterations following cafeteria regimen and renal mass reduction

Regarding cardiac parameters, we observe that heart weight indexed to tibia length was higher in cafeteria rats with or without subtotal nephrectomy (Table 2). The cardiac hypertrophy was mainly due to left ventricular hypertrophy. Interestingly, the rise in heart weight index in nephrectomised rats was significantly enhanced in rats fed cafeteria diet when compared to those fed standard rat chow. Mean arterial pressure and pulsed pressure were not affected by the cafeteria diet in intact rats. Both pressures were elevated in nephrectomised groups and the rise was lower in rats fed the cafeteria diet (Table 2).

As illustrated in Fig. 2(A–B), Sirius red staining of the left ventricle was significantly higher in cafeteria fed rats without renal mass reduction (intact rats). Subtotal nephrectomy increased the area of Sirius red staining in cafeteria fed rats whereas it devoid of significant effect in standard chow fed rats. Of note, Sirius red staining area was also significantly larger in 5/6th (a/I) fed cafeteria diet compared to 5/6th (a/I) rats on the standard chow.

Concerning markers of inflammation, we observed an elevation of CD68 staining only in rats fed cafeteria diet that have undergone renal mass reduction (Fig. 2C–D).

mRNA Expression of markers of cardiac fibrosis

Markers of cardiac fibrosis expression were shown in Fig. 3A–B. We observed a tendency to elevation of Gal-3 and GDF-15 following high fat diet, and also in 5/6th (a/I) Group (Fig. 3 A–B). As reported in Fig. 3C, a tendencies decrease of IL-33 was observed following nephrectomy, independently of regimen (no significant). Contrastingly, we did not observed elevation of ST2L or sST2 (Fig. 3D–E).

Endothelial to mesenchymal transition implication during heart fibrosis

We performed co-immunostaining between endothelial marker CD31 and smooth muscle marker α -SMA. Quantification analyse of percentage of cells with co-staining showed a significant increase of cells with endothelial to mesenchymal transition in rats group with cafeteria and 5/6th (a/I) treatments (Fig. 4A–B).

Further, we observed an increase of collagen 1 and nestin mRNA expression levels in rats which underwent renal mass reduction, independently of regimen (Fig. 4C–D). Otherwise, we did not observe variations of mRNA expression levels influenced by cafeteria diet or renal mass reduction for the other markers of EndMT (Table 3).

Discussion

In this study we evaluated if cardiac remodeling associated with CKD is worsened when metabolic alterations are already present in rat model of metabolic syndrome. We show that cardiac fibrosis and inflammation associated with CKD are enhanced in our model of metabolic dysfunction profile. We also suggest that EndMT phenomenon is implied in cardiac fibrosis process.

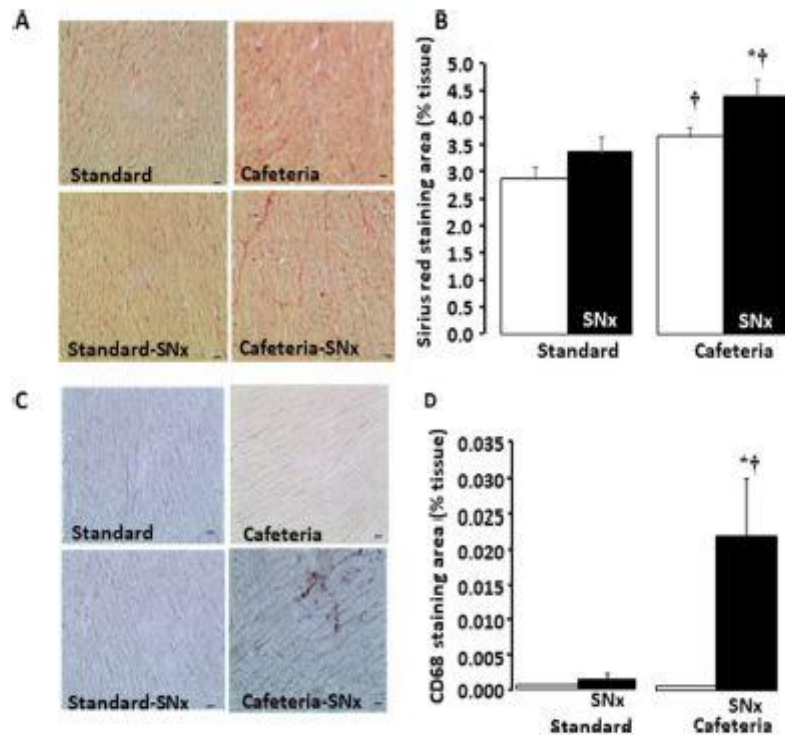
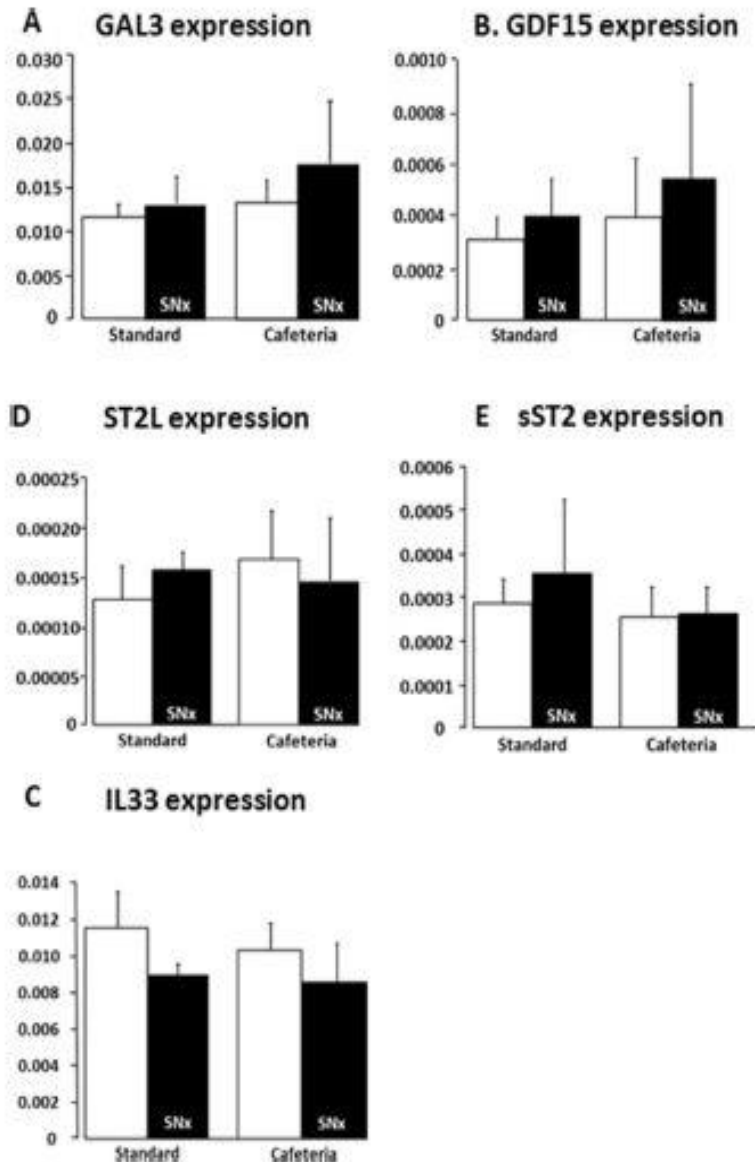


Fig.2 Effect of cafeteria diet on heart fibrosis and inflammation during CKD. **A** Sirius red staining area. **B** Histological observation of fibrosis using Sirius red staining (X400). **C** CD68 staining area. **D** Histological observation of macrophages infiltration using CD68 staining (X400). Scale bar 40 μ m. 5/6th (a/I); SNx. * indicates $P < 0.05$ vs intact group in the same diet (5/6th (a/I) effect); † indicates $P < 0.05$ vs Standard group with the same renal surgery (diet effect)

In the present model, the cafeteria type diet administered was rich in fat (lard), sugar (soda) and also in salt (cheese). This type of diet closely reproduces western diet type and obviously led to obesity and insulin resistance in the present rats as reported in Wistar rats [23]. To obtain a chronic kidney disease, 5/6th Renal ablation and infarction (a/I) surgery was realized and as expected, creatinine level was elevated. Although significance level was not achieved, the rise of plasma creatinine induced by 5/6th (a/I) was tendency increased in rats fed cafeteria diet compared to those on regular rat chow. In patients who had undergone unilateral nephrectomy, proteinuria and renal insufficiency were mostly observed in obese patients [24]. Collectively, these observations confirm that pre-existing obesity and insulin resistance may worsen the deterioration of renal function in chronic kidney disease.

Besides its impact of renal function, metabolic disturbance per se may also affect cardiac structure. Associated with metabolic changes, the cafeteria diet resulted in cardiac hypertrophy even in absence of renal mass reduction, thus suggesting a direct effect of the diet. This is in accordance with the increase of LV mass reported in mice fed high fat diet [25] and in Wistar rats fed hypercaloric western-type diet, a regime similar to that used in the present study [26]. On the other hand, heart weight was increased in rats with subtotal nephrectomy as already reported in children [27] and in rats [28]. Yet, the influence of a pre-existing metabolic syndrome on the cardiac alterations consecutively observed in CKD has not been investigated and it represents the main objective of the present study. Rats fed cafeteria diet with higher body weight and HOMA-IR exhibited a more marked increase in heart weight index when submitted to a subsequent impairment of renal function. Taken together, our results confirm that Western-type diet, i.e. insulin resistance and obesity, induces per se a cardiac remodeling and demonstrate that it aggravates the impact of CKD on heart structure.

Fig. 3 Fibrosis signalling during CKD and cafeteria diet. Expression levels of mRNA (relative to RPLP0) for (A) Gal3, (B) GDF15, (C) IL33, (D) ST2L, and (E) sST2. 5/6th (a/D): SNx. No significant change was detected between groups.



Similarly, cardiac fibrosis evidenced by Sirius red staining was enhanced by subtotal nephrectomy (non-significant) or the cafeteria diet (significantly) by themselves and further increased when renal failure occurred in presence of metabolic disturbances. Fibrosis contributes significantly to cardiac remodeling, but promoters involved in these phenomena are still unclear. The ST2L/IL-33 axis showed anti-inflammatory and cardioprotective effects by reducing cardiac hypertrophy and myocardial injury [29], whereas the circulating soluble form of ST2 (sST2) acts as a decoy receptor and has emerged as a prognostic biomarker in patients with heart failure and chronic kidney disease [30, 31]. Furthermore, sST2 is increased in severe obesity and plays an important role in the vascular remodeling associated with obesity [32]. In the present study, ST2L and sST2 expressions were not affected by the regime or renal mass reduction, thus suggesting that they are not involved at this stage. In contrast, the expression of IL-33 appears to be tendencies diminished in 5/6th (a/I) groups. In contrast, Galectin-3 (Gal-3), a member of the lectin family with profibrotic properties [33, 34] seems to be slightly higher in the group with rich diet and 5/6th (a/I) but not significantly. Therefore, the more marked fibrosis in CKD rats previously fed a Western type diet may also result from imbalance between pro- and anti-fibrotic cardiokines.

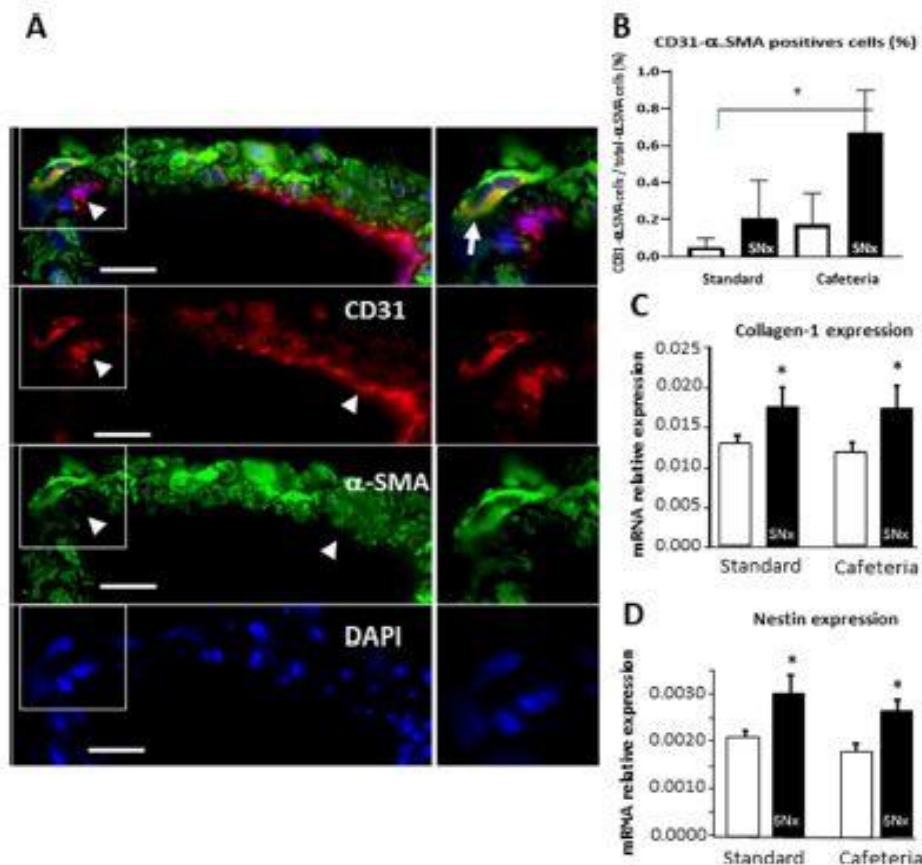


Fig. 4 Involvement of EndMT in Cardiac Fibrosis in the Cafeteria-IRC Diet Model. (A) Immunofluorescence staining on heart slices with anti-CD31 (red), DAPI (blue) and anti- α -SMA (green). Arrow-head showed endothelial cell and arrow showed cell in EndMT. (B) Quantification of co-staining CD31- α -SMA cells/total α -SMA cells

on blood vessels (in percentage). Expression levels of mRNA (relative to RPLP0) for (C) collagen 1 and (D) nestin. 5/6h (a/I): SNx. * indicates $P < 0.05$ vs intact group in the same diet (5/6h (a/I) effect); † indicates $P < 0.05$ vs Standard group with the same renal surgery (diet effect). Scale bar 40 μ m

Table 3 mRNA expression levels of various markers of EndMT after 6 months of Standard or Cafeteria feeding without (Intact) or with later (at 4 months) subtotal nephrectomy (5/6h (a/I))

Diet surgery	Standard intact	Standard 5/6h (a/I)	Cafeteria intact	Cafeteria 5/6h (a/I)
Vimentin	0.05978 ± 0.00585325	0.04881 ± 0.00172646	0.05204 ± 0.00673197	0.05637 ± 0.00558286
Elastin	0.00266 ± 0.00028775	0.00291 ± 0.00022756	0.00336 ± 0.00059862	0.00406 ± 0.00040284
S100A	0.00218 ± 0.0002141	0.00228 ± 0.0000963	0.00230 ± 0.00012959	0.00333 ± 0.00061206
α -SMA	0.06399 ± 0.00573091	0.04868 ± 0.00230651	0.05095 ± 0.00438947	0.05596 ± 0.00569581
Cadherin	0.03825 ± 0.00107763	0.03732 ± 0.00098593	0.03385 ± 0.00725532	0.03833 ± 0.0029136
CD31	0.01580 ± 0.0070668	0.01635 ± 0.0049294	0.01579 ± 0.0070617	0.01699 ± 0.0053729
TGF- β	0.00717 ± 0.00092	0.00835 ± 0.00060	0.00711 ± 0.00146	0.00697 ± 0.00228

No significant difference in any markers was found among groups

In the present model of obesity and insulin resistance, an elevation of arterial pressure does not appear to be a major determinant of cardiac hypertrophy and fibrosis. Conversely, a rise in arterial pressure accompanied cardiac hypertrophy and fibrosis in CKD rats on regular chow. Yet, enhancement of cardiac alterations observed in cafeteria fed rats submitted to later subtotal nephrectomy were not related to an additional rise in arterial pressure. It can be therefore concluded that the worsening of cardiac alterations seen when both pathologies are present is not predominantly due to an increase of arterial hypertension. Inflammation is another factor that could promote cardiac fibrosis. Indeed,

we observe a high CD68 staining, indicator of macrophages infiltrates in CKD rats fed cafeteria type diet. In addition, expression of GDF-15, an inflammatory factor of the TGF- β 1 superfamily [35], had a tendency to increase in the animals which combined rich diet feeding and 5/6th (a/I).

In contrast, in our study, no increase of TGF- β 1 expression was observed in CKD rats, independently of the diet, a finding that does not favour its role in cardiac fibrosis in this situation. The absence of implication of TGF- β 1 pathway remains to be confirmed and regulatory phenomena that may interfere after its expression should be investigated. Of note, BMP-7 may oppose TGF-beta-induced fibrogenesis [36], and administration of exogenous BMP-7 inhibits cardiac fibrosis progression [37]. Since our results were collected at the end of 6 months of cafeteria diet and 2 months of renal mass reduction, an earlier stimulation of TGF- β 1 cannot be excluded and may have participated in cardiac alterations. Taken together, our results suggest that cardiac inflammation associated with a chronic kidney disease is aggravated in presence of obesity and insulin resistance phenotype.

The predominant cellular mediators of cardiac fibrosis are thought to be myofibroblasts. The origin of these myofibroblasts may result from fibroblastic cells transdifferentiated from cells of endothelial origin. By Immunofluorescence experiments, we confirmed this hypothesis. We observed a co-staining with the endothelial marker CD31 and the smooth muscle marker α -SMA especially in rats with nephrectomy and cafeteria diet. These results suggested an implication of the transdifferentiation process during cardiac fibrosis in this model. Interestingly, the EndMT was increased in the condition with high inflammation state (CD68 staining). We reported also an elevation of expression of collagen 1 and nestin in CKD rats, independently of the diet. The EndMT is a complex process and results a reduction in cell junctions objectified by a down regulation of endothelial markers, such as VE-cadherin and CD31, and an increase in cell motility and expression of mesenchyme markers, such as α -SMA and FSP-1 [38]. EndMT plays an important role in development, regeneration and pathological processes, such as fibrosis and tumour stroma formation. Previous studies reported that myofibroblasts involved in reparative and reactive fibrosis are characterized by nestin expression [39–41]. Nestin is an intermediate filament protein that could form heterodimers with other intermediate filament proteins including vimentin and desmin [42]. Several studies reported that nestin was highly expressed during embryogenesis and downregulated or absent in the adult rat [43, 44]. Other studies reported that heart may contain nestin resident progenitor cell population implicated in cardiac remodeling and/or regeneration [45]. Nestin rather α -SMA expression may represent a more appropriated marker of activated fibroblast during reactive fibrosis [46]. Indeed, the preponderance of collagen type 1(+) cells coexpressing nestin rather than α -SMA suggests that α -SMA expression was not required to initiate the reactive fibrotic response [40]. TGF- β 1 is also known to stimulate the myofibroblast transdifferentiation [47]. Despite the lack of increase at the end of our protocol, its participation to EndMT in the initial phase cannot be excluded. According to the results obtained and to the best of our knowledge, we can suggest for the first time that EndMT phenomenon is implied in cardiac fibrosis process in CKD; however, these results have to be confirmed, especially in a kinetic study. To resume, our study shows that a pre-existing metabolic disease amplifies CKD induced-cardiac remodeling, and suggests that endothelial to mesenchymal transition phenomena is implied in the cardiac fibrosis process.

Author contributions

JB, RF, wrote the main manuscript text MP, NG, AC, LJ, RF performed experiments All authors reviewed the manuscript.

Data Availability

All data will be available upon reasonable request by sending an email to: fabrice.raynaud1@umontpellier.fr

Declarations

Competing interests The authors declare that there is no conflict of interest regarding the publication of this paper. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, Saran R, Wang AY, Yang CW (2013) Chronic kidney disease: global dimensions and perspectives. *Lancet* 382:260–272
2. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C (2004) Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 351:1296–1305
3. Stevens LA, Li S, Wang C, Huang C, Becker BN, Bombardieri AS, Brown WW, Rios Burrows N, Jurkovic CT, McFarlane SI, Norris K C, Shlipak M, Whaley-Connell AT, Chen SC, Bakris GL, McCullough PA. Prevalence of CKD and comorbid illness in elderly patients in the United States: results from the Kidney Early Evaluation Program (KEEP) *American Journal of Kidney Diseases*. 2010;55:S23–S33
4. Ronco C, Haapio M, House AA, Anavekar N, Bellomo R (2008) Cardiorenal syndrome. *J Am Coll Cardiol* 52:1527–1539
5. Nishida K, Kyoji S, Yamaguchi O, Sadoshima J, Otsu K (2009) The role of autophagy in the heart. *Cell Death Differ* 16:31–38
6. Dorn GW (2009) Apoptotic and non-apoptotic programmed cardiomyocyte death in ventricular remodeling. *Cardiovasc Res* 81:465–473
7. Krenning G, Zeisberg EM, Kalluri R (2010) The origin of fibroblasts and mechanism of cardiac fibrosis. *J Cell Physiol* 225:631–637
8. Lopez B, Gonzalez A, Diez J (2010) Circulating biomarkers of collagen metabolism in cardiac diseases. *Circulation* 121:1645–1654
9. Sun Y, Kiani MF, Postlethwaite AE, Weber KT (2002) Infarct scar as living tissue. *Basic Res Cardiol* 97:343–347
10. Hinz B (2007) Formation and Function of the myofibroblast during tissue repair. *J Invest Dermatol* 127:526–537
11. Pira-Velazquez S, Jimenez SA (2019) Endothelial to Mesenchymal Transition: Role in Physiology and in the Pathogenesis of Human Diseases. *Physiol Rev* 99:1281–1324
12. Jover B, Reynes C, Rugale C, Reboul C, Jeanson L, Tournier M, Lajoix AD, Desmetz C (2017) Sodium restriction modulates innate immunity and prevents cardiac remodeling in a rat model of metabolic syndrome. *Biochim Biophys Acta* 1863:1568–1574
13. Leask A (2015) Getting to the heart of the matter: new insights into cardiac fibrosis. *Circ Res* 116:1269–1276

14. Parikh NI, Hwang SJ, Larson MG, Meigs JB, Levy D, Fox CS (2006) Cardiovascular disease risk factors in chronic kidney disease: overall burden and rates of treatment and control. *Arch Intern Med* 166:1884–1891
15. Kannel WB, McGee DL. Diabetes and Glucose Tolerance as Risk Factors for cardiovascular Disease: The Framingham Study. *Diabetes Care* 1979;2:120–126.
16. Kurniawan AL, Hsu CY, Rau HH, Lin LY, Chao JC (2019) Association of kidney function-related dietary pattern, weight status, and cardiovascular risk factors with severity of impaired kidney function in middle-aged and older adults with chronic kidney disease: a cross-sectional population study. *Nutr J* 18:27
17. Muga MA, Owili PO, Hsu CY, Rau HH, Chao JC-J (2016) Association between dietary patterns and cardiovascular risk factors among middle-aged and elderly adults in Taiwan: a populationbased study from 2003 to 2012. *PLoS ONE* 11:e0157745
18. Paterson EN, Neville CE, Silvestri G, Montgomery S, Moore E, Silvestri V, Cardwell CR, MacGillivray TJ, Maxwell AP, Woodside JV, McKay GJ (2018) Dietary patterns chronic kidney disease: a cross sectional association in the Irish Nun eye study. *Sci Rep* 8:6654
19. Spoto B, Pisano A, Zoccali C (2016) Insulin resistance in chronic kidney disease: a systematic review. *Am J Physiol Renal Physiol* 311:1087–1108
20. Kaesler N, Babler A, Floege J, Kramann R (2020) Cardiac Remodeling in Chronic Kidney Disease. *Toxins* 12:161
21. Cordaillat M, Rugale C, Casellas D, Mimran A, Jover B (2005) Cardiorenal abnormalities associated with high sodium intake: correction by spironolactone in rats. *Am J Physiol Regul Integr Comp Physiol* 289:1137–1143
22. Oudot C, Lajoix AD, Jover B, Rugale C (2013) Dietary sodium restriction prevents kidney damage in high fructose-fed rats. *Kidney Int* 83:674–683
23. Johnson AR, Wilkerson MD, Sampey BP, Troester MA, Hayes DN, Malowski L (2016) Cafeteria diet-induced obesity causes oxidative damage in white adipose. *Biochem Biophys Res Com* 473:545–550
24. Praga M, Hernandez E, Herrero JC, Morales E, Revilla Y, Diaz- Gonzalez R, Rodicio JL (2000) Influence of obesity on the appearance of proteinuria and renal insufficiency after unilateral nephrectomy. *Kidney Int* 58:2111–2118
25. Ceylan-Isik AF, Kandadi MR, Xu X, Hua Y, Chicco AJ, Ren J, Nair S (2013) Apelin administration ameliorates high fat diet-induced cardiac hypertrophy and contractile dysfunction. *J Mol Cell Cardiol* 63:4–13
26. Goncalves N, Silva AF, Goncalves Rodrigues P, Correia E, Moura C, Eloy C, Roncon-Albuquerque R Jr, Falcao-Pires I, Leite- Moreira AF (2016) Early cardiac changes induced by a hypercaloric Western-type diet in “subclinical” obesity. *Am J Physiol Heart Circ Physiol* 310:H655–H666

27. Mitsnefes MM, Betoko A, Schneider MF, Salusky IB, Wolf MS, Juppner H, Warady BA, Furth SL, Portale AA (2018) FGF23 and Left Ventricular Hypertrophy in Children with CKD. *Clin J Am Soc Nephrol* 13(1):45–52
28. Lin YP, Yu WC, Hsu ME, Tsai HC, Liao CC, Lin CH (2015) Comparative proteomic analysis of rat left ventricle in a subtotal nephrectomy model. *J Chin Med Assoc* 78(4):218–228
29. Kakkar R, Lee RT (2008) The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Drug Discov* 7(10):827–840
30. Bayes-Genis A, de Antonio M, Galan A, Sanz H, Urrutia A, Cabanes R et al (2012) Combined use of high-sensitivity ST2 and NTproBNP to improve the prediction of death in heart failure. *Eur J Heart Fail* 14(1):32–38
31. Plawecki M, Morena M, Kuster N, Chenine L, Leray-Moragues H, Jover B, Fesler P, Lotierzo M, Dupuy AM, Klouche K, Cristol JP (2018) sST2 as a new biomarker of chronic kidney disease-induced cardiac remodeling: Impact on risk prediction. *Mediators Inflamm* 8(2018):3952526
32. Martinez-Martinez E, Miana M, Jurado-Lopez R, Rousseau E, Rossignol P, Zannad F, Victoria Cachafeiro V, Natalia Lopez- Andres N. A role for soluble ST2 in vascular remodeling associated with obesity in rats. *PLoS ONE*, 2013 Nov 12;8(11).
33. Ochieng J, Furtak V, Lukyanov P (2002) Extracellular functions of galectin-3. *Glycoconj J* 19:527–535
34. Sharma UC, Pokharel S, Van Brakel TJ, van Berlo JH, Cleutjens JPM, Schroen B, Andre S, Crijns HJGM, Gabius HJ, Maessen J, Pinto YM (2004) Galectin-3 marks activated macrophages in failure-prone hypertrophied hearts and contributes to cardiac dysfunction. *Circulation* 110:3121–3128
35. Stahrenberg R, Edelmann F, Mende M, Kockskamper A, Dungen HD, Luers C, Binder L, Herrmann-Lingen C, Gelbrich G, Hasenfuss G, Pieske B, Wachter R (2010) The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. *Eur J Heart Fail* 12(12):1309–1316
36. Weiskirchen R, Meurer SK (2013) BMP-7 counteracting TGFbeta1 activities in organ fibrosis. *Front Biosci* 18:1407–1434
37. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, Chandraker A, Yuan X, Pu WT, Roberts AB, Neilson EG, Sayegh MH, Izumo S, Kalluri R (2007) Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 13:952–961
38. Van meeteren LA, ten Dijke P. Regulation of endothelial cell plasticity by TGF- β . *Cell Tissue Res*. 2012;347:177–186.

39. Beguin PC, Gosselin H, Mamarbachi M, Calderone A (2011) Nestin expression is lost in ventricular fibroblasts during postnatal development of the rat heart and re-expressed in scar myofibroblasts. *J Cell Physiol* 227(2): 813–820
40. Chabot A, Hertig V, Boscher E, Nguyen QT, Boivin B, Chebli J, Bissonnette L, Villeneuve L, Brochiero E, Dupuis J, Calderone A (2015) Endothelial and epithelial cell transition to a mesenchymal phenotype was delineated by nestin expression. *J Cell Physiol* 1601–1609
41. Sakairi T, Hiromura K, Yamashita S, Takeuchi S, Tomioka M, Ideura H, Maeshima A, Kaneko Y, Kuroiwa T, Nangaku M, Takeuchi T, Nojima Y (2007) Nestin expression in the kidney with an obstructed ureter. *KI* 72:307–318
42. Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, Boheler KR, Wobus AM (2004) Nestin expression: A property of multi-lineage progenitor cells? *Cell Mol Life Sci* 61:2510–2522
43. Oikawa H, Hayashi K, Maesawa C, Masuda T, Sobue K (2010) Expression profiles of nestin in vascular smooth muscle cells in vivo and in vitro. *Exp Cell Res* 1993(106):1291–1300
44. Sejersen T, Lendahl U (1993) Transient expression of the intermediate filament nestin during skeletal muscle development. *J Cell Sci* 106:1291–1300
45. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal- Ginard B, Kajstura J, Leri A, Anversa P (2002) Chimerism of the transplanted heart. *N Engl J Med* 346:5–15
46. Hertig V (2017) Nestin expression is upregulated in the fibrotic rat heart and is localized in collagen-expressing mesenchymal cells and interstitial CD31(+)-cells. *PLoS One* 12(4):e0176147
47. Sappino AP, Schurch W, Gabbiani G (1990) Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. *Lab Invest* 63:144–161
48. Bujak M, Frangogiannis NG (2006) The role of TGF- β signaling in myocardial infarction and cardiac remodeling. *Cardiovas Res* 74:184–195
49. Cacho J, Sevillano J, de Castro J, Herrera E, Ramos MP (2008) Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am J Physiol Endocrinol Metab* 295:E1269–1276
50. Mizushige K, Yao L, Kiyomoto H, Yu Y, Hosomi N, Ohmori K, Matsuo H (2000) Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model. *Circulation* 101:899–907