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Sodium restriction modulates innate immunity and prevents cardiac remodeling in a rat model of metabolic syndrome

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ABSTRACT

In the view of the relationships between excessive sodium intake, immunity and target organ damage, we hypothesized that reduction in dietary sodium would be beneficial in the prevention of cardiac alterations through a restrained local immunity response in a rat model of metabolic syndrome. Sprague-Dawley rats were fed a 60% fructose diet with either a normal sodium (0.64% NaCl) or a low sodium content (<0.01% NaCl) for 8 weeks. After 4 weeks, rats were infused or not with angiotensin II (200 $ng \cdot kg^{-1} \cdot min^{-1}$, sc) for 4 weeks. Tail-cuff blood pressure was determined in conscious rats. Heart and left ventricle weight, cardiomyocyte size, and cardiac fibrosis were evaluated. We performed a transcriptomic analysis in order to identify differentially regulated cardiac mRNAs between normal and low sodium diets. We validated those results using qPCR and immunohistochemistry.

Angiotensin II-induced blood pressure rise was blunted (~50%) in the low-sodium fed rats while cardiac hypertrophy and fibrosis were prevented. Transcriptomic analysis revealed 66 differentially regulated genes including 13 downregulated genes under the low sodium diet and implicated in the innate immune response. This was confirmed by reduced cardiac macrophages infiltration under the low sodium diet.

Dietary sodium restriction prevents structural alterations of the heart of rats with fructose-induced insulin resistance and angiotensin II-hypertension. The reduction of cardiac inflammation and macrophage infiltration suggests that innate immunity has an important role in the beneficial effect of sodium restriction on cardiac remodeling.

1. Introduction

The metabolic syndrome is defined as a cluster of hemodynamic and metabolic abnormalities [1,2] associated with an increased risk for development of cardiovascular disease, and insulin resistance as a key factor [3,4]. Excessive dietary fructose consumption contributes to obesity in humans [5] and to insulin resistance in rodents [6]. In the rat model, a high fructose diet is associated with abnormal myocardial architecture [7] and cardiac hypertrophy [8] a preclinical disease strongly predictive of cardiovascular morbidity and mortality [9]. In humans [10], insulin resistance is associated with an increase in cardiac collagen content and diastolic dysfunction. Similar alterations are present in the heart of hypertensive patients [11] and rats [12]. In addition, enhanced angiotensin II and insulin resistance interact in the development of cardiovascular disease [13]. Reduction of left ventricular mass was reported to improve the risk of subsequent complications [14] and can be achieved with non-pharmacological maneuvers including a reduction in dietary sodium. Cardiac remodeling was obliterated by dietary sodium restriction in various models of hypertension [15–17] as well as in hypertensive patients [18]. Moreover, the beneficial effect of sodium reduction on the heart was reported in insulin resistance [17] and in a new model of metabolic syndrome, the DahlS.*Z-Lepr*^{fa}/*Lepr*^{fa} rat [19], an effect involving a reduction of oxidative stress [15–17].

In insulin resistance, inflammatory cells infiltrate the heart [20] and cardiac mRNA expression of inflammation factors are enhanced [21], and macrophages are a major source of proinflammatory cytokines, such as TNF-α, IL-6 and IL1-β [22]. In hypertension, inflammatory signals are released through monocytes and the recruitment of lymphocytes into cardiac tissue initiates the fibrosis cascade [10]. Adaptive immunity is also involved in cardiac remodeling, as demonstrated by the blunting of the deleterious effects of Angiotensin II (AngII)-induced hypertension in immune deficient RAG-$1^{-/-}$ [23] and SCID [24] mice.

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We previously reported that sodium restriction reduced macrophage infiltration and the levels of TNF-α and IL-6 in the kidney and adipose tissue of the fructose fed rat [25]. Therefore, in insulin resistance as in hypertension, innate immunity participates in organ damage, and its modulation may explain the beneficial effect of the nutritional intervention. Yet, the implication of immunity deserves further investigation in the context of hypertension, insulin resistance and more widely of metabolic syndrome [26].

In the present study, we hypothesized that local innate immune response accompanies the beneficial effect of dietary sodium restriction on cardiac alterations in a rat model of metabolic syndrome that mimics the human situation [5]. Rats fed a high fructose diet were chronically infused with AngII to induce a clear hypertension, superimposed to insulin resistance. We performed a transcriptomic analysis on left ventricles to evaluate the influence of dietary sodium withdrawal.

2. Methods

Full description of experimental procedures is available in Materials and Methods in the online-only Data Supplement. Two groups of 40 male Sprague-Dawley rats were fed for 8 weeks a 60% fructose diet with either a normal sodium (NS: 0.64% NaCl) or a low sodium content (LS: <0.01% NaCl). After four weeks, twenty rats from each regimen were infused subcutaneously with angiotensin II (AngII, 200 ng·kg^{-1}·min^{-1}) for the remaining four weeks.

The statistical evaluation was 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. If the data were not normally distributed, statistical evaluation was performed by using ANOVA (Kruskall-Wallis) and Mann-Whitney *U* test. Differences were considered significant when $p < 0.05$.

For transcriptomic analysis, statistical analysis was performed using R software version 3.3.0 along with the Limma package. The method used for background correction was based on the normal-exponential convolution model with the saddle-point approximation to maximum likelihood. Normalization was performed using cyclic loess method. Only probes whose signal was considered as higher than background in at least four out of six replicates in at least one condition were selected for further analysis. Within-array replicate probes were replaced with their average. The assessment of differentially expressed mRNAs between NS diet and LS diet was performed using the Limma GLM (Generalized Linear Model) method followed by Benjamini Hochberg correction for multiple testing. Genes with a corrected p value lower than 0.1 were selected for further investigation.

3. Results

3.1. Low sodium diet influences metabolic parameters

After four weeks of fructose regimen, i.e. before AngII infusion, body weight was significantly lower in rats submitted to dietary sodium restriction ($p = 0.0003$). This effect of low sodium persisted during the four next weeks on AngII infusion. Yet, body weight gain and final body weight were similar between hypertensive and normotensive rats on the same diet (Table 1). Food intake was significantly lower (by 10–12%) in both LS groups compared to control NS groups (Table 1). Fasting plasma glucose was not significantly different between groups (Table 1). The response to insulin injection induced a large fall in blood glucose in all rats. However, plasma glucose and AUC decreased to a larger extent in control LS fructose rats compared to the three other groups, which were not different from each other (Supplementary data, Fig. S1).

3.2. Low sodium diet influences cardiovascular parameters

Before AngII-infusion, i.e. after 4 weeks on the fructose diet, systolic tail-cuff pressure was similar in rats on the NS and LS diet (Fig. 1A). As expected, AngII led to an important rise in blood pressure that was significantly reduced in the LS compared to the NS fed group (Fig. 1B, percent change of 22 ± 3 vs 41 ± 4%, respectively).

3.3. Cardiac morphological and histological alterations are prevented by low sodium diet

As expected, heart weight and left ventricle weight as well as HW index (HWI) and left ventricle weight index were significantly higher in rats infused with AngII (Table 1). Yet, the effect of AngII was significantly less marked in LS rats (5–6% increase) compared to rats on NS diet (18–24%). Similarly, cardiomyocyte size was significantly smaller in AngII-infused, LS fructose rats compared to their NS counterparts (Fig. 1C). When the linear relationship between body weight and heart weight was examined, the steepening of the slope in AngII, NS fructose rats was prevented in LS rats (Fig. 1D).

Sirius red area staining of cardiac tissue was not significantly different in LS vs. NS fructose control groups (4.8 ± 0.9 vs 3.9 ± 0.7%, Fig. 1E). AngII infusion was associated with a larger stained area in rats fed the NS but not LS diet (6.5 ± 1.2 vs 4.97 ± 0.8%). Cardiac mRNA expression of TGF-β and SMAD2 were significantly reduced in LS Fructose rats compared to the respective NS Fructose rats infused or not with AngII (Fig. 1F).

3.4. Transcriptomic analysis of cardiac tissue from NS and LS rats

To identify genes involved in the prevention of cardiac alterations under the LS diet, we compared transcriptomes based on the sodium criteria, i.e. from LS and NS ($n = 6$ in each) left ventricles using microarrays. Following data processing and statistical analysis, we obtained a list of 66 transcripts differentially regulated (21 upregulated, 45 downregulated) between NS and LS rats. We subjected this list to Gene Ontology (GO) classification and enrichment analysis, which describes genes in terms of their associated biological processes, molecular function and cellular component. In Fig. 2, we show the classification in terms of biological process with fold enrichment when significant. Genes implicated in localization, transport, development process, immune system process, adhesion, vesicle-mediated transport,

Table 1
Influence of AngII infusion and low sodium diet on experimental parameters in fructose fed rats.

Groups	NS fructose CT	NS fructose + AngII	LS fructose CT	LS fructose + AngII
Final body weight (g)	420 ± 8	418 ± 8	348 ± 8[b]	342 ± 7[b]
BW change from week 4 (g)	56.7 ± 3.0	60.0 ± 4.4	38.6 ± 9.5[b]	32.6 ± 3.3[b]
3-day mean food intake (g/24 h, 8th week)	20.3 ± 0.7	21.6 ± 0.6	17.8 ± 0.5[b]	18.3 ± 0.5[b]
Heart weight (g)	1.17 ± 0.04	1.39 ± 0.04[a]	0.98 ± 0.03	1.02 ± 0.07[b]
Heart weight index (mg/gBW)	2.80 ± 0.06	3.30 ± 0.06[a]	2.78 ± 0.03	3.00 ± 0.07[b]
LV weight (g)	0.89 ± 0.03	1.03 ± 0.03[a]	0.70 ± 0.02[b]	0.75 ± 0.03[b]
LV weight index (mg/gBW)	2.05 ± 0.03	2.48 ± 0.05[a]	2.07 ± 0.04	2.17 ± 0.05[b]
Plasma glucose, mg/dL	123 ± 7	125 ± 4	127 ± 4	120 ± 4

BW: Body Weight; LV: Left Ventricle. Data are mean ± SE ($n = 20$ per group).
[a] $p < 0.05$ vs. the respective control group.
[b] $p < 0.05$ vs. the corresponding NS Fructose group.