SALT-DEPENDENT HYPERTENSION: A COMPARISON OF THE RENAL PROTEOME BETWEEN WISTAR AND SPRAGUE-DAWLEY RATS IN RESPONSE TO HIGH SODIUM DIET

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INTRODUCTION AND AIMS

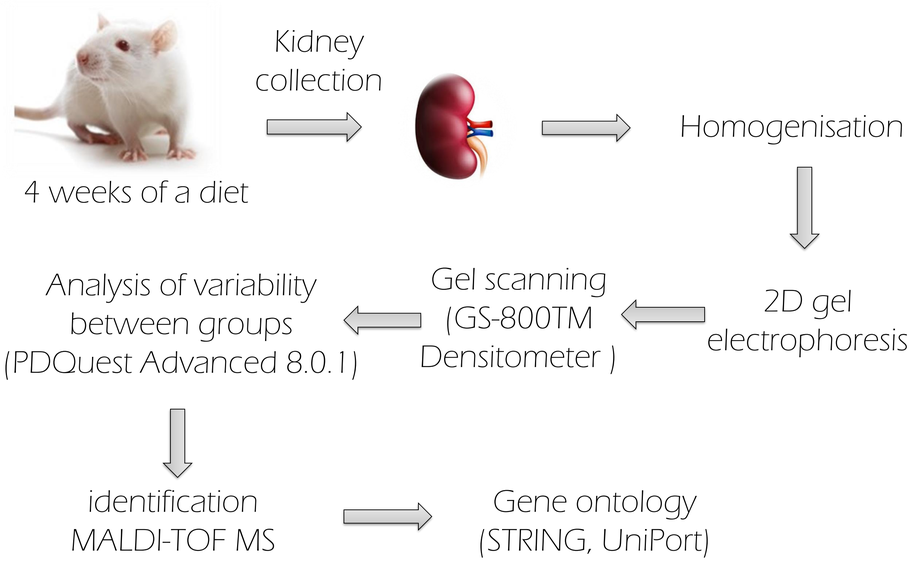
High salt intake constitutes one of the major risk factor for arterial hypertension. There is vast evidence that high sodium intake causes differential blood pressure responses in individual subjects: some can be described as salt-sensitive and some as salt-resistant. However, the background of this variability is still unknown.

Our earlier experiments (unpublished data, Fig.1) showed that 21 days' exposure to high salt diet (HS) induced a significant blood pressure elevation (tail cuff method) in normal Wistar (W) but not in Sprague-Dawley (S-D) rats. These unexpected results inspired us to investigate the subject further and to employ the proteomic approach. In hope to unravel the basis of variable susceptibility to sodium. We have undertaken: first, to identify the potential differences in basal protein content, especially in renal medulla (the region allegedly crucial for blood pressure control) between S-D and Wistar rats fed standard diet (0.25% Na, STD). Second, to determine the possible changes in renal protein expression in response to high sodium diet (4% Na, HS) in both strains.

METHODS

The experimental groups were as follows:
W/STD Wistar rats fed standard diet
S-D/STD Sprague-Dawley rats fed standard diet
W/HS Wistar rats on high sodium diet
S-D/HS Sprague-Dawley rats on high sodium diet

After four weeks' exposure to either diet the animals were sacrificed and the kidneys were excised and weighed; the renal medulla and the cortex were dissected and frozen separately (-80°C). Medullary proteins were separated using two-dimensional electrophoresis, followed by the identification of statistically valid protein spots with the aid of MALDI-TOF mass spectrometry.



CONCLUSIONS

The results of our previous and present proteomic studies suggest that Wistar rats are more susceptible to high sodium intake compared to Sprague-Dawley rats. One of the reasons for S-D rats' resistance to salt loading might be more effective mobilization of proteins acting against oxidative stress and decreased AT1-R availability, due to the activity of hemopexin.

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RESULTS

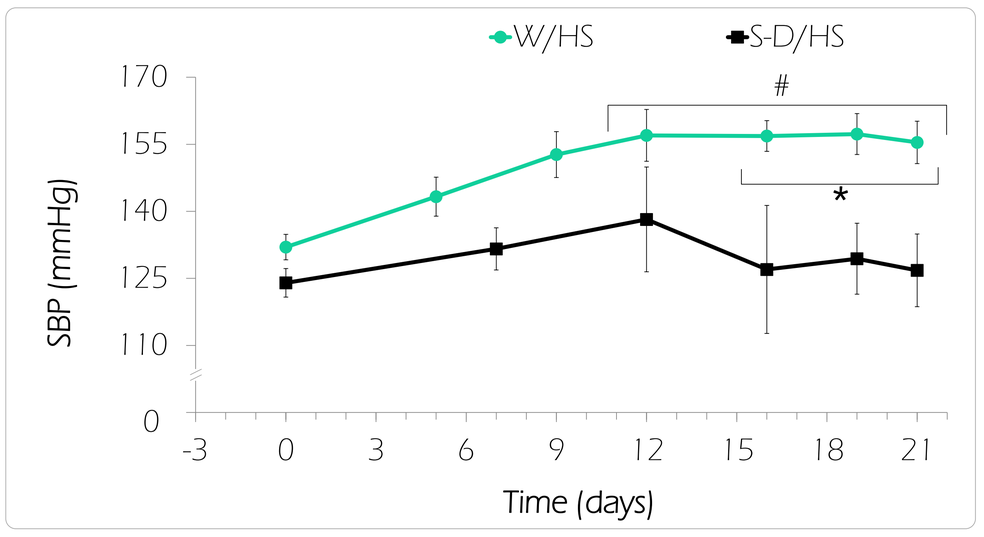

Fig.1: **Systolic blood pressure (SBP)** of Wistar (W) and Sprague-Dawley (S-D) rats fed high sodium diet (HS) for 21 days; * significant for W/HS versus S-D/HS group, # significant in comparison to the value on the day „0" for W/HS group; ANOVA with repeated measurements, followed by Duncan *post-hoc* test (STATISTICA, version 10.0, StatSoft Inc.).

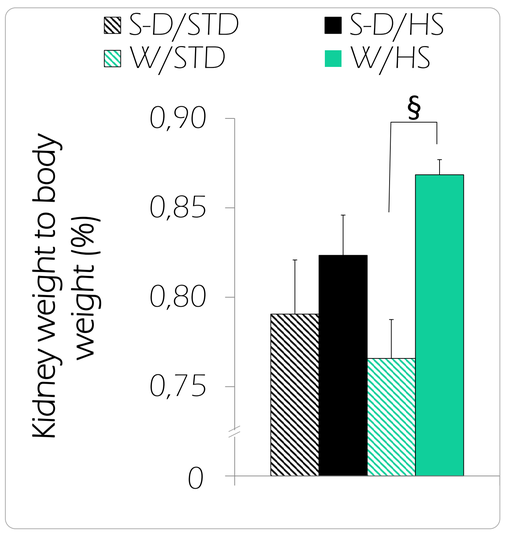

Fig.2: **Kidney weight to body weight ratio (%)** of Wistar (W) and Sprague-Dawley (S-D) rats fed high sodium (HS) or standard diet (STD) for 28 days; **§** significant for W/HS in comparison to W/STD group, one-way ANOVA.

✓ Analysis revealed 47 differentially expressed proteins in Wistar rats fed HS diet (29 down-regulated and18 up-regulated) out of which 28 were identified, whereas in S-D/HS group we observed only 28 (16 down-regulated and 12 up-regulated) out of which 11 was identified

Accession no UniProt/NCBI	Protein name	STD vs. HS Wistar	STD vs. HS Sprague-Dawley
Energetic metabolism-related proteins			
P04639	Apolipoprotein A-I	0.71	1.26
P02650	Apolipoprotein E	0.59	0.29
P10959	Carboxylesterase 1C	0.49	0.47
O88989	Malate dehydrogenase, cytoplasmic	0.34	1.15
P48500	Triosephosphate isomerase	0.06	1.17
P10719	ATP synthase subunit beta, mitochondrial	2.15	0.80
P04764	Alpha-enolase	1.39	1.15
P07335	Creatine kinase B-type	1.85	1.41
P08461	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	1.64	1.42
P56574	Isocitrate dehydrogenase [NADH], mitochondrial	1.86	1.33
Q641Y2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial	1.12	1.66
P49432	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial	1.79 2.18	1.02 0.86
Other proteins			
XP_008767869	Antithrombin-III isoform X1	0.60	0.46
P04897	Guanine nucleotide-binding protein G(i) subunit alpha-2	0.63	0.43
NP_001100276	Glucosidase 2 subunit beta precursor	0.72	0.35
P20059	Hemopexin	0.48 0.50	0.79 1.19
EDM18039	Hemopexin, isoform CRA_f	0.47	0.63
P05544	Serine protease inhibitor A3L	0.47	0.42
Q9EQT5	Tubulointerstitial nephritis antigen - like	1.24	0.36
P55260	Annexin A4	1.33	0.74
Q62667	Major vault protein	2.07	1.77
Q63798	Proteasome activator complex subunit 2	1.26	1.61
P46462	Transitional endoplasmic reticulum ATPase	2.09	0.52

Accession no UniProt/NCBI	Protein name	STD vs. HS Wistar	STD vs. HS Sprague-Dawley
Cell redox homeostasis and response to stress			
P62260	14-3-3 protein epsilon	0.75	0.66
P34058	Heat shock protein HSP 90-alpha	0.48	0.94
P61980	Heterogeneous nuclear ribonucleoprotein K	0.98	0.57
P04785	Protein disulfide - isomerase	0.62	0.49
P11598	Protein disulfide – isomerase A3	0.46	0.49
Q5XIM9	T-complex protein 1 subunit beta	0.45	1.83
Q920J4	Thioredoxin-like protein 1	0.82	0.28
P97532	3-mercaptopyruvate sulfurtransferase	1.03	3.50
P54311	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	1.39 1.32	1.04 1.42
P63018	Heat shock cognate 71 kDa protein	1.85	1.26
Q3KR86	MICOS complex subunit Mic60 (fragment)	1.85	0.96
Q66HF1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	1.46 2.26	0.81 0.99
Q63081	Protein disulfide-isomerase A6	0.74	1.34
P67779	Prohibitin	1.31	2.06
Cytoskeletal and related proteins			
Q63610	Tropomyosin alpha-3 chain	0.50	0.85
P09495	Tropomyosin alpha-4 chain	0.72	0.52
P60711	Actin cytoplasmic 1	5.34	1.02

✓ In S-D/HS group, among proteins upregulated in response to HS diet were the molecules involved in the response to oxidative stress: **prohibitin** (gene name: Phb), 3-mercaptopyruvate sulfurtransferase (Mpst) or NADH dehydrogenase [ubiquinone] iron-sulfur protein 2 (mitochondrial; Ndufs2). In Wistar rats the expression of these proteins was not elevated after exposure to high sodium intake.

✓ Interestingly, **hemopexin**, an acute phase protein, was down-regulated after high-sodium intake only in Wistar rats. Recent study shows that active hemopexin might be considered as a potential determinant of Ang II responsiveness, therefore it might influence the Ang II-mediated hypertension by decreasing AT1-R availability. The effects of a high-salt diet are related to the function of the renin-angiotensin system, which is normally suppressed by a high-salt diet, however the exact mechanisms remain unclear. Possibly, hemopexin decreases AT1-R availability in S-D rats, supporting the maintenance of normal blood pressure level, which makes S-D rats more resistant to sodium overload.

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