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Effect of Short Term High Dietary Salt on Insulin sensitivity in the Peripheral Tissues

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- **Sodium**
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Abstract

It has been noted that high salt intake is allied with the risk of renal failure and cardiovascular diseases. Effects of the salt intake on insulin sensitivity were extensively studied, but findings were changeable and somewhat contradictory. Collectively, the mechanism of salt has modulated insulin sensitivity still so far ambiguous. The current study was designed to evaluate the effect of different sodium diets on insulin sensitivity, adipokines and free radicals in the adipose tissues and skeletal muscles. In this article, rat were distributed into three groups whether received normal sodium (0.45% NaCl, NS), Low sodium (0.02% NaCl, LS) or high sodium diet (8% NaCl, HS) for a period of two weeks. Results demonstrated a remarkable increase in the body weight and fat content of LS in comparison to HS group. Moreover, the LS treated group showed increased level of fasting blood glucose and plasma insulin. Contrariwise HS diet increased adiponectin and reduced the leptin gene expression, as well, the level of angiotensin converting enzyme (ACE). There was no change in nitric oxide (NO) in the skeletal muscle among all groups, while ROS were increased only in the LS group. These data offered the HS intake as another modulator of insulin sensitivity in the insulin sensing tissues. HS regulate insulin sensitivity by modulation of ACE, adiponectin and appetite via reduction of leptin levels in the peripheral tissue.

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Introduction

A battle on the effects of dietary salt on insulin sensitivity draws a mixed overview, as some researchers have reported an increase, others have demonstrated a decreased sensitivity (1). Important to note that researchers found that high dietary sodium intake rise plasma leptin and increases mass of adipose tissue in rats, the same as reported in obese experimental models (2).

It seems that obesity and salt are tightly associated and regulated may use the same or different mechanisms. Obesity is a main risk factor for development of several metabolic disorders including type-2 diabetes, cardiovascular diseases and hypertension (3-7). Third National Health and Nutrition Examination Survey in the United States (NHANES) disclosed that high blood pressure and the increment of BMI are tightly linked as risk factors for hypertension (8, 9). Lipolysis the adipose tissue accompanied by release of higher levels of blood free fatty acids (FFAs) and glycerol. Therefore, increase blood lipid profile increasing the risk of hyperlipemia and nonalcoholic fatty liver (NAFLD) (10).

Adipose tissue as a reservoir for fats secrete a broad spectrum of molecules include hormones, cytokines, and other proinflammatory and inflammatory bioactive compounds (11, 12). In this context, restriction of dietary sodium for a short-term has been reported to rise adipose tissue mass as well as leptin in rodents (2).

The major characteristic features of Type 2 diabetes (T2D) are the high blood insulin levels and insulin resistance in the peripheral tissues, this associated with upregulated levels of the renin-angiotensin system (RAS), which increase prevalence of hypertension, nonalcoholic fatty liver disease (NAFLD) and cardiovascular diseases (13, 14). Accordingly, RAS inhibition impairs insulin signaling, up-regulates NF-kB pathway, and diminishes bioavailability of nitric oxide (NO) leading to vasoconstriction and consequently insulin resistance and impairment of endothelial dysfunction. Thus, the RAS, insulin resistance, and sodium regulation are tightly associated and amalgamate each other (15-17).

We hypothesize that high sodium intake (HS) have positive effects on insulin sensitivity and lipid storage, which can modulate levels of the nitric oxide (NO), reactive oxygen species (ROS) and renin-angiotensin- system (RAS).

Material and Methods

Animals

Thirty male Albino rats were purchased from Qena Breeding Center (Qena, Egypt) at 10 weeks of age. Each group contains 10 animals which whether fed standard rodent diet containing normal sodium (0.45% NaCl, NS) as a control group, Low sodium (0.02% NaCl, LS) or high sodium diet (8% NaCl, HS) for 2 weeks. The rats were kept in cages with average temperature (25°C). Food and water were obtainable ad libitum during the whole study. Treatment of the study groups were continued for 21 days. All treated group were fed Normal sodium diet for 24 hours before slaughtering.

Body composition

Carcasses were individually detached, wrapped and frozen at -20°C. Later on, each carcass was cut into eight equal sized sections and weighed exactly. These sections were dried to constant

weight (4–5 d, typically) as described earlier with minor modifications (18). Briefly, each carcass treated with enough salt and dried at 45 °C, then carcass was individually wrapped in Whatman paper, placed into a protective cotton sack and placed into absolute Ethanol, and placed in boiling water path for 6-8 h. This duration was sufficient to remove all lipids from the carcass.

Each carcass was then thoroughly dried of Methanol and reweighed. Weight loss after being dried was recorded as water weight. Weight loss after being treated with methanol was recorded as carcass lipid weight.

Measurement of biochemical parameters

Angiotensin-I converting enzyme activity (ACE) in the serum was measured spectrophotometrically using a commercial kit from FAR diagnostics according to the manufacturer instructions.

Bilirubin was assessed by measuring bilirubin as previously reported using a commercial kit. Plasma glucose concentration were measured using a handheld glucometer (Contour next; Bayer Vital Co., Basel, Switzerland).

Plasma Insulin

Plasma insulin were measured using the ELISA kits according to the manufacturers' instructions. Absorbance was read on BioTek ELx800 (BioTek Instruments Inc., USA) at 450 nm. Change in fold expression computed by cutoff values of the control group.

Detection of ROS in skeletal muscles

Levels of ROS in the soleus muscles were assessed as previously described. In Brief, 2',7'-dichlorofluorescein (H2DCF) dye was used as a probe, the fluorescence was detected at 490/520 nm and the optical density [a relative fluorescence unit (RFU)] were adjusted to samples protein concentrations and were defined as RFU/μg protein (19).

RNA isolation

Tissue total RNA was extracted using phenol/chloroform method (20), dissolved in dimethyl pyrocarbonate treated water and stored at -80°C for further usage. The RNA concentration and quality of samples were using Nanodrop and a ratio of about 2.0 was considered.

Determination of mRNA levels by real-time RT-PCR

Gene expression for mRNA abundance in the white adipose tissue (WAT) were quantified by real-time RT-PCR using in triplicate in Stratagene Gene Amp PCR system 400 thermal cycler (Perkin-Elmer Corp., Norwalk, CN, USA). Used primer pairs were previously described elsewhere, for Adiponectin 5ʻ-GAGAGAAGGGAGACGCAGGT-3ʻ and 5ʻ-GAACATTGGGGACAGTGACG-3ʻ, and for leptin 5ʻ- CTCAGCATT CAG GGCTAA GG-3ʻ and 5ʻ- AAG CCTCGCTCTACTCCACA-3ʻ. Activity of inducible nitric oxide (iNOS) mRNA expression was quantified in the soleus muscles using the following primers 5ʻ-CACATGCAGAATGAGTACCGG-3ʻ and 5ʻ-AGGCTGCCCGGAAGGTTTGTA-3ʻ, and for β-actin 5ʻ-TGTCACCAACTGGGACGATA-3ʻ, and 5ʻ-GGGGTGTTGAAGGTCTCAAA-3ʻ (2, 21, 22). All data were normalized to fold changes, using $2^{-\Delta\Delta C}$, compared to control.

Statistical analyses

Data were analyzed by parametric statistics (ANOVA and repeated measures ANOVA and t-tests, with Turkey's test used as appropriate) as described for each experiment, with set at $P<0.05$, two-tailed.

RESULTS

Effect of dietary salt on body weight and internal fat pads

Metabolic syndromes often associated with different variables includes high body weight and increased body fat mass. Table 1 presents body weight of the different treatment group compared to the control. Salt-treated groups exhibit similar weight with a light deviation but did not showed significant difference, although all three groups are initially matched weight and age. In contrast high sodium group (HS) showed significant lower body weight in comparison to the Low sodium (LS) but not to normal sodium group (NS) $P\leq0.0228$.

As expected, higher body weight was correlated with more body fat as shown in Table 1. The highest fat content found in the LS group ($P\leq0.0236$), while the lowest fat found in the HS group ($P\leq0.0087$). Calibration fat content to the NS group showed a significant reduction fat content ($P<0.0006$) as shown in Figure 1.

However, basal FFA levels were comparable across NS and HS groups ($P> 0.05$). Although basal FFA levels were higher in LS when compared to NS ($P\leq 0.0034$), and the internal comparison between both treated groups LS vs. HS showed moderate change ($P\leq0.0201$) as shown in Table 1.

Effect of dietary salt on blood glucose, plasma Insulin and Free Fatty acids

Change in fasting blood glucose between NS and LS group was significant ($P\leq0.0075$), but it was not the case for HS group which showed no change in the level of glucose. On the other hand, in contrast to what expected aligned LS data with HS showed less significant value ($P\leq 0.0383$). The same observation were recorded for plasma insulin which was much higher in LS in comparison to NS and lower for HS regarding LS respectively with value of $P\leq 0.0095$ and $P\leq 0.0135$.

The homeostasis model assessment (HOMA) calculated as an Insulin resistance index as prior reported (23). Data showed that LS group was much resistant for insulin in comparison for NS or HS with P-value more than 0.01 as shown in Table 2.

HS change NO gene expression and ROS in the skeletal muscles

It has been shown that the higher fat body content the highest reactive oxygen species production and raise of free radical load in the tissue (24, 25). In this experiment, that hypothesis was test in all experimental group, as expected LS group showed higher ROS in comparison to NS and LS ($P\leq 0.05$) as depicted in Figure 2b.

Nitric oxide (NO) is a momentous short lived free radical, with a wide range of cellular functions include vasodilation, regulate sodium transport and insulin uptake in the peripheral tissues (26-28). Dietary salt different loads has not significant effects on the levels of inducible nitric oxide

synthase (iNOS) value among all groups (P>0.05). These data contrasted others who observed a downregulation of NO activity during high salt load in the kidney and heart (29, 30). Unchangeable levels of NO in the skeletal muscles may explained by high level of ROS in the LS group which able to rapidly oxidize endothelium-derived NO (31).

Dietary salt modulate gene expression levels of adiponectin and leptin in WAT tissue

The reduction in body and white adipose tissue weight were accompanied by a significant increase in adiponectin gene expression. In adipose tissue of rats maintained on a high- salt load increase of about 16% in adiponectin mRNA level was found (P<0.023). A significant reduction in adiponectin mRNA levels approximately 23% were found between rats maintained on low salt diet and those fed normal salt diet (P<0.002) as shown in Fig.3a. Relative expression of adiponectin between LS and HS was highly significant with approximately 39% increase in case of HS (P< 0.0001).

Increase in the level of adiponectin in white adipose tissue of LS group was associated with comparable increase in leptin mRNA expression of about 15% (P≤ 0.02). Corresponding decrease about 24% in leptin gene expression in case of HS feeding group in comparison to LS group was observed as shown in Figure 3b, these observations are in line with previously reported data (2).

Effect of dietary salt modulation on level of plasma ACE Activity

The renin-angiotensin- system (RAS), is mainly regulate sodium homeostasis and blood pressure (26). Moreover, it has been postulated that high-salt diet increases plasma adiponectin levels through RAS and consequently activation of adenosine monophosphate dependent kinase (AMPK), PPAR-α, and may other unaddressed signaling pathways (32, 33). Current study tested that hypothesis by measuring the Angiotensin Converting Enzyme (ACE), the rate limiting step in angiotensin I conversion into angiotensin II (34). Data revealed that high salt load reduces ACE activity in the Adipose tissue 25.3% in comparison to LS group (P≤ 0.001). Moreover, LS group ACE level was increased 19% in comparison to NS (P≤ 0.01) as shown in figure 4a. Furthermore, ACE is positively correlated with leptin values obtained for rats on the HS diet (r= 0.2709, p< 0.0091) and with Adiponectin (r= 0.3596, p< 0.002) as shown in figure 4b. These data showed that HS inhibits ACE activity.

Table 1. Body weight, fat weight and FFAs Level.

Variables	NS	LS	HS
Body Weight (gm)	303.6 ± 24.05	317.44 ± 28.54	288.27 ± 17.26#
Carcass fat (mg)	51.65± 2.62	57.08 ± 3.17*	45.38 ± 2.05†
FFAs (mmol/L)	0.32 ± 0.45	0.42 ± 0.06**	0.34± 0.05

Body weight, carcass fat content and plasma free fatty acids. All data represented as mean ± SD. *P<0.05 and †P < 0.01 and #P<0.05 compared to LS

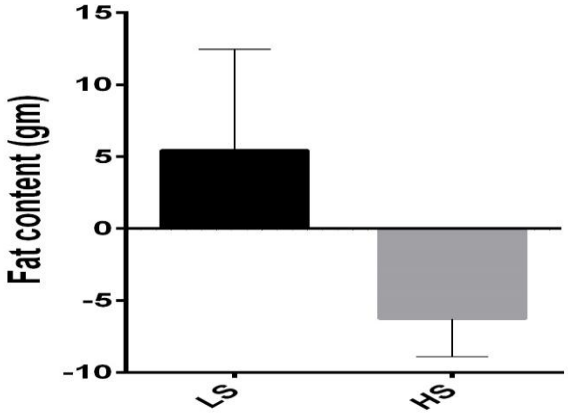

Figure 1. Change in fat content from LS to HS diet.

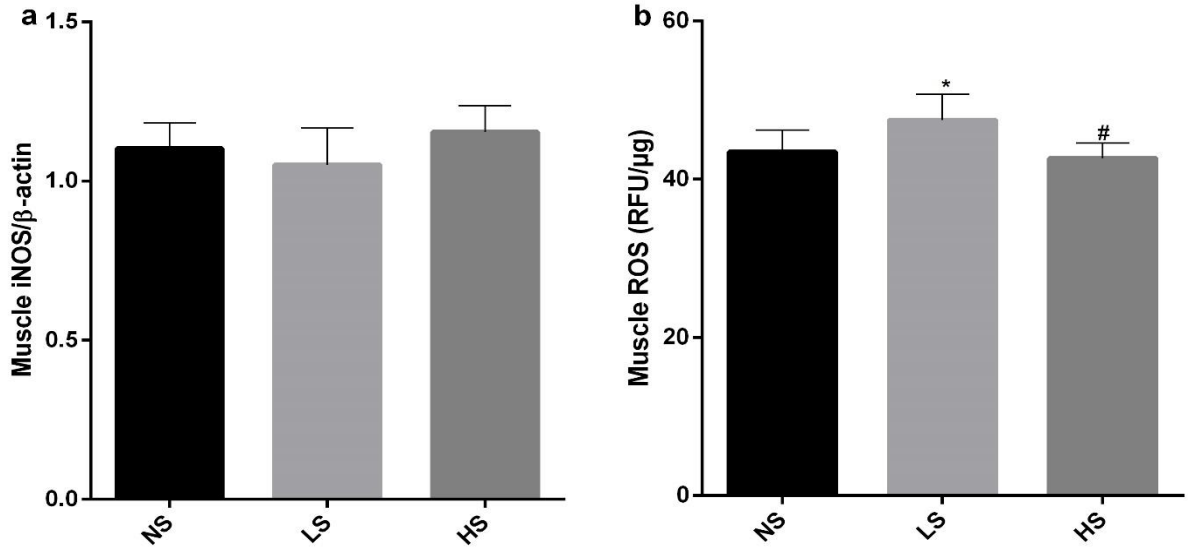

Figure 2. Reactive Oxygen Species (ROS) and inducible Nitric Oxide (iNOS) in skeletal muscles. Expression of the relative fluorescence units of (ROS) Reactive Oxygen Species (a) and (iNOS) the inducible Nitric Oxide gene expression(b) in the skeletal muscles. Data were represented as mean ± SD. Significant data shown by *P<0.05 compared to control whereas #P<0.05 compared to LS.

Table 2. Blood glucose, plasma insulin level and HOMA-IR.

Variables	NS	LS	HS
Plasma Glucose (mg/dL)	104.83 ± 2.83	113.19 ± 2.71*	106.79 ± 4.68##
Plasma Insulin (ng/dL)	0.81 ± 0.012	0.85 ± 0.026†	0.81 ± 0.014#
HOMA-IR	2.014 ± 0.04	2.29 ±0.09†	2.06 ±0.10##

Plasma insulin, and Blood glucose represented as mean ± SD. Significant data shown by *P<0.05 and †P < 0.01 compared to control whereas #P<0.05 and ##P<0.01 compared to LS.

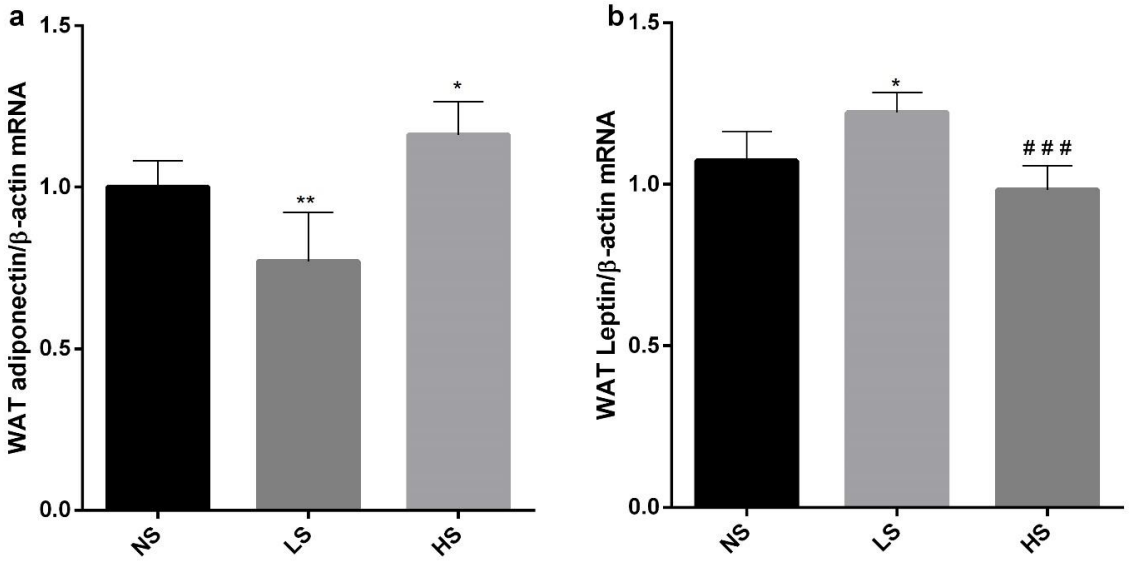

Figure 3. Adiponectin and leptin gene expression in the white adipose tissue. Relative gene expression of Adiponectin (a) and leptin (b) in WAT. Data were represented as mean ± SD. Significant data shown by *P<0.05; **P<0.051 compared to control whereas ###P<0.001 compared to LS.

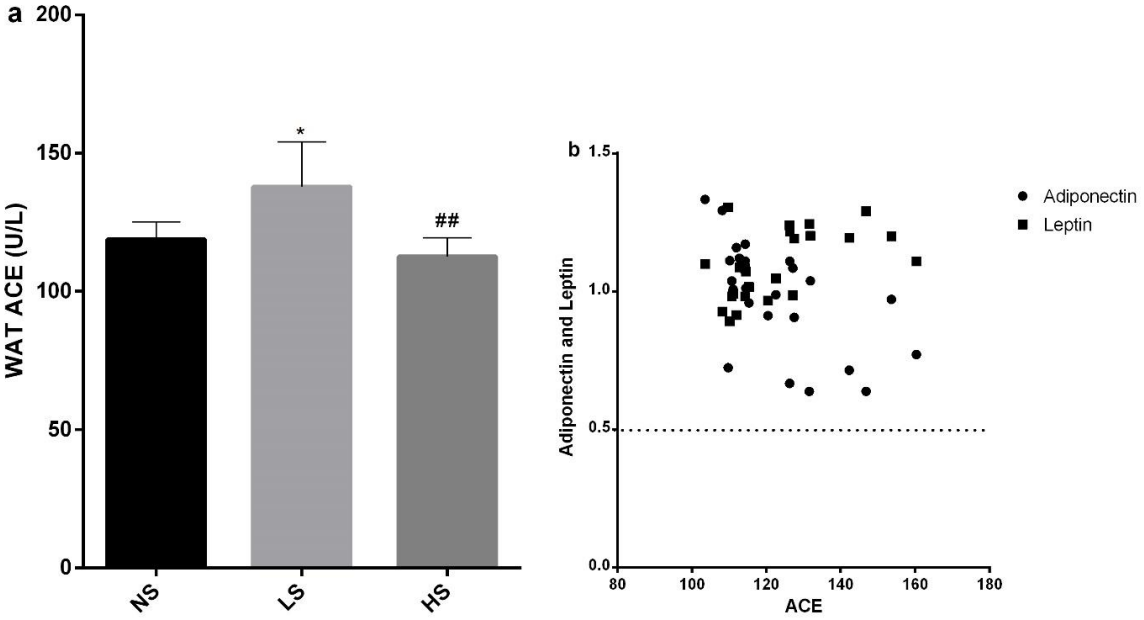

Figure 4.Angiotensin converting Enzyme and Correlation with Adiponectin and leptin. Levels of ACE in the WAT (a). Calculation of the correlation between values of leptin and adiponectin in relation to ACE (b). Data were represented as mean ± SD. Significant data shown by *P<0.05 compared to control whereas ##P<0.01 compared to LS.

Discussion

The metabolic syndromes include several disorders, among these are the insulin resistance and

hypertension that are the most associated ones. Several studies showed that about 50% of individuals with high blood hypertension had hyperinsulinemia and insulin resistance, whereas approximately 80% of patients with type 2 diabetes suffer hypertension (34, 35). These observation notions that a tight link between hypertension and progress of insulin resistance. The conflict of data about the effects of dietary salt on insulin sensitivity had made a sundry summary, which enforce researchers for more studies explaining such remarkable clue.

In the present study, rat received different amounts of dietary salt for a period of two weeks, several parameters have been assessed and recorded to evaluate effect of different doses on the phenotypical and physiological body parameters.

Results showed a significant increase in the body weight and proportional body fat content in the low salt treated group (LS). This increase in body weight was correlated with higher circulating free fatty acids, Blood glucose did not altered in HS rat, but increased in the LS group. As well, the LS group showed higher plasma insulin among all other groups. Moreover, computed HOMA-IR proved that LS had the highest insulin resistance among the different groups. These data in consent with previous study found that low salt diet rise plasma Fatty Acids and rises insulin, this elevation in FFAs levels impair endothelium-dependent vasodilation (36). These data suggest that low salt diet impaired insulin sensitivity and increases insulin resistance in healthy subjects (37).

Association between high body weight and much produced reactive oxygen species (ROS) has been proven in several studies (24, 25). In this experiment, LS group showed the highest ROS in the skeletal muscles in comparison to NS and LS groups. It was surprising not to found an effect on the NO level among treated groups. As the insulin sensitivity is correlated with higher levels of NO in the peripheral tissues, but here it was not the case where different loads of dietary salt showed a comparable level of NO, these data contrasted others who observed a downregulation of NO activity during high salt load in different organs (29, 30). Furthermore, others found that HS promotes nitric oxide (NO) production in skeletal muscles, enhanced glucose uptake (34). The consistent level of NO in the skeletal muscles is may due to the high oxidation rate of the endothelium-derived NO by the ROS in the LS group (31).

Previous study showed that high salt intake rises adiponectin mRNA levels through activation of the renin-angiotensin-aldosterone system (32). Assessment of adiponectin level in the white adipose tissue (WAT) showed a significant decrease in the LS group, while an increase in the case of HS group. Change between HS and LS group was significantly higher, this increment of delta change was about 39% increase in adiponectin in case of HS group. These data in agreement with others mentioned that insulin sensitivity dependent adiponectin mediates through binding adiponectin receptors, eventually activates the adenosine monophosphate dependent kinase (AMPK) pathway (33).

Serum leptin and adiponectin concentrations were shown to correlate with insulin resistance (38). Adiponectin has various effects on cellular physiology ranged from decreasing lipolysis

throughout increasing eNOS to increasing glucose uptake and insulin secretion (39). Moreover, multiple functions of leptin include regulation of food intake and expenditure, appetite beside its proinflammatory and proangiogenic properties (21, 40). Collectively, these data specify both the decrease in WAT leptin gene expression and the increase in adiponectin level by high dietary salt intake may be displaying beneficial effects of salt load on insulin sensitivity.

Insulin sensitizing effects mediates through AMPK pathway activation through activation of the renin-angiotensin- system (RAS). Current study tested the angiotensin converting enzyme activity (ACE), which converts Ang I into Ang II exerting its inhibitory effects on the PI3K pathway activation (34). Current data revealed that high salt load reduces ACE activity in the Adipose tissue while low salt diet increased ACE activity. Linking ACE assessment data with phenotypical and physiological data proven that a tight correlation between slat load, ACE and insulin sensitivity.

Conclusion

The high salt load mediates insulin sensitivity through inhibition of RAS which promotes PI3K pathway in the insulin-sensitizing tissues. In addition, high salt promotes adiponectin level in the WAT and reduces the gene expression of leptin. It seems that the reduction in the levels of nitric oxide (NO) in the HS group may be due to its oxidation by the reactive oxygen species (ROS) in the skeletal muscles. This article indicated a substantial role of salt balance on the insulin sensitivity, but other studies should addressed the long-term effects of salt load on integrity of most salt-sensitizing organs histology and physiology.

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