

Changes in cardiac Na⁺/K⁺-ATPase expression and activity in female rats fed a high fat diet

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Abstract

The aim of this study was to investigate whether the presence of endogenous estradiol alters the effects of a high fat (HF) diet on activity/expression of the cardiac Na⁺/K⁺-ATPase, via PI3K/IRS and RhoA/ROCK signalling cascades in female rats. For this study, female Wistar rats (8 weeks old, 150-200g), were fed a standard diet or a HF diet (balanced diet for laboratory rats enriched with 42% fat) for 10 weeks. The results show that rats fed a HF diet exhibited a decrease in phosphorylation of the α_1 subunit of Na⁺/K⁺-ATPase by 30% (p<0.05), expression of total α_1 subunit of Na⁺/K⁺-ATPase by 31% (p<0.05), and association of IRS1 with p85 subunit of PI3K by 42% (p<0.05), while the levels of cardiac RhoA and ROCK2 were significantly increased by 84% (p<0.01) and 62% (p<0.05), respectively. Our results suggest that a HF diet alters cardiac Na⁺/K⁺-ATPase expression via molecular mechanisms involving RhoA/ROCK and IRS-1/PI3K signaling in female rats.

Key words: High fat diet, estradiol, Na⁺/K⁺-ATPase, obesity, RhoA/ROCK signaling, female

Abbreviations: Akt, protein kinase B; CD36, cluster of differentiation 36; CVD, cardiovascular disease; T2DM, type 2 diabetes mellitus; ER α , estrogen receptor- α ; HF diet, high fat diet; HOMA-IR, HOMA-index of insulin resistance; HOMA- β , HOMA-index of β -cell function; iNOS, inducible nitric oxide synthase; INS, insulin; IR, insulin resistance; IRS, insulin receptor substrate; Na⁺/K⁺-ATPase, sodium/potassium-adenosine-triphosphatase; PI3K, phosphatidylinositol 3-kinase; RhoA, Ras homolog gene family, member A; ROCK, Rho kinase.

Introduction

Obesity is defined as an excessive presence of fat in the body and if uncorrected, contributes to the onset and development altered glucose, lipid and energy metabolism, insulin resistance (IR) and variety of cardiovascular diseases (CVD) [1,2]. Rodents fed a high fat diet (HF diet) rapidly develop hyperinsulinemia, hyperglycaemia, whole body IR, and are a valuable research model since they can provide insight into the mechanisms underlying IR in obese individuals with impaired glucose tolerance or type 2 diabetes mellitus (T2DM) [3,4]. In biomedical research, it has become increasingly apparent that female sex hormones, primarily estradiol, have a favourable effect on insulin (INS) sensitivity and that men are more susceptible to IR, T2DM, metabolic syndrome and CVD when compared to premenopausal women [5-9]. However, estradiol production and action may be disrupted by a HF diet, which may be the reason why cardioprotective effects of estradiol are blunted in obesity and IR [10].

Sodium/potassium-adenosine-triphosphatase (Na^+/K^+ -ATPase) is an integral membrane protein that transports K^+ ions into the cell and Na^+ ions out of the cell two using the energy derived from hydrolysis of ATP. It is composed of a 112 kDa catalytic α subunit, a heavily glycosylated 35 kDa β subunit [11,12], and a regulatory subunit called FXYD proteins, which are often referred to as γ -subunits [13]. The α -subunit catalyses ATP hydrolysis and exists in four separate isoforms, α_1 – α_4 . The α_1 and α_2 subunits are expressed in rat heart [14]. Na^+/K^+ -ATPase regulates smooth muscle reactivity and is proposed to be involved in development of systemic hypertension, while the reduction in the transsarcolemmal Na^+ gradient, established and maintained by Na^+/K^+ -ATPase, leads to heart hypertrophy and failure [14,15]. Furthermore, obesity is associated with the reduction of Na^+/K^+ -ATPase activity in heart, skeletal muscle and liver, probably due to the development of IR since it has been shown that INS induces translocation of Na^+/K^+ -ATPase subunits from intracellular stores to plasma membrane by a phosphatidylinositol 3-kinase (PI3K) dependent pathway [16-19]. In addition, gender-specific regulation of Na^+/K^+ -ATPase exist, and estradiol exerts its cardioprotective effects partially by up-regulating Na^+/K^+ -ATPase activity/expression [20-22]. It has been shown that estradiol increases synthesis of new α subunits and Na^+/K^+ -ATPase activity by a mechanism involving PI3K signalling [23,24,22]. Despite the importance of obesity induced reduction in Na^+/K^+ -ATPase activity in the pathogenesis of several diseases including T2DM and CVD, the association between obesity/ Na^+/K^+ -ATPase/CVD is still poorly understood. It has been reported that a HF diet upregulates Ras homolog gene family, member A (RhoA)

and its downstream effector Rho kinase (ROCK) in the heart of diabetic rats [25]. Moreover, RhoA/ROCK pathway was demonstrated to down-regulate PI3K/protein kinase B (Akt) signalling [26,27]. ROCK exists in two widely expressed isoforms, ROCK1 and ROCK2, and the ability of ROCK to interfere with INS signalling appears to be isoform-dependent and tissue-specific [28].

We have previously shown that obesity, when accompanied with IR and hypertension, reduces the activity/expression of the cardiac Na⁺/K⁺-ATPase by a mechanism involving activation of RhoA and reduction of PI3K/Akt activity. We have also demonstrated that estradiol treatment restores the function of Na⁺/K⁺-ATPase in heart of obese male rats [29]. In this study, we examine whether the decreased ability of endogenous estradiol to stimulate Na⁺/K⁺-ATPase pump activity in obesity is due to an alteration in the PI3K/insulin receptor substrate (IRS) and RhoA/ROCK signaling cascade.

Material and Methods

2.1. Materials

Ether was purchased from Lek (Ljubljana, Slovenia). Luminol and p-coumaric acid were obtained from Sigma Aldrich Corporation (St. Louis, MO, USA). Protease (Complete, Ultra Mini, EDTA-free) and phosphatase inhibitor cocktails (PhosStop), were purchased from Roche (Mannheim, Germany). The rabbit polyclonal antibodies (anti-Rho A, anti-phospho- α_1 Na⁺/K⁺-ATPase (Ser²³) and anti- α_1 Na⁺/K⁺-ATPase) and monoclonal (anti-PI3K p85 α) were obtained from Abcam (Cambridge, UK). The rabbit polyclonal (anti-ROCK2) and monoclonal (anti-PI3K p110 α) antibodies were purchased from Cell Signalling Technology (CST, USA). The goat polyclonal anti- α_2 Na⁺/K⁺-ATPase antibody, rabbit polyclonal mouse anti-actin monoclonal antibody, and the secondary anti-mouse and anti-rabbit antibodies conjugated to alkaline phosphatase (ALP) or to horseradish peroxidase (HRP) and BCIP/NBT (5-bromo-4-chloro-3-indoyl phosphate/nitro blue tetrazolium chloride), were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Animals

Adult female Wistar rats (8 weeks old, 150-200g), bred at the Institute of Nuclear Sciences (Vinca, Belgrade) were used in this study. The animals were divided into 2 groups: control females (Control) and high-fat diet fed females (Obese). The animals were kept under a 12:12 h light: dark cycle at 22±2°C with. Over the next 10 weeks control females had *ad libitum*

access to standard laboratory chow composed of 20% proteins, 8% cellulose, 13% moisture, 1% calcium, 0.90% lysine, 0.75% methionine+cystine, 0.5% phosphorus, 0.15 – 0.25% sodium, vitamin mixture (A 10000 IU/kg, D₃ 1600 IU/kg, E 25 mg/kg, B₁₂ 0.02 mg/kg), mixture of minerals (in milligrams per kilogram: zinc 100, iron 100, manganese 30, copper 20, iodine 0.5, selenium 0.1), antioxidants 100 mg/kg, and digestible/metabolizable energy 11 MJ/kg (prepared by “D. D. Veterinarski zavod Subotica”, Subotica, Serbia), while obese females were fed a standard laboratory chow enriched with 42% fat. At the end of the experiment all animals were euthanized under deep ether anesthesia, hearts were excised and quick frozen in liquid nitrogen at -80°C and stored until further experiments. The results related to the body mass, levels of insulin, glucose, the HOMA-index of insulin resistance (HOMA-IR) and HOMA-index of β -cell function (HOMA- β) in control and obese female rats are already published [30], and demonstrate that our obese female rats did not develop IR (depicted by unchanged levels of HOMA-IR and HOMA β). Experimental protocols were approved by the Vinca Institute’s Ethical Committee for Experimental Animals.

2.3. Heart lysate preparation

To isolate heart lysate proteins we homogenized approximately 200 mg of rat heart tissue on ice with an Ultra-Turrax homogenizer in lysis buffer (pH 7.4) containing: 10 mM Tris, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1% Triton X-100, 2 mM sodium orthovanadate, phosphatase and protease inhibitor cocktails. Homogenates were incubated for 1h at 4°C and centrifuged at 4°C at 100,000 \times g for 20 min. The supernatants (containing proteins) were obtained and concentration of proteins was determined by Lowry method [31]. The final lysate was stored at -80°C until further experiments.

2.4. Heart plasma membrane protein extraction

To isolate membrane proteins pieces of rat heart (200 mg) were incubated for 30 min in a high-salt solution (20 mM HEPES, 2 M NaCl, and 5 mM sodium azide, pH 7.4) at 4°C. This was followed by centrifugation for 5 min at 1000 \times g, and rehomogenation of the pellet on ice with an Ultra-Turrax homogenizer in TES-buffer (pH 7.4) containing: 20 mM Tris, 250 mM sucrose, and 1 mM EDTA, 2 mM sodium orthovanadate, phosphatase and protease inhibitor cocktails. The homogenate was centrifuged for 5 min at 1000 \times g while the resulting pellet was then rehomogenized in a TES-buffer and recombined the supernatant obtained in previous centrifugation. Afterwards the homogenate was centrifuged for 10 min at 100 \times g, and the obtained supernatant was additionally centrifuged for 10 min at 5000 \times g. The final

pellet (referred to as the “plasma membrane fraction”) was resuspended in TES buffer and stored at -80°C for further analysis. Protein concentrations were determined by the Lowry method [31].

2.5. Measurement of cardiac Na^+/K^+ -ATPase activity

The Na^+/K^+ -ATPase activity in heart plasma membrane protein fraction was determined with the modified spectrophotometric procedure [32,33]. Briefly, the reaction medium (20 mM Tris-HCl, 40 mM NaCl, 8 mM KCl, 2 mM MgCl_2 , pH 7.4) and 0.125 mg/ml protein were pre-incubated for 10 min at 37°C . Addition of 2 mM ATP started the reaction and after 15 min, the reaction was terminated by the addition of ice-cold 3 M perchloric acid and the samples were then cooled on ice. A corresponding set of samples was prepared the same way but with additional 2 mM ouabain. The Na^+/K^+ -ATPase activity represents the difference in the amount of inorganic orthophosphate released from the hydrolysis of ATP, between the samples with or without ouabain. Inorganic orthophosphate concentration was measured by addition of 0.2 M ammonium heptamolybdate in 30% (w:v) sulfuric and a drop of 132 mM stannous chloride. After incubation on room temperature for 15 min, the absorbance was measured at 690 nm. Na^+/K^+ -ATPase activity was calculated using a phosphate standard calibration curve and the results were expressed as mmol phosphate/min/mg protein.

2.6. SDS-PAGE and Western blotting

Equal amounts of either total protein lysates or plasma membrane protein extracts (80 $\mu\text{g}/\text{lane}$) were separated by 10% or 12% SDS-polyacrylamide gel electrophoresis [34] and transferred to polyvinylidene difluoride (PVDF) membranes as previously described [35,36]. The membranes were blocked with 5% bovine serum albumin and probed with antibodies directed against p85 and p110 subunits of PI3K, RhoA and ROCK2 for total protein lysates and with antibodies directed against α_1 phosphorylated at Ser^{23} and non-phosphorylated forms of α_1 and α_2 Na^+/K^+ -ATPase for membrane proteins. After washing, membranes were incubated with the appropriate secondary antibodies and used for subsequent detection with either BCIP/NBT or with the electrochemiluminescence (ECL) method. In order to insure that protein loading was equal in all samples, all blots were probed with anti-actin antibody and appropriate secondary antibody. Signals on membranes were quantified using ImageJ 1.45s software (National Institutes of Health, USA, <http://rsb.info.nih.gov>).

2.7. Co-Immunoprecipitation of IRS and p85 proteins

For immunoprecipitation, 500 µg of cellular protein was incubated overnight with 2 µg of anti-insulin receptor substrate-1 (IRS-1) antibody at 4°C. Immunocomplexes were collected with protein A/G-sepharose overnight at +4°C and then recovered by centrifugation (2500 *xg*; 5 min) and washed three times with TBS. Proteins were separated by SDS-PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane, and probed with an anti-p85 antibody (in a dilution of 1:1000). After washing, membranes were incubated with the HRP conjugated secondary antibodies and used for subsequent detection with ECL method.

2.8. Statistical Analysis

Values are expressed as mean ± SEM. Statistical analyses of data were evaluated with a Student's *t*- test using Microsoft Excel program for Windows. A two-tailed $P < 0.05$ was considered significant.

3. Results

3.1. Effects of a HF diet on cardiac α_1 and α_2 subunits of Na^+/K^+ -ATPase and Na^+/K^+ -ATPase activity in obese female hearts

Since the reduced activity and expression of Na^+/K^+ -ATPase is a key event leading to the development of various forms of CVD [37], we first examined the effects of a HF diet on the level of α_1 subunit phosphorylation at Ser²³, and the level of total α_1 and α_2 subunit of Na^+/K^+ -ATPase in female rats. The results show that a HF diet led to a decrease in the level of α_1 subunit phosphorylation by 30% ($p < 0.05$) (Fig. 1a) and total α_1 subunit expression by 31% ($p < 0.05$) in obese females compared with their control (Fig. 1b and c). The density ratio between the phosphorylated and total forms of the Na^+/K^+ -ATPase α_1 subunit in cardiac tissue was not observed to be different between obese and control female rats (Fig. 1d). In addition, a HF diet did not alter the level of α_2 subunit (Fig. 1e) or in the Na^+/K^+ -ATPase activity (Fig. 2).

3.2. Effects of HF diet on RhoA and ROCK2 protein expression in rat heart

To examine the mechanism by which cardiac expression of Na^+/K^+ -ATPase may be regulated in female rates fed a HF diet, we next assessed the effects of a HF diet on expression of RhoA

and ROCK2. The results show that in obese females the expression of RhoA was increased by 84% ($p < 0.01$) while the level of ROCK2 was increased by 62% ($p < 0.05$) compared with controls (Fig. 3).

3.3. Effects of HF diet on expression of p85 and p110 subunits of PI3K and association of IRS1 and p85 in rat heart

Obesity is associated with the reduction of Na^+/K^+ -ATPase activity in heart, likely due to the development of IR. We have previously shown that estradiol induces changes in cardiac Na^+/K^+ -ATPase activity/expression in male rats by a PI3K dependent pathway [29]. Here we explored the effects of a HF diet on the expression of the p85 and p110 subunits of PI3K in the heart of female rats fed a HF diet. The results revealed no significant change in the level of p85 or p110 subunits of PI3K compared to controls (Fig. 4).

3.4. Effects of HF diet on association of IRS1 and p85 subunit of PI3K in rat heart

Since HF diet-feeding increases phosphorylation of IRS at Ser³⁰⁷, which in turn has been shown to reduce the interaction of IRS with the p85 subunit of PI3K, thereby limiting PI3K activation and impairing estradiol signalling [38], we next examined association between IRS-1 and p85 in the hearts of female rats fed a HF diet. The results of co-immunoprecipitation of IRS-1 and p85 proteins revealed reduced association of IRS-1 protein with p85 subunit of PI3K by 42% ($p < 0.05$) in obese compared with control female rats (Fig. 5).

4. Discussion

We have previously reported that a HF diet, despite causing the development of an obese phenotype in both male and female rats, only induces hyperlipidaemia, hyperglycaemia, and IR in male rats [39,30,40]. Furthermore, we have reported that in male rats, obesity accompanied with IR decreases cardiac Na^+/K^+ -ATPase activity/expression, while estradiol administration as bolus injection achieved contrary effects. In this study we assessed whether the presence of endogenous estradiol in female rats, prevents HF diet induced alterations in the translocation of the cardiac Na^+/K^+ -ATPase activity/expression, since it is still unknown to what extent obesity compromises cardioprotective effects of estradiol. Our results indicate that a HF diet causes up-regulation of RhoA/ROCK protein expression, decreases the IRS-1 protein association with p85 subunit of PI3K and reduces the expression of the α_1 subunit of Na^+/K^+ -ATPase in female rats. However despite these changes, the activity and expression of

α_2 subunit of Na^+/K^+ -ATPase in cardiac tissue were unaltered under the influence of a HF diet in female rats.

In cardiac muscle, Na^+/K^+ -ATPase plays a crucial role in the regulation of cardiac electrophysiology and cardiomyocytes contractility, while various cardiac disorders including cardiac hypertrophy and hypertension, which commonly occur as a consequence of obesity, are associated with reduction of Na^+/K^+ -ATPase activity and expression [18,19,41,37]. We have previously reported that in the hypertrophic heart of male rats a HF diet reduces the activity and expression of the α_1 and α_2 subunits of Na^+/K^+ -ATPase, while estradiol treatment reduced heart hypertrophy and increased Na^+/K^+ -ATPase expression/activity [42,39]. Earlier, Dzurba et al. reported that pretreatment of ovariectomized female dogs with estradiol increased Na^+/K^+ -ATPase activity in the myocardium [43]. Later, Palacios et al. demonstrated that treatment of aortic rings isolated from ovariectomized female rats with estradiol restored activity of Na^+/K^+ -ATPase similar to the values observed in aortic rings from intact rats [44]. In addition, the same authors show that estradiol treatment of male rat aortic rings increased expression of α_2 subunit mRNA, and also that α_2 subunit expression is greater, while the α_1 subunit is lower in untreated arterial vessels of female rats compared with males. Here we show that in female rats a HF diet reduces the expression of the α_1 subunit of Na^+/K^+ -ATPase, but does not affect the expression of α_2 subunit or the Na^+/K^+ -ATPase activity. Numerous studies show significant gender differences in the relative amount of α_1 and α_2 catalytic isoforms and the activity of Na^+/K^+ -ATPase [44,24,45,46]. In our study, unaltered Na^+/K^+ -ATPase activity despite the reduced content of its α_1 subunit may be explained by the fact that rodent α_1 -subunit isoform has a very low affinity to ouabain, and ouabain-sensitive methods largely reflect the α_2 -subunit content [47,48]. Michea et al. reported that a diminished expression of α_1 subunit does not affect Na^+/K^+ -ATPase activity, whereas the reduction of α_2 protein accounted for the reduction of total Na^+/K^+ -ATPase activity of diabetic animals [49]. Recently, Correll et al show by overexpressing α_1 and α_2 *tg* mice, that only overexpression of α_2 subunits of the Na^+/K^+ -ATPase reduced cardiac hypertrophy and remodeling [50]. Furthermore, distribution of α_1 and α_2 isoforms varies in heart in a region-specific manner [51], even in the same single cell [52]. All these findings potentially suggest that the α_2 subunit of Na^+/K^+ -ATPase is capable of substituting the role of α_1 subunit in terms of regulating Na^+/K^+ -ATPase activity. Our data support this hypothesis, since in our study the protein expression of α_1 subunit was reduced, while the activity of Na^+/K^+ -ATPase was unchanged. We also assume that the stimulatory effect of estradiol on

Na⁺/K⁺-ATPase activity is sufficient to maintained normal function of Na⁺/K⁺-ATPase during reproductive period in HF fed female rats, even it was shown that obesity permanently reduce expression and activity of Na⁺/K⁺-ATPase in heart.

There is strong evidence that a HF diet increases the expression of RhoA and ROCK in various tissues, and that alterations in activity of downstream targets can enhance vascular smooth muscle cells contractility which can eventually lead to the development of several pathological conditions, including hypertension, atherosclerosis and heart failure [53-55]. Soliman et al. reported that feeding mice a HF diet for 17 weeks significantly increases the expression of cardiac RhoA and ROCK2 [56]. Similarly, in our study a HF diet augmented both RhoA and ROCK2 expression in obese female rat hearts. This may be related to the activation of the renin–angiotensin system, which is common in obesity and is characterized by increased level of Ang II [57]. This in turn induces a hypertrophic response in cardiomyocytes through various signal transduction pathways, including activation of RhoA [58,59]. Furthermore, it has been shown that both inducible nitric oxide synthase (iNOS) and the RhoA/ROCK pathway are activated in hearts of streptozotocin-induced diabetes as well as that iNOS may be a contributing factor in the RhoA/ROCK-mediated contractile dysfunction by increasing the total pool of RhoA available for activation [56,25]. In addition, we have previously demonstrated that a HF diet in the same rats used in this study caused an increase in cardiac iNOS mRNA and protein levels by a mechanism involving increased activation of Akt [60]. The mechanism by which iNOS may regulate RhoA expression in the heart appears to be a combination of transcriptional and translational upregulation of the RhoA gene and decreased degradation of the RhoA protein [56,25,61,62]. A number of studies demonstrated that upregulation of iNOS leads to RhoA phosphorylation at Ser¹⁸⁸ thereby protecting it from ubiquitin/proteasome-mediated degradation, while iNOS inhibition was associated with a decrease in RhoA mRNA and protein expression in the aorta and pulmonary artery [63,62,61].

Obesity decreases Na⁺/K⁺-ATPase activity and expression through dysregulation of multiple signalling cascades, and one of the mechanisms includes the IRS-1/PI3K signalling pathway [29]. Depending on the cell type, estradiol signaling can involve the PI3K signaling pathway. We have previously reported that PI3K is involved in estradiol regulation of the sodium pump in heart tissue [22,29]. Estradiol has been shown to activate PI3K through binding of phosphotyrosine-containing proteins such as IRS-1 and insulin receptor substrate-2 (IRS-2)

and the association of p85 with IRS-1 in different types of cells [64,65]. However, obesity induced IR is associated with serine phosphorylation of IRS-1 which attenuates tyrosine phosphorylation of IRS-1 in response to estradiol stimulation [66,67]. It has been shown that a HF diet increases phosphorylation of IRS at Ser³⁰⁷, which is located near the phosphotyrosine-binding domain of IRS-1, and its phosphorylation has been shown to reduce the interaction of IRS with the p85 subunit of PI3K, thereby limiting PI3K activation and impairing estradiol and INS signalling [38,68]. Results from our study reveal that a HF diet does not influence the expression of p85 and p110 subunits of PI3K, but significantly reduces the association of p85 subunit of PI3K and IRS-1 in the heart of obese female rats. This may be a consequence of enhanced RhoA/ROCK signalling, since it has been shown that both partial deletion of ROCK2 and its inhibition by fasudil prevents Ser³⁰⁷ phosphorylation of IRS-1 in mice fed a HF diet for 17 weeks [56]. In addition, we have previously reported that in the same rats used in this study, a HF diet altered the expression of cardiac cluster of differentiation 36 (CD36) and fatty acid metabolism [60], leading to the accumulation of intramyocellular lipids, which in turn may activate serine kinases such as protein kinase C and mammalian target of rapamycin to consequently induce serine phosphorylation of IRS-1 [69,70].

Even though healthy premenopausal women are naturally protected from CVD, partially via ER α signalling in the vasculature [71], several lines of evidence show that beneficial effects of ER α signalling are blunted in obesity and IR conditions [72] as well as that HF diet reduces concentration of estradiol and alters the expression of estrogen receptors [73-75]. We have previously demonstrated that a HF diet decreases serum estradiol level, as well as cardiac estrogen receptor- α (ER α) signalling and believe that due to the lack of beneficial action of estradiol, some of HF diet effects on female heart are similar to those observed in male rats and they include the up-regulation of cardiac iNOS expression [60] and consequential stimulation of RhoA/ROCK signalling and decrease in IRS-1/PI3K association and the expression of α_1 subunit of Na⁺/K⁺-ATPase. However, why reduced estradiol affected the level of cardiac Na⁺/K⁺-ATPase α_1 subunit expression but not α_2 subunit expression remains to be elucidated. Although there has been much research into the role of estradiol in regulating Na⁺/K⁺-ATPase activity and its contribution to the pathogenesis of cardiovascular disease in obesity and T2DM, the molecular mechanisms that control these processes are poorly understood. The research outlined in our study provide new information on the molecular basis of a Na⁺/K⁺-ATPase regulation by endogenous estradiol in the diabetic female rat heart and its role in the control of the estradiol-regulated Na⁺/K⁺-ATPase activity.

A greater understanding of how obesity impairs Na⁺/K⁺-ATPase activation will provide important insights into preventing and reducing CVD in the female population.

Conflict of interest

None declared.

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FIGURE LEGENDS

Fig. 1 Effects of a HF diet on expression of cardiac α_1 and α_2 subunits level of Na^+/K^+ -ATPase (a) Phosphorylation of the α_1 subunit of Na^+/K^+ -ATPase at Ser^{23} . (b) Expression of total α_1 subunit of Na^+/K^+ -ATPase. (c) Ratio of phospho- α_1 Na^+/K^+ -ATPase and total α_1 Na^+/K^+ -ATPase. (d) Expression of total α_2 subunit of Na^+/K^+ -ATPase. The results are expressed relative to the value obtained for the control and represent mean \pm SEM (n=4-5; * $p < 0.05$; N.S.-not significant). Representative western blots of phosphorylation of α_1 subunit of Na^+/K^+ -ATPase at Ser^{23} , expression of total α_1 and α_2 subunit of Na^+/K^+ -ATPase and corresponding β -actin proteins are shown.

Fig. 2 Effects of a HF diet on cardiac Na^+/K^+ -ATPase activity in female rats. Specific activities of Na^+/K^+ -ATPase are expressed in $\text{mmol P}_i/\text{h}/\text{mg}$ of protein and represent mean \pm SEM (n=6-8).

Fig. 3 Effects of HF diet on RhoA and ROCK2 protein expression in female rat heart (a) Expression of RhoA protein in lysate. (b) Expression of ROCK2 protein in lysate. The results are expressed relative to the value obtained for control and represent mean \pm SEM (n=5-6; * $p < 0.05$, ** $p < 0.01$). Representative western blots for RhoA, ROCK2 and corresponding β -actin proteins in control and obese female rats are shown.

Fig. 4 Effects of HF diet on p85 and p110 subunits of PI3K expression in heart lysates (a) Expression of p85 protein in lysate. (b) Expression of p110 protein in lysate. The results are expressed relative to the value obtained for the control and represent mean \pm SEM (n=4-5; N.S.-not significant). Representative western blots for p85 and p110 subunits and corresponding β -actin proteins in control and obese female rats are shown.

Fig. 5 Effects of HF diet on association of IRS1 and p85 subunit of PI3K in rat heart Association of IRS1 and p85 in lysate. Results are expressed relative to the value obtained for control and represent mean \pm SEM (n=4; * $p < 0.05$). Representative western blots of IRS1 association with p85 subunit of PI3K in cardiac lysates are shown.

Figure 1.

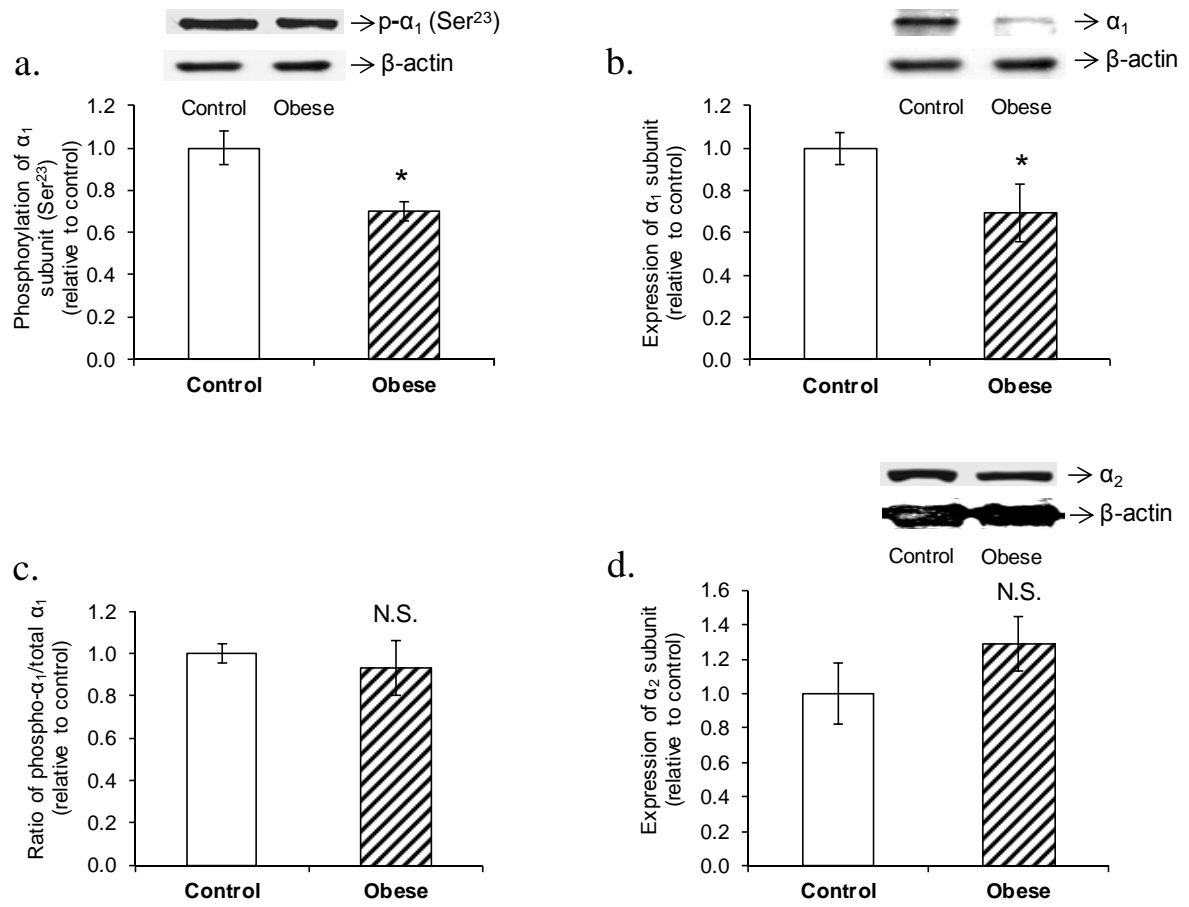


Figure 2.

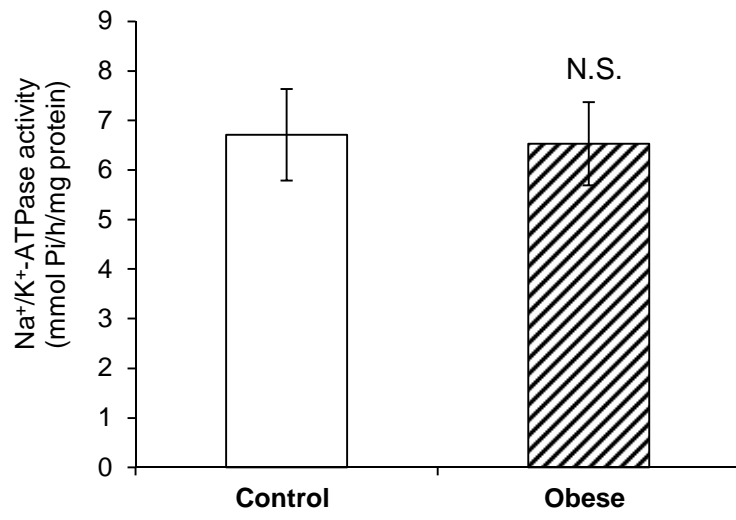


Figure 3.

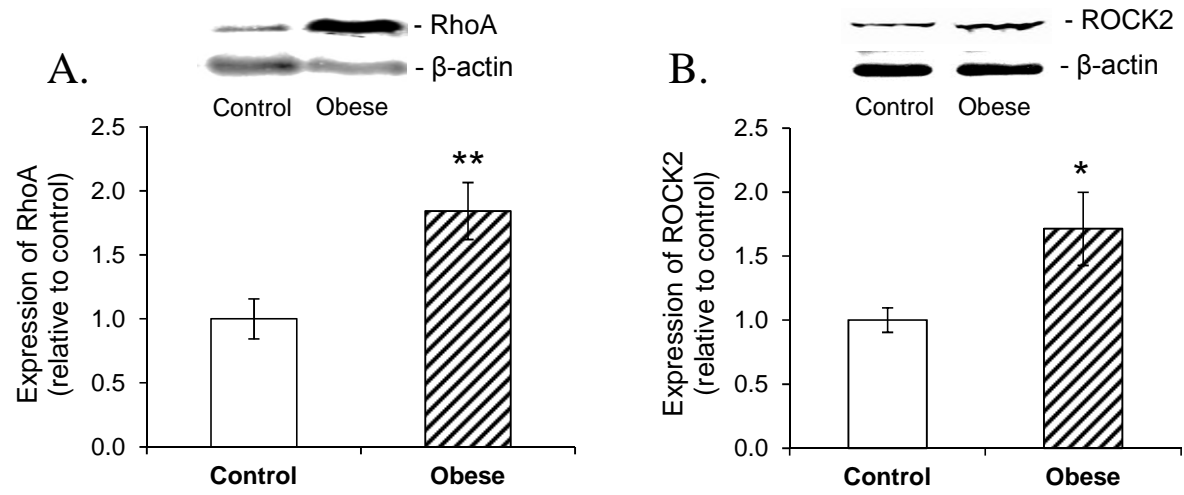


Figure 4.

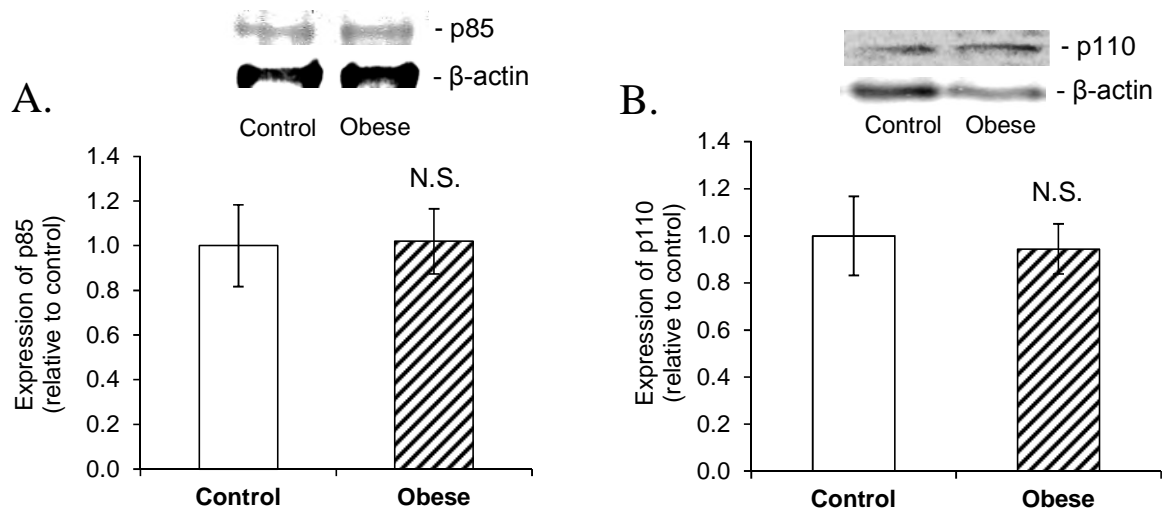


Figure 5.

