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Caloric restriction attenuates aging-induced cardiac insulin resistance in male Wistar rats through activation of PI3K/Akt pathway

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Abstract *Background and Aim:* Caloric restriction (CR) improves insulin sensitivity and is one of the dietetic strategies most commonly used to enlarge life and to prevent aging-induced cardiovascular alterations. The aim of this study was to analyze the possible beneficial effects of caloric restriction (CR) preventing the aging-induced insulin resistance in the heart of male Wistar rats.
Methods and results: Three experimental groups were used: 3 months old rats (3m), 24 months old rats (24m) and 24 months old rats subjected to 20% CR during their three last months of life (24m-CR). After sacrifice hearts were mounted in a perfusion system (Langendorff) and heart function in basal conditions and in response to accumulative doses of insulin (10^{-9}-10^{-7} M), in the presence or absence of Wortmannin (10^{-6} M), was recorded. CR did not attenuate the aging-induced decrease in coronary artery vasodilation in response to insulin administration, but it prevented the aging-induced downregulation of cardiac contractility (dp/dt) through activation of the PI3K/Akt intracellular pathway. Insulin stimulated in a greater extent the PI3K/Akt pathway vs the activation of the MAPK pathway and increased the protein expression of IR, GLUT-4 and eNOS in the hearts of 3m and 24m-CR rats, but not in the hearts of 24m rats. Furthermore, CR prevented the aging induced increase in endothelin-1 protein expression in myocardial tissue.
Conclusion: In conclusion CR partially improves cardiac insulin sensitivity and prevents the aging induced decrease in myocardial contractility in response to insulin administration through activation of PI3K/Akt pathway.

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Introduction

Aging is considered the major risk factor for the development of cardiovascular diseases, which represent the leading cause of death in developed countries [1]. Among the different factors, alterations in cardiovascular insulin sensitivity could be one of the mechanisms implied in this condition, as insulin resistance is a main factor in the pathogenesis of cardiovascular diseases [2] and reduced insulin sensitivity is a common finding in the elderly [3].

Insulin has several biological functions in addition to its role in the regulation of plasma glucose levels. In the cardiovascular system insulin is reported to induce arterial vasodilatation through the release of nitric oxide (NO) by the vascular endothelium [4]. In the myocardium insulin exerts a positive inotropic effect which seems to be independent, at least in part, from the stimulation of glucose uptake by cardiomyocytes [5]. The insulin-induced increase of heart contractility is reported to be mediated by PI3K activation [5], enhanced Ca2+influx through L-type Ca2+ channels [6] and facilitation of sarcoplasmic reticular calcium transport [7].

It is well known that insulin binding to insulin receptor (IR) activates two intracellular pathways; the phosphoinositide 3-kinase (PI3K)/Akt pathway, which in skeletal muscle and adipose tissue activates the translocation of glucose transporter 4 (GLUT-4) from the cytoplasm to the cellular membrane and mediates the metabolic effects, and the mitogen activated protein kinase (MAPK) pathway, which promotes cell proliferation and differentiation [8]. In the endothelium the activation of the PI3K/Akt pathway derives in eNOS phosphorylation and NO production whereas the activation of MAPK pathway induces the release of the vasoconstrictor peptide endothelin-1 [9].

It is reported that PI3K/Akt and MAPK pathways are differently affected by insulin resistance, with the activation of PI3K/Akt being significantly reduced and the activation of the MAPK pathway remaining unaltered in this condition [4,10]. This imbalance results in a predominance of the vasoconstrictor and proliferative actions of insulin, which contributes to the impairment of cardiovascular function [9]. Most of the studies that have analyzed insulin effects on the cardiovascular system have focused on the arterial vasodilating actions, existing relatively few studies that had analyzed the insulin-induced inotropic effect in the heart [11].

Caloric restriction is an effective intervention for preventing both insulin resistance and aging-induced cardiovascular alterations [12–14], including aging-induced insulin vascular resistance [15]. The aim of this study was to analyze the possible protective effects of CR in aging-induced insulin resistance in the heart of male Wistar rats, which may be relevant for the treatment/prevention of aging-induced cardiac alterations in the elderly.

Methods

Animals

3month-old (3m; n = 12) and 24month-old (n = 24) male Wistar rats were used in this study. Animal handling was performed according to European Union laws (Directive 2010/63/EU) and experimental procedures were approved by the Institutional and Regional Ethic Research Committees.

Rats were housed in temperature and humidity-controlled quarters and were subjected to a 12 h light/dark cycle, and free access to both food (standard chow; Eurorodent Diet 14%, Labdiet, St. Louis, MO, USA) and water. Before sacrifice, animals were subjected to 12 h fasting. Afterwards they were injected with heparin (1000 UI; i.p) and killed by an overdose of anesthesia (sodium pentobarbital; 100 mg/kg i.p) followed by decapitation.

Caloric restriction

At the age of 21 months half of the 24m rats (n = 12) were subjected to a moderate caloric restriction protocol for three months. For that purpose, animals were placed in individual cages and were daily fed an amount of chow equivalent to 80% of normal food intake. Previous data show that at the end of this protocol restricted animals show a reduction of approximately 20–25% in body weight, without compromising their nutritional status, and a reduction in the adiposity index even below the adiposity index of young animals [16,17]. Therefore, this protocol of CR can be considered a bearable intervention comparable to dietetic protocols prescribed for losing weight in humans in a healthy way.

Perfused heart technique (Langendorff)

After sacrifice, hearts were removed from the rats and immediately the ascending aorta was cannulated. Hearts were then subjected to retrograde perfusion in a non-recirculating Langendorff perfusion system with Krebs-Henseleit buffer (115 mM NaCl, 4.6 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 2.5 mM CaCl2, 25 mM NaHCO3 and 11 mM glucose) equilibrated with 95% oxygen and 5% carbon dioxide to a pH of 7.3–7.4. Perfusion was initiated at a constant flow rate of 11–15 ml/min to provide a basal perfusion pressure of approximately 70 mmHg. Both the heart and the perfusion solution were maintained at 37 °C throughout the entire process.

After a 20 min equilibration period with constant flow perfusion, effects on coronary perfusion pressure, left intraventricular pressure and heart rate were recorded in response to accumulative doses of insulin added to the perfusion solution (10^{-9} -10^{-7} M) in the presence/absence of Wortmanin (10^{-6} M) (preincubation for 30 min before insulin administration). Each dose of insulin was administered every ten minutes and the highest dose (10^{-7} M) was administered continuously for half an hour in order to induce significant changes in protein expression. To compare the effects of insulin administration in cardiac function half of the hearts from each experimental group were perfused with Krebs-Henseleit buffer during the same time without adding insulin (control hearts).

Coronary perfusion pressure was measured through a lateral connection in the perfusion cannula and left

ventricular pressure was measured using a latex balloon inflated to a diastolic pressure of 5–10 mmHg, both of them connected to Statham transducers (Statham Instruments, Los Ángeles, CA, EE.UU). Left ventricular developed pressure was recorded and used to calculate both the heart rate and the first derivative of the left ventricular pressure curve (dP/dt) as an index of heart contractility. All these parameters were recorded on a computer using the PowerLab/8e data acquisition system (ADInstruments, Colorado Springs, CO, EE.UU).

After the functional studies both control and insulin treated hearts from the three experimental groups were collected and kept frozen (−80 °C) to further analyze the effects of insulin administration in the protein expression of different markers related to insulin sensitivity in the myocardium.

Western Blot

100 mg of myocardial tissue was homogenized using RIPA buffer. After centrifugation (12000 rpm, 4°C, 20min), supernatant was collected and total protein content was measured by the Bradford method (Sigma–Aldrich, St. Louis, MO, EE.UU). In each assay, the same amount of protein was loaded in each well (100 μg). After electrophoresis using resolving acrylamide SDS gels (8–10%) (Bio-Rad, Hércules, CA, EE.UU), proteins were transferred to polyvinylidine difluoride (PVDF) membranes (Bio-Rad, Hércules, CA, EE.UU). Transfer efficiency was determined by Ponceau red dyeing (Sigma–Aldrich, St. Louis, MO, EE.UU). Membranes were then blocked with Tris-buffered saline (TBS) containing 5% (w/v) non-fat dried milk and incubated with the appropriate primary antibody; Akt (1:1000) (Merck Millipore, Darmstadt, Germany); Phospho-Akt (Ser473) (1:500) (Cell signaling Technology, Danvers, MA, EE.UU); MAPK (1:1000) (Merck Millipore, Darmstadt, Germany); Phospho-Erk1/2(Thr185/Tyr187) (1:500) (Merck Millipore, Darmstadt, Germany); IR (1:500) (Abcam, Cambridge, UK); eNOS (1:200) (Abcam, Cambridge, UK), GLUT-4 (1:500) (Abcam, Cambridge, UK), ET-1 (1:250) (Abcam, Cambridge, UK)GAPDH (1:1000) (Sigma–Aldrich, St. Louis, MO, EE.UU).

Membranes were washed and incubated with the secondary antibody conjugated with peroxidase (1:2000; Pierce, Rockford, IL, USA). Peroxidase activity was visualized by chemiluminescence and quantified by densitometry using BioRad Molecular Imager ChemiDoc XRS System (Hércules, CA, EE.UU). All membranes were finally incubated with GAPDH (Sigma–Aldrich, St. Louis, MO, EE.UU) to normalize each sample for gel-loading variability. For each sample relative protein expression levels were calculated in relation to protein expression levels in samples from 3m rats.

Statistical analysis

Data of cardiac function were analyzed by repeated measures ANOVA. Protein expression data was analyzed by two-way ANOVA considering the experimental group (3m, 24m or 24m-CR) as one factor and insulin administration as other factor. In case of interaction between the two factors, one-way ANOVA was carried out. The post-hoc analysis was performed by the Newman Keuls test. A p value of <0.05 was considered significant.

Results

Body and heart weights

CR prevented the aging-induced increase in body weight and attenuated the aging-induced decrease in heart relative weight (Table 1).

Hemodynamic parameters of perfused hearts in basal conditions

In basal conditions, 24m rats showed lower heart rate compared to 3m rats, without significant changes in coronary pressure, developed intraventricular pressure or dP/

Table 1 Body weight, relative heart weight, basal perfusion pressure, left ventricular developed pressure and heart rate in 3 month-old rats (3m), 24 month-old rats fed "*ad libitum*" (24m) and 24 month-old rats subjected to caloric restriction during their three last months of life (24m-CR).

	3m control	3m + wortmannin	24m control	24 m + wortmannin	24m-CR control	24m-CR + wortmannin
Body weight (g)	401 ± 9.4	–	679.2 ± 25.3 ***	–	558.5 ± 9.8 ***, ###	–
Heart (mg/100 g bw)	332 ± 0.009	–	258 ± 0.013 ***	–	288 ± 0.011 #	–
% Coronary perfusion, pressure (mm Hg)	95 ± 5	45 ± 2 $$$	76 ± 7	69 ± 6	90 ± 6	65 ± 12
Left ventricular developed pressure (mm Hg)	73 ± 8	95 ± 5 $	92 ± 18	99 ± 1	91 ± 12	972 ± 16
dp/dt (mm Hg/s)	1794 ± 159	2711 ± 198 $$	2177 ± 408	2436 ± 426	2115 ± 289	1743 ± 343
Heart rate (beats/min)	234 ± 10	215 ± 1	186 ± 7 *	178 ± 23	195 ± 11*	177 ± 16

*P < 0.05 vs 3 months; ***P < 0.001 vs 3 months ###P < 0.001 vs 24 months.
Data are represented as mean ± SEM (n = 7–9 rats/group).

dt. CR did not modify any of the hemodynamic parameters (Table 1).

Hemodynamic parameters of perfused hearts in response to insulin

Insulin administration to perfused hearts induced changes in coronary perfusion pressure, developed intraventricular pressure and dP/dt, that were significantly different between the doses of insulin and also between experimental groups.

Figure 1 shows how insulin administration to the perfusion system induced vasodilatation of coronary arteries at the concentrations of 10^{-9} and 10^{-8} M and vasoconstriction at the concentration of 10^{-7} M in 3m rats. On the contrary, vasodilatation in response to insulin (10^{-9} and 10^{-8} M) was abolished and contraction in response to insulin 10^{-7} M was significantly increased in coronary arteries from 24m rats compared to 3m rats (Fig. 1). CR did not prevent the aging induce alterations in coronary arteries in response to insulin administration.

Figure 2 shows how insulin administration (10^{-9} and 10^{-8} M) increased intraventricular pressure (Fig. 2A) and dP/dt (Fig. 2B) in the hearts of 3m rats but not in the hearts of 24m rats. CR partially restored the aging induced decrease both in intraventricular pressure and dP/dt. Heart rate was not significantly modified in response to insulin in any experimental group (data not shown).

Hemodynamic parameters of perfused hearts in response to insulin after blockade of the IP3K by Wortmannin

In basal conditions, pretreatment with the PI3K antagoinst Wortmannin (10^{-6} M) reduced basal coronary perfusion pressure ($P < 0.001$) and increased basal dP/dt ($P < 0.01$) and developed intraventricular pressure ($P < 0.05$) in perfused hearts from 3m rats. On the contrary, preincubation with Wortmannin did not modify any of the hemodynamic parameters in the perfused hearts of 24m and 24m-CR rats (Table 1).

Wortmannin preincubation blocked insulin induced coronary vasodilatation in the hearts from 3m rats, but it did not modify insulin-induced vasoconstriction in the hearts from 24m rats (Fig. 1). Likewise, Wortmannin abolished the insulin-induced increase of developed intraventricular pressure (Fig. 2A) and dP/dt (Fig. 2B) both in 3m rats and in 24m-CR rats.

Activation of IP3K/Akt y MAPK pathways by insulin in perfused hearts

Both factors, experimental group and insulin, induced a significant effect on the p-Akt/Akt ratio ($P < 0.05$ and $P < 0.001$ respectively; Fig. 3A) with no interaction between them. The post-hoc analysis revealed no significant differences among experimental groups in basal conditions. Insulin administration significantly increased the p-Akt/Akt ratio in the heart of 3m ($P < 0.001$), 24m ($P < 0.01$) and 24m-CR ($P < 0.001$) rats, with this increase being significantly lower in the heart of 24m rats.

There was an interaction between the two factors in the p-MAPK/MAPK ratio ($F = 4.96$; $P < 0.05$; Fig. 3B). In absence of insulin this ratio was significantly decreased in the hearts of 24m-CR rats compared to young rats ($P < 0.01$). In response to insulin, the activation of MAPK was reduced in the hearts of 3m rats ($P < 0.02$), not modified in the hearts of 24m rats and significantly increased in the hearts of 24m-CR ($P < 0.01$).

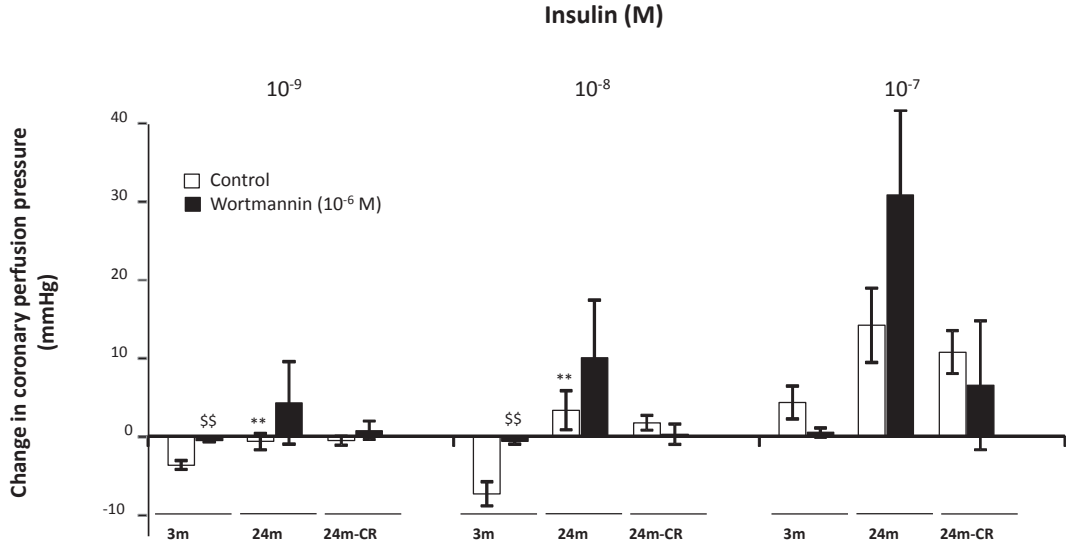

Figure 1 Coronary vasodilatation (negative changes in coronary perfusion pressure) and coronary vasoconstriction (positive changes in coronary perfusion pressure) in response to insulin (10^{-9} -10^{-7} M) in perfused hearts from 3 (3m) – or 24-months-old rats fed *at libitum* (24m), or 24-months-old rats after 3 months of caloric restriction (24m-CR), in the absence/presence of the PI3K inhibitor Wortmannin (10^{-6} M). **$P < 0.01$ difference between 3m and 24m. $$ $P < 0.01$ difference between hearts in the presence or absence of Wortmannin (10^{-6} M). Values are represented as mean ± S.E.M; $n = 7–9$ rats/experimental group.

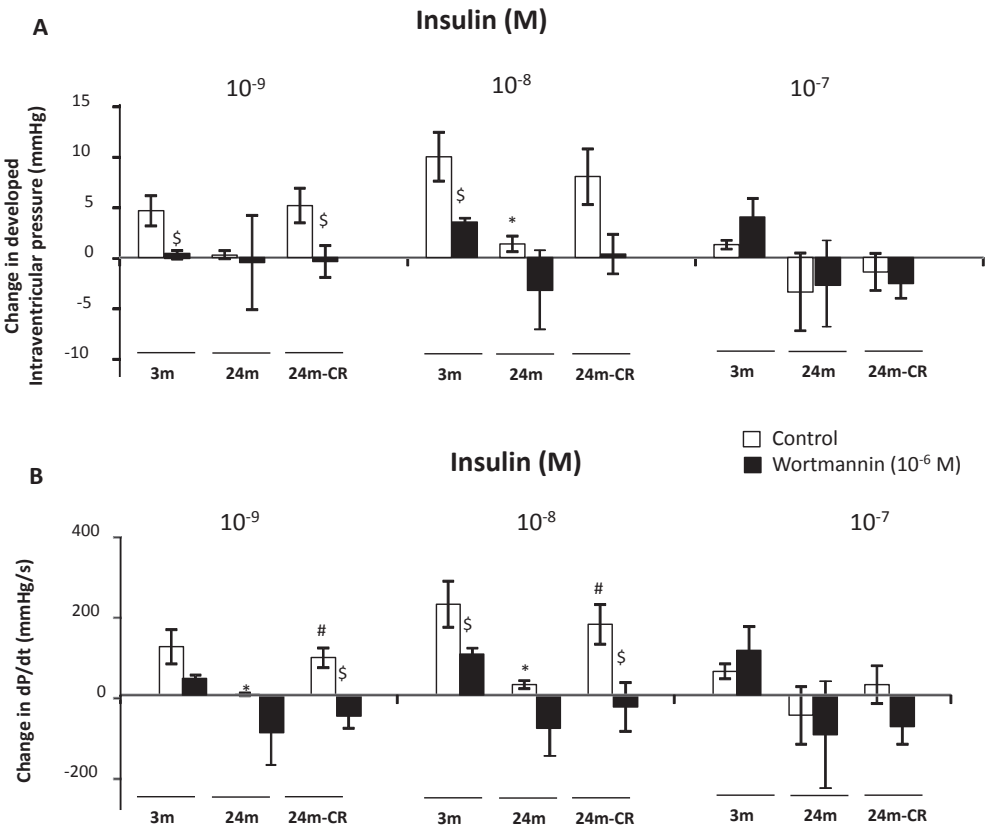

Figure 2 Changes in developed left intraventricular pressure (A) and dP/dt (B) in response to insulin (10^{-9} -10^{-7} M) in perfused hearts from 3 (3m) – or 24-months-old rats fed *at libitum* (24m), or 24-months-old rats after 3 months of caloric restriction (24m-CR), in the absence/presence of the PI3K inhibitor Wortmannin (10^{-6} M). *P < 0.05 difference between 3m and 24m; #P < 0.05 difference between 24m and 24m-CR; $ P < 0.05 difference between hearts in the presence or absence of Wortmannin (10^{-6} M). Values are represented as mean ± S.E.M; n = 7–9 rats/experimental group.

Finally, the rationale between the p-Akt/Akt and p-MAPK/MAPK ratios is shown in Fig. 3C. Both factors, experimental group and insulin, induced a significant effect on this ratio (P < 0.05 and P < 0.001 respectively; Fig. 3C) with no interaction between them. In basal conditions hearts of 24m-CR rats showed an increase in the Akt/MAPK ratio compared to young rats (P < 0.01). After insulin administration, a significant increase was found in the hearts of 3m (P < 0.001) and 24m-CR (P < 0.01) rats but not in the hearts of 24m rats.

Effects on insulin in GLUT-4, IR, ET-1 and eNOS levels in perfused hearts

Figure 4 shows the protein levels of eNOS (4A), IR (4B), GLUT-4 (C) and ET-1 (D) in hearts from 3m, 24m and 24m-CR rats in absence/presence of insulin administration.

There was no interaction between factors in eNOS, IR and GLUT-4 protein levels. In all cases, hearts perfused with vehicle showed no significant changes among experimental groups. In response to insulin eNOS, IR and GLUT-4 protein content was significantly up-regulated in hearts from 3m to 24m-CR rats (P < 0.05 for all) but not in hearts from 24m rats.

Regarding ET-1 protein expression in myocardial tissue, a significant interaction between the two factors was found (F = 6.27; P < 0.05). In basal conditions, hearts from 24m rats showed increased levels of ET-1 compared to 3m rats (P < 0.05). Insulin administration did not change ET-1 protein content in the hearts of 3m and 24m-CR rats but it significantly downregulated its protein levels in the hearts of 24m rats (P < 0.05).

Discussion

This study shows that insulin has direct effects on the heart, both in the myocardium and in the coronary circulation and that these effects are altered in response to both aging and CR. To our knowledge, this is the first study reporting the beneficial effects of CR in aging-induced cardiac insulin resistance and may have a strong relevance in the treatment and/or prevention of aging induced cardiovascular alterations.

Our results show that in the hearts of young rats, insulin produces vasodilatation in the coronary circulation at low concentrations and vasoconstriction at the highest concentration studied (10^{-7} M). Likewise insulin has been reported to exert vasodilatory effects in different vascular beds such as the aorta [18], the mesenteric [19], the

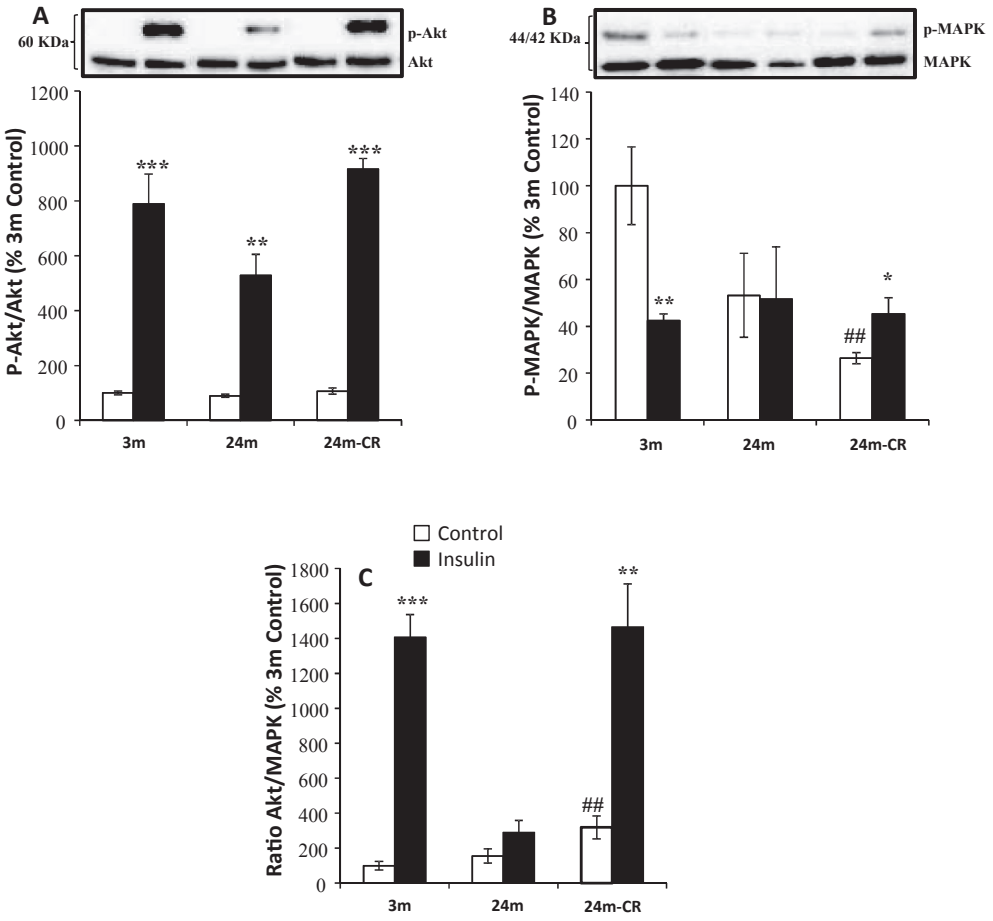

Figure 3 A) Relation of protein levels of phosphorylated Akt (p-Akt) to total Akt in perfused hearts from 3 (3m) – or 24-months-old rats fed *at libitum* (24m), or 24-months-old rats after 3 months of caloric restriction (24m-CR), incubated either with vehicle (control) or with insulin for 30 min. B) Relation of protein levels of phosphorylated MAPK (p-MAPK) to total MAPK in perfused hearts from 3 (3m), 24-months-old rats fed *at libitum* (24m), and 24-months-old rats after 3 months of caloric restriction (24m-CR), incubated either with vehicle (control) or with insulin for 30 min. C) Relation between Akt and MAPK activation in perfused hearts from 3 (3m), 24-months-old rats fed *at libitum* (24m), and 24-months-old rats after 3 months of caloric restriction (24m-CR), incubated either with vehicle (control) or with insulin for 30 min $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ difference between hearts incubated with insulin and control. ##P < 0.01 difference compared to 3m group. Values are represented as mean ± S.E.M; n = 7–9 rats/experimental group.

cerebral [20] and the coronary [21]circulations. Our results also show that insulin increased myocardial contractility in the hearts of the 3m rats, as indicated by the developed intraventricular pressure and dP/dt, which is in agreement with previous findings by Stehr et al., 2007 [22] and Ren et al., 1999, [23].

Insulin-induced coronary vasodilatation in the heart of young rats seems to be mediated by the activation of the PIK3/Akt pathway, as this effect was abolished by the preincubation with the PIK3 antagonist Wortmannin. This pathway is reported to mediate insulin-induced vasodilatation in coronary arteries [24] and the release of nitric oxide by endothelial cells [25]. Likewise, our results show that PI3K activation is also responsible for the insulin-induced inotropic effects in the myocardium, as previously described [26]. However, the vasoconstriction of coronary arteries in response to a high dose of insulin (10^{-7} M) was not affected by Wortmannin blockade, which indicates that other mechanism different than PI3K activation, may act in this condition. This mechanism most likely involves the activation of the MAPK pathway and the subsequent production of ET-1 [27] and could be related with the increased incidence of cardiovascular diseases in situations of hyperinsulinemia [4], as the activation of this pathway promotes proliferative and vasoconstrictor effects [8]. In this regard, our results show that myocardial ET-1 levels in response to insulin 10^{-7} M increase in young rats but not in aged rats, regardless of being subjected or not to CR, whereas the vasoconstrictor effect is evident in all experimental groups. These results would contradict our previous hypothesis that insulin induced-vasoconstriction of coronary arteries is mediated by ET-1. However, it is important to point out that ET-1 expression has been measured in the myocardium and not specifically in coronorary arteries, so to address this issue further investigations that include the quantification of ET-1 in coronary arteries are required.

Previous studies have confirmed that aging is associated with decreased insulin sensitivity in terms of glucose

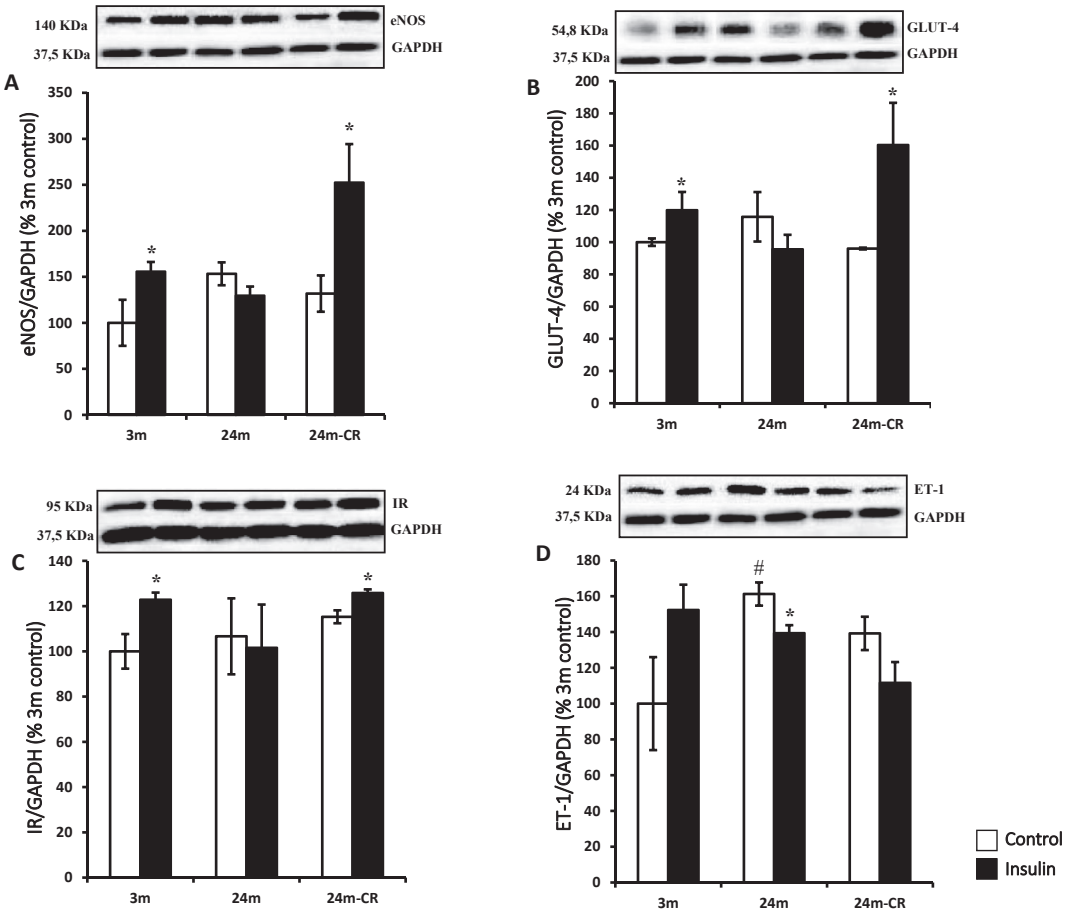

Figure 4 Protein expression of endothelial nitric oxide synthase (eNOS) (A), Insulin receptor (IR) (B) Glucose transporter 4 (GLUT-4) (C), and endothelin-1 (D) in perfused hearts from 3 (3m), 24-months-old rats fed *at libitum* (24m), and 24-months-old rats after 3 months of caloric restriction (24m-CR), incubated either with vehicle (control) or with insulin for 30 min *P < 0.05 difference between hearts incubated with insulin and control. #P < 0.05 ##P < 0.01 difference compared to 3m. Values are represented as mean ± S.E.M; n = 7–9 rats/experimental group.

uptake by cardiomyocytes [28,29]. In this study we did not measure glucose uptake, which may constitute a limitation of the study. However, both the coronary vasodilatation and the myocardial inotropic effect in response to insulin were abolished in the hearts of 24m rats, which indicates a state of cardiac insulin resistance. Likewise, other studies have found that ageing is associated with decreased insulin sensitivity in the myocardium [23]. This is probably due to an impairment of the PIK3/Akt pathway, as the ratios pAkt/Akt and Akt/MAPK were increased in response to insulin in a less extent in the hearts of 24m rats compared to the hearts of 3m rats. Although the protein expression of IR and GLUT-4 was similar in the hearts of young and old rats, insulin significantly activated the protein expression of both proteins in the myocardium of young rats but not in the myocardium of aged rats fed *ad libitum*. The effect of insulin on GLUT-4 is mediated by activation of PIK3/Akt [30], so these results clearly show that aging is associated with an impairment of all the effects dependent of this pathway.

Aging-induced insulin resistance in coronary arteries is most likely due to impairment in nitric oxide synthesis, as eNOS protein expression was upregulated in response to insulin in the hearts of young rats but not in the hearts of aged rats fed *ad libitum*.

The vasoconstrictor peptide ET-1 exerts opposite effects to nitric oxide, and its enhanced activity and/or production by the endothelium is involved in cardiovascular insulin resistance [9,31]. In the present study, the basal expression of ET-1 was significantly increased in 24m rats, in agreement with previous studies [32], but it was not modified in response to insulin administration neither in young nor in aged rats. Therefore the exact role of ET-1 in the reduced insulin-induced vasodilatation in this experimental model requires further investigation.

CR has emerged as an effective procedure to prevent some of the vascular and cardiac dysfunctions associated with aging [33]. The present model of CR has been shown to attenuate insulin resistance in other organs and tissues [13] including the aorta [15]. Likewise, in this study we have found that CR improved partially or completely the aging-induced insulin resistance in coronary arteries vasodilation, myocardial contractility and the gene expression of IR, GLUT-4 and eNOS in myocardial tissue. In addition, although the basal expression of ET-1 was not reduced by CR, in response to insulin the protein levels of

this vasoconstrictor peptide were significantly reduced, with this effect not being observed in aged rats fed *at libitum*.

The beneficial effect of CR preventing the aging-induced decrease in myocardial contractility seems to be mediated by a decreased activation of MAPK pathway and an increased activation of the PI3K/Akt pathway in response to insulin as it is indicated by the Akt/MAPK ratio. It is reported that insulin-induced activation of PI3K/Akt pathway is responsible for the inotropic effect and for the vasodilation of coronary arteries through eNOS activation and the subsequent release of NO by vascular endothelium, whereas activation of the MAPK pathway promotes deleterious effects such as cardiomyocyte hypertrophia and vasoconstriction of coronary arteries due to the release of ET-1 [4]. It is also reported that in physiological conditions the activation of the PI3K/Akt pathway predominates over the activation of the MAPK pathway. On the contrary, in situations of insulin resistance the activation of the PI3K/Akt pathway decreases and the activation of the MAPK pathway increases or remains unchanged in response to insulin which justifies, at least in part, the decreased contractility of myocardium in aged rats and its prevention by CR.

Conclusions

In conclusion, CR may be an effective dietetic intervention to improve the aging-induced impairment of cardiac function due to decreased myocardial insulin sensitivity.

Conflicts of interest

Authors declare no conflict of interests.

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