Cardiovascular Pharmacology

Heart dysfunction induced by choline-deficiency in adult rats: The protective role of L-carnitine

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Choline is a B vitamin co-factor and its deficiency seems to impair heart function. Carnitine, a chemical analog of choline, has been used as adjunct in the management of cardiac diseases. This study investigates the effects of choline deficiency on myocardial performance in adult rats and the possible modifications after carnitine administration. Wistar Albino rats (n=24), about 3 months old, were randomized into four groups fed with: (a) standard diet (control-CA), (b) choline deficient diet (CDD), (c) standard diet and carnitine in drinking water 0.15% w/v (CARN) and (d) choline deficient diet and carnitine (CDD+CARN). After four weeks of treatment, we assessed cardiac function under isometric conditions using the Langendorff preparations [Left Ventricular Developed Pressure (LVPD-mmHg), systolic force, assessed by (+) dp/dt, showed no statistical difference between groups. A significant increase in serum BNP concentration was found in the CDD group (P<0.004) which was attenuated by carnitine (P<0.05), whereas homocysteine presented contradictory results (higher in the CDD+CARN group). Heart histopathology revealed a lymphocytic infiltration of myocardium and valves in the CDD group that was reduced by carnitine. In conclusion, choline deficiency in adult rats impairs heart performance; carnitine acts against these changes.

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1. Introduction

Choline is a B vitamin co-factor and, although it is not by strict definition a vitamin, it is considered as an essential nutrient (Zeisel and Bluszczyn, 1994). It is almost always found in the form of phosphatidylcholine or sphingomyelin in most foods (Zeisel et al., 2003). A choline deficient setting, due to inadequate dietary intake, is established in about two weeks (Pomfret et al., 2010) and little is known about the effects observed in older rodents. Carnitine, a tertiary amino-acid, is considered as a chemical analog of choline (Pieklik and Guynn, 1975). It is synthesized in liver and kidneys and is mainly stored in the skeletal and cardiac muscle (Dayanand et al., 2011). L-carnitine, the biologically active enantiomer, mediates the transport of long-chain fatty acids into the mitochondrial matrix for beta-oxidation and has proved to be of protective role in myocardium contractility compared to control (P<0.01), as assessed by LVPD, was noted along with a significantly impaired diastolic left ventricular function, as assessed by (-) dp/dt (P=0.02) that were prevented by carnitine. Systolic force, assessed by (+) dp/dt, showed no statistical difference between groups. A significant increase in serum BNP concentration was found in the CDD group (P<0.004) which was attenuated by carnitine (P<0.05), whereas homocysteine presented contradictory results (higher in the CDD+CARN group). Heart histopathology revealed a lymphocytic infiltration of myocardium and valves in the CDD group that was reduced by carnitine. In conclusion, choline deficiency in adult rats impairs heart performance; carnitine acts against these changes.

Choline seems to have, among other traits, a major physiological role in the development and function of the cardiovascular system in rodents and choline deficiency has been associated with significant cardiovascular morbidity or even mortality (Kesten et al., 1945; Newberne and Salmon, 1963; Repetto et al., 2010; Wilgram et al., 1954; Wilgram, 1957; Williams, 1960). Furthermore, choline is precursor of acetylcholine, which is a basic neurotransmitter of the autonomous nervous system that regulates chronotropic and dromotropic responses of the heart. However, most of the data regard weanling and young rats (Kesten et al., 1945; Wilgram et al., 1954, Wilgram, 1957; Repetto et al., 2010) and little is known about the effects observed in older rodents.

Carnitine is a chemical analog of choline (Pieklik and Guynn, 1975). It is synthesized in liver and kidneys and is mainly stored in the skeletal and cardiac muscle (Dayanand et al., 2011). L-carnitine, the biologically active enantiomer, mediates the transport of long-chain fatty acids into the mitochondrial matrix for beta-oxidation and has proved to be of protective role in myocardium contractility compared to control (P<0.01), as assessed by LVPD, was noted along with a significantly impaired diastolic left ventricular function, as assessed by (-) dp/dt (P=0.02) that were prevented by carnitine. Systolic force, assessed by (+) dp/dt, showed no statistical difference between groups. A significant increase in serum BNP concentration was found in the CDD group (P<0.004) which was attenuated by carnitine (P<0.05), whereas homocysteine presented contradictory results (higher in the CDD+CARN group). Heart histopathology revealed a lymphocytic infiltration of myocardium and valves in the CDD group that was reduced by carnitine. In conclusion, choline deficiency in adult rats impairs heart performance; carnitine acts against these changes.
possess anti-oxidant and anti-inflammatory activity (Calò et al., 2006; Gómez-Amores et al., 2006; Mingorance et al., 2011; Silvério et al., 2011), in contrast to a choline deficient state that is associated with increased oxidative stress (Repetto et al., 2010). Due to its pharmacologic properties, carnitine has been recognized as a nutritional supplement in cardiovascular disease (Flanagan et al., 2010) and it is currently used as an adjunctive therapy in various heart conditions with promising results (Dayanand et al., 2011; Krim et al., 2012).

Carnitine is metabolized much more rapidly in choline-deficient rats than in normal rats (Mehlman et al., 1978; Tsai et al., 1975). In addition, choline can serve as methyl donor for the synthesis of carnitine from lysine and methionine (Griffith, 1987) and choline deficiency has been accompanied by carnitine deficiency (Corredor et al., 1967; Dodson and Sachan, 1996; Sheard and Krasin, 1994). This fact could further impair myocardial function (Zaugg et al., 2003) since cardiac muscle utilizes fatty acids as its primary energy source (Dayanand et al., 2011).

The present study was designed (a) to investigate the short-term effects of dietary choline deprivation on heart function and histology of healthy adult rats and (b) to evaluate the effects of carnitine administration on the eventual changes of the aforementioned parameters in a choline deficient state.

2. Materials and methods

2.1. Animals and diets

Male Wistar Albino rats \((n=24)\), three months old \((350+/−30\text{ g body weight})\), purchased from the Greek National Center of Scientific Research 'Democritos', were used. After seven days of acclimatization at constant environmental conditions \((\text{room temperature 25}+/−1 \text{ C, humidity 45% and light/dark cycle 12/12 h})\), the rats were randomly assigned into 4 groups according to the following dietary pattern: (a) rats receiving standard diet \((\text{control-CA})\), (b) rats receiving standard diet and carnitine in drinking water \(0.15\text{% w/v}\), (c) choline-deprived rats receiving choline deficient diet \((\text{CDD})\) and (d) choline-deprived rats receiving choline deficient diet and carnitine in drinking water \(0.15\text{% w/v}\). The number of animals in each group was six. They were housed separately in stainless steel cages, while food and water were provided \(\text{ad libitum}\). Diets were purchased from AnaLab Ltd., Athens, Greece and L-carnitine was obtained by Vianex SA, Athens, Greece. The mean daily dose of L-carnitine used was \(200\text{ mg/kg body weight}\). The analytical composition \((\text{g/kg})\) of the choline deficient diet was: sugar 413, starch 110, dextrose 110, hydrogenated vegetable oil 100, pea protein 90, soya protein isolate 60, corn oil 50, mineral mix 35, vitamin mix 10, cellulose 10, vitamin free casein 10, L-cystine 2. The standard diet was enriched by choline \((1.1 \text{ g/kg})\) at the expense of sucrose. The dietary intervention was imposed for four weeks in order to explore the early effects of dietary choline deprivation in the heart function of adult rats. All animal procedures were carried out in accordance with EEC-86/609 regulations under the authority of the relevant project license obtained from the Prefecture of Athens and were approved by the Institutional Animal Care and Use Committee of the University of Athens for Medical Sciences. The number of rats and the suffering were kept to a minimum as possible.

2.2. Isolated heart preparation

A non-working isolated rat heart preparation was perfused at a constant flow according to the Langendorff technique. An intraventricular balloon allowed measurement of left ventricular pressure under isovolumic conditions. Left ventricular balloon volume was adjusted to produce an average initial left ventricular end-diastolic pressure of 6–8 mmHg in all groups and was held constant thereafter throughout the experiment. Mean coronary perfusion pressure was adjusted between 67 and 72 mmHg in all experiments during the first 5 min of stabilization and kept constant thereafter. This resulted in mean coronary flow of 15.5 ml/min. No difference in coronary flow was found between groups. Intraventricular balloon was made from a flexible balloon catheter and latex membrane (Pantos et al., 2003,2007,2009). Since the balloon was not compressible, left ventricular contraction was isovolumic. As intraventricular volume was maintained at a constant value, diastolic fiber length, which represented preload, did not change. Thus, the left ventricular peak systolic pressure and the left ventricular developed pressure (LVPDP), defined as the difference between left ventricular peak systolic pressure and left ventricular end-diastolic pressure, represented indexes of systolic function obtained under isometric conditions.

Rats were anaesthetized with ketamine HCl \((100\text{ mg/kg body weight})\) and heparin 1000 IU was given intravenously before thoracotomy. Ketamine was chosen, because it minimally affects contractile properties of the myocardium (Pantos et al., 2003,2007,2009). The hearts were rapidly excised, placed in ice-cold Krebs-Henseleit buffer \((\text{composition in mmol/L: sodium chloride 118, potassium chloride 4.7, potassium phosphate mono-basic 1.2, magnesium sulfate 1.2, calcium chloride 1.4, sodium bicarbonate 25, and glucose 11})\) and mounted on the aortic cannula of the Langendorff perfusion system. Perfusion with oxygenated \((95\% \text{ O}_2/5\% \text{ CO}_2)\) Krebs-Henseleit buffer was established within 60 s after thoracotomy. The perfusion apparatus was heated to ensure a temperature of \(37^\circ\text{C}\) throughout the course of the experiment. Sinus node was removed after excision of the right atrium. All hearts were paced by epicardial placement of a platinum lead in the intraventricular septum at the base of the heart. Voltage and pulse duration of the pacemaker were adjusted at 9 V and 0.5 ms respectively, while the heart rate was set at 330 beats per min. This value of heart rate is within normal limits for rat species (Van Zutphen et al., 2001). The water filled balloon, connected to a pressure transducer and coupled to a Gould RS 3400 recorder was advanced into the left ventricle through an incision in the left atrium. Pressure signal was transferred to computer using data analysis software \((\text{IOX, Emka Technologies})\) which allowed continuous monitoring and recording.

Left ventricular function was assessed by recording the left ventricular developed pressure \((\text{LVPDP-mmHg})\) and the positive and negative first derivatives of LVPDP \((+)/\text{dp/dt and } (-)/\text{dp/dt})\). \((+)/\text{dp/dt and } (-)/\text{dp/dt} (\text{mmHg/s})\) are the sensitive indices of contractile function with respect to the rate of increase and rate of decrease of intraventricular pressure respectively. All preparations were perfused for 30 min and measurements were performed at the end of this period. All preparations included in this study were stable for at least the last 10 min of the perfusion period. LVPDP as well as \((+)/\text{dp/dt and } (-)/\text{dp/dt} \text{ values were recorded as mean values over a period of 30 s}.

2.3. Histopathologic analysis

Following the evaluation of the mechanical heart function, the hearts were fixed after incubation in 10% formalin solution and then embedded in paraffin. Six sequential tissue sections, 4 mm in thickness, were taken from each heart, at a distance approximately of 2 mm from each other. Histological evaluation of the hearts was performed using Eosin-Hematoxylin and Masson stains. Randomly selected fields were examined under light microscopy. Myocardial inflammation, valvular inflammation, cardiac interstitial fibrosis, perivascular fibrosis and cardiac interstitial edema were separately graded in a scale from 0 to 3 \((0=\text{absent}, 1=\text{mild}, 2=\text{moderate}, 3=\text{severe})\).
3 = severe) by two independent experienced pathologists in a blinded manner. Consensus was achieved in all specimens; re-evaluation was necessary in only 8 out of 112 slides examined.

2.4. Homocysteine and BNP assay

Blood was collected after sternotomy from the right atrium. Serum was stored at −80 °C until analysis. Homocysteine levels in serum were measured by using Fluorescent Polarization Immunoassay kit obtained from Abbot SA (AxSYM System, USA). The reference range of the kit is 6–15 μmol/L. BNP quantification was performed by the use of the AssayMax rat BNP-45(rBNP-45) Elisa Kit (AssayPro), using a polyclonal antibody specific for BNP-45. The minimum detectable dose of rat BNP-45 is typically ~0.03 ng/ml. Intra-assay and inter-assay coefficients of variation were 4.3% and 7.4% respectively.

2.5. Statistical analysis

The statistical tests have been performed by the Statistical package SPSS 19 (Academic license). Prior to any statistical test, the normality of the studied variables was evaluated by the Kolmogorov–Smirnov normality test, according to which the variables LVDP, (+) dp/dt, (−) dp/dt and homocysteine were found to follow a normal distribution whereas the rest did not. Furthermore, the four groups (CA, CARN, CDD, CDD+CARN) had equal variances, according to Levene’s test.

ONE WAY ANOVA test and Kruskal Wallis test were performed for all variables with normal and non-normal-distribution respectively. Statistical significance was considered for P values of <0.05. Whatever the P value of these tests was lower than 0.05 the statistical significance between the groups was checked, one by one, by performing t-test for independent samples and non-parametric Mann Whitney test respectively.

3. Results

3.1. Evaluation of the mechanical properties of the heart

Results of the mechanical evaluation of the myocardium are demonstrated in Fig. 1.

Left ventricular function, as assessed by LVDP values, showed a slight but statistically significant deterioration of 17% in the CDD group compared to the control group [102 (8.71) mmHg vs. 123 (13.5) mmHg, P=0.01]. Carnitine administration prevented impairment of left ventricular function; LVDP was increased by 15.7% in the CDD+CARN group compared to the CDD group [121 (6.5) mmHg in the CDD+CARN group vs. 102 (8.71) mmHg in the CDD group, P < 0.001]. (+) dp/dt, representing the left ventricular systolic force, was not statistically different between groups. On the contrary, diastolic left ventricular function, assessed by (−) dp/dt, was significantly impaired in the CDD group compared to the control group, resulting in a decrease of 17.3% [1911 (229.5) mmHg/s vs. 2311 (329) mmHg/s, P=0.002]. However, carnitine administration prevented diastolic dysfunction in rats under choline deficient diet since (−) dp/dt was increased by 15.1% in the CDD+CARN group compared to the CDD group, approaching the LVDP values of the control group [2250 (141.7) mmHg/s in the CDD+CARN group vs. 1911 (229.5) mmHg/s in the CDD group, P=0.01].

3.2. Heart pathology

The results of the histopathologic analysis are shown in Fig. 4.

The inflammatory infiltration consisted mainly of lymphocytes; immunohistochemical labeling for macrophages with anti-CD 68 antibody was negative.

Myocardial inflammation: Focal inflammatory infiltration of the myocardium was mostly observed in rats consuming choline deficient diet (Fig. 4a) compared to rats consuming standard diet, alone or in combination with carnitine. Carnitine administration, although attenuated, was unable to prevent these myocardial inflammatory lesions in CDD rats (Fig. 2).

Valvular inflammation: The grade of valvular inflammation was higher in rats consuming choline deficient diet (Fig. 4b) compared to rats consuming standard diet, alone or in combination with carnitine. Administration of carnitine prevented the development of valvular inflammation in CDD rats (Figs. 2 and 4d).

Cardiac interstitial fibrosis: The cardiac interstitial fibrosis has been considered as a specific histopathologic finding since it was...
observed only in the groups receiving choline deficient diet (Fig. 4c). Moreover, its severity was reduced by carnitine administration (Figs. 3 and 4d).

Perivascular fibrosis and cardiac interstitial edema: These histopathological findings are not considered to be absolutely specific since they were observable at some degree in all groups. However, significant differences, concerning their extent and severity, were detected among the various examined groups being considerably more severe in the CDD group. Furthermore, carnitine administration in rats under standard diet was associated with some worsening of the perivascular fibrosis and cardiac interstitial edema (Fig. 4e) whereas in the CDD rats a striking reduction of the severity of these lesions was noticed (Figs. 3 and 4d).

3.3. Serum homocysteine and brain Natriuretic peptide (BNP) levels

Homocysteine levels were not statistically different between the control and the CDD groups (17.2 ± 2.7 μmol/L in the CDD vs. 14.5 ± 1.3 μmol/L in the CA). Among groups, rats consuming choline deficient diet and carnitine in the drinking water (CDD+CARN) had the highest homocysteine levels (31.15 ± 11.02 μmol/L) compared to every other group (vs. 14.5 ± 1.3 μmol/L in CA, P=0.01, vs. 14 ± 1.4 μmol/L in CARN group, P=0.01 and vs. 17.2 ± 2.7 μmol/L in CDD group, P=0.03). Fig. 5 depicts the observed changes in serum homocysteine concentration among the groups.

In the CDD group a statistically significant increase of BNP (0.73 ± 0.01 ng/ml) was found compared either to CA (0.07 ± 0.01 ng/ml, P=0.004) or to CARN (0.08 ± 0.01 ng/ml, P=0.004) group. BNP was significantly reduced in the CDD+CARN group compared to CDD (0.71 ± 4 x 10^-2 ng/ml vs. 0.73 ± 0.01 ng/ml, P=0.026) but the levels remained very high compared to control group.

4. Discussion

The present study clearly demonstrates the early detrimental effects of dietary deprivation of choline on myocardial performance of adult rats and the beneficial effects of carnitine in this setting. Our study involved isolated hearts in which there was no autonomic stimulation, thus the possible impact of choline deficiency and carnitine administration on cardiac autonomic neurotransmission could not be evaluated.

Choline deprivation has been reported to cause a constellation of cardiac manifestations including myocardial fibrosis, restrictive pericarditis, myocardial necrosis, fatty deposits inside the myocardium, mononuclear cell myocardial infiltration, pericardial effusions and artery thrombosis (Hove et al., 1957; Kesten et al., 1945; Newberne and Salmon, 1963; Repetto et al., 2010; Wilgram et al., 1954, Wilgram, 1957; Williams 1960). The severity of the lesions in experimentally induced choline deficiency is species, sex and diet dependent with heart morbidity being more severe in male rodents.
following consumption of high fat diets (Hove et al., 1957; Newberne and Salmon, 1963; Wilgram et al., 1954).

In the present study, the findings of myocardial monocyte infiltration in the choline deficient group, along with the cardiac interstitial edema and fibrosis, are in accordance to previous reports (Kesten et al., 1945; Repetto et al., 2010; Wilgram et al., 1954; Wilgram, 1957; Williams, 1960). However, the deleterious effect of choline deficiency on the cardiac valves, involving valvular inflammation and fibrosis, to our knowledge, has not been described before. In contrast to the previous studies, we did not observe any fat deposits in the myocardium, arterial thromboses or pericardial effusions. The above discrepancies, according

Fig. 4. Histopathologic lesions in the valves and the myocardium of the studied groups.
(a) Evident lymphocytic infiltration within and around edematous myocardial fibers of a specimen from the group receiving choline deficient diet (CDD group), (H-E, original magnification × 400).
(b) Presence of mononuclear inflammatory cells in a valve of a specimen from the group receiving choline deficient diet (CDD group), (H-E, original magnification × 100).
(c) Evidence of fibrosis in a valve of a specimen from the group receiving choline deficient diet (CDD group), (Masson, original magnification × 100).
(d) Minimal inflammatory-fibrotic changes in a valve of a specimen from the group receiving choline deficient diet plus carnitine (CDD+CARN group), (Masson, original magnification × 100).
(e) Mild pathologic lesions are implied in this specimen from the group receiving standard diet plus carnitine (CARN group), (Masson, original magnification × 100).

Fig. 5. Serum homocysteine concentration from rats under standard diet (CA), choline deficient diet (CDD), carnitine (CARN), choline deficient diet and carnitine (CDD+CARN). Values are expressed as mean ± S.E.M. (6 animals per group). Asterisks represent the statistical significant differences between the indicated groups, *P < 0.05, **P < 0.01, ***P < 0.001.
to the existing literature, may be due to the different animal species, the duration of the dietary manipulation and the variability of fat type and concentration in the choline deficient diets used (Kesten et al., 1945; Newberne and Salmon, 1963; Williams, 1960).

The pathologic changes in the hearts of the CDD group were accompanied by concordant functional changes. The myocardium of the CDD rats showed impaired left ventricular contractility (as demonstrated by the LVDP values) and diastolic function [as demonstrated by the (−) dp/dt values] indicating that adult rats under a choline deficient state develop prominent early diastolic dysfunction probably due to reduced compliance of the myocardium. This is the first study demonstrating the choline-deficiency impact on the functional parameters of the heart.

The myocardial dysfunction is linked with the inflammatory reaction observed in the hearts of CDD rats, which in turn may be linked with the increased oxidative stress and decreased antioxidant concentrations observed in a choline deficient setting (Marcinek, 2004; Petrossilo et al., 2007; Repetto et al., 2010; Vrablic et al., 2001).

In a choline deficient setting, homocysteine cannot be converted to methionine due to lack of methyl groups and is accumulated. Hyperhomocysteinemia has been reported to adversely affect myocardial compliance and heart contractility (da Costa et al., 2005; Herrmann et al., 2006; Joseph et al., 2003). However, in the present study, although the homocysteine serum levels in choline deprived rats were higher than the control, the difference did not reach statistical significance. This can be attributed to the relatively short duration of the dietary intervention (Devì et al., 2006) and the age of the rats. Indeed, hyperhomocysteinemia has been observed in weaning rats, in which the immature renal function and the development of renal failure favor the accumulation of homocysteine (Ossani et al., 2006).

Administration of L-carnitine attenuated and even prevented valve inflammatory infiltration and reduced fibrosis, while it had a non-significant effect on the inflammatory infiltration of the myocardium. This discrepancy could probably be related to the impaired endothelium function that has been shown to precede the inflammation and fibrosis in a choline deficiency setting (Pasarin et al., 2012). Thus, the inflammatory lesions noted in the valves could be attributed to the endothelium dysfunction which is commonly associated with increased reactive oxygen species and reduced NO (nitric oxide) bioavailability (Durbin and Gotlib, 2002; Leask et al., 2003). The beneficial role of carnitine in this case is due to its ability to ameliorate the proper function of endothelium and increase NO production (De Marchi et al., 2012).

Moreover, it facilitates the restoration of myocardial energy reserves (Dayanand et al., 2011), in accordance to the results of the heart mechanical evaluation. As far as the myocardial inflammation is concerned, the reported hyperhomocysteinemia in the CDD+CARN group indicates the presence of oxidative stress in the respective area, implying that carnitine failed to substantially reduce it; the underlying mechanisms, though, remain obscure and further studies are needed to elucidate this issue.

However, the improvement of heart histology was accompanied by restoration of the functional properties of the myocardium to control levels. Carnitine may preserve myocardial function by modulating the activity of xanthine oxidase (Derin et al., 2004), nitric oxide synthase (Derin et al., 2004) and monoamine oxidase (Savitha et al., 2007), thus reducing reactive oxygen species production (Strauss and Porras, 2007). In addition, carnitine has been shown to exert significant anti-fibrotic action by reducing angiotensin-II collagen release in heart fibroblasts (Chao et al., 2010), decreasing endothelin-1 (Chao et al., 2010) and collagen expression (Orlandi et al., 2007) and modulating the equilibrium between metalloproteases and metalloprotease inhibitors (Martínez et al., 2009).

Surprisingly, while L-carnitine administration had no effect on serum homocysteine levels in the standard diet group, it increased homocysteinaemia in the group receiving choline deficient diet. However, this adverse effect did not seem to influence the myocardial contractile response since heart mechanical evaluation in the CDD+CARN group was comparable to that of the control group. Taking into account the short duration of the experimental procedure, the eventual maintenance of the myocardial performance is in corroboration with the study of Walker et al. (2004) demonstrating that a relatively short-term exposure to hyperhomocysteinemia does not necessarily impair heart mechanics properties. The role of carnitine in the homocysteine metabolism in a choline deficiency setting has not yet been fully elucidated. Our data imply that the interaction between choline and carnitine is more complicated than acting as a substitute to one another.

Nevertheless, the groups fed with choline deficient diet (CDD, CDD+CARN) demonstrated a remarkable increase of BNP probably related to the myocardial diastolic dysfunction (Cingolani et al., 2003) and the presence of inflammatory lesions (Ogawa et al., 2008). Furthermore, the increase of BNP could also be related to the impaired mitochondrial fatty acid oxidation observed in this setting as has been previously reported (Guéant Rodriguez et al., 2012). Consequently, the significant decrease in serum BNP concentration noted in the CDD+CARN group compared to the CDD group could be attributed to the anti-oxidant and anti-inflammatory capacity of carnitine. Moreover, carnitine prevents myocardial cell apoptosis by inhibiting ceramide generation (Abadie et al., 1999), preventing mitochondrial cytochrome c release (Oyanagi et al., 2011) and enhancing prostacycline synthase and peroxisome proliferators activator receptor alpha activities (Chao et al., 2011; Jing et al., 2011). The co-existence of increased levels of homocysteine and BNP in the CDD+CARN group along with the maintenance of heart functional properties is in line with the study of Herrmann et al. (2007) where no adverse cardiac remodeling was observed either.

An interesting finding was the mild pathologic lesions shown in the carnitine only treated group (CARN), even though these lesions were not able to impair the heart performance. The mean daily dose of L-carnitine used was 200 mg/kg body weight which, in humans, is equivalent to 2.3 g for a 70 Kg person (Reagan-Shaw et al., 2008), corresponding to a therapeutic dose ranging between 1 and 4 g/day. Although the beneficial effects of L-carnitine on organ function have been observed in several diseases including alcoholic myocardopath (Jing et al., 2011), myocardial ischemia and reperfusion (Orlandi et al., 2007), chronic heart failure (Orlandi et al., 2007), doxorubicin-induced heart damage (Abadie et al., 1999; Chao et al., 2011; Strauss and Porras, 2007), cisplatin-induced renal fibrosis (Martínez et al., 2009), chronic renal failure (Sener et al., 2004) and bleomycin-induced lung damage (Sayed-Ahmed et al., 2004), the effects of carnitine administration at physiologic or pharmacologic doses in healthy subjects, have never been thoroughly investigated (Broad et al., 2011). The finding that carnitine may imply heart toxicity under normal conditions merits additional investigation and it may be of clinical importance.

5. Conclusions

In conclusion, we demonstrated that choline deficient diet administered for four weeks in adult rats can lead to adverse cardiac pathologic changes that are accompanied by significant functional impairment of the heart. L-carnitine supplementation seems to be able to offset the deleterious effect of choline deficiency on the myocardial performance managing to restore heart mechanical properties.
6. Clinical perspectives

Choline deficiency has been reported in various clinical settings such as in patients treated with methotrexate (Cassano et al., 2011), anticonvulsant drugs (Biai, 2011) and parenteral nutrition (Zeisel, 2005), as well as in patients with bipolar disorder (Stoll et al., 1996), depression (Stoll et al., 1996), history of gastric-bypass surgery (Biai, 2011), chronic renal failure (Zeisel, 2005) and in postmenopausal women (Fisher et al., 2010). However, the data regarding the clinical impact of choline deficiency are very limited (Zeisel et al., 2001) and the possible impact of this nutritional deficiency on the heart function has not been established. According to the present study, one could assume that choline deficiency in the clinical setting could affect the overall recovery of a patient since not expected heart problems could arise. A deterioration of 17% in contractile function in a patient (e.g. fall in ejection fraction from 60% to 45%) has clear biological value since it is characterized as mild heart failure. Carnitine seems to have a compensatory potential in a choline deficient state. Further investigation is necessary to delineate the interaction of choline and carnitine in humans.

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Appendix A. Supporting information
Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2013.03.025.

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