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Journal of Nutritional Biochemistry 15 (2004) 80–89

THE JOURNAL OF Nutritional Biochemistry

# Opposite effect of methionine-supplemented diet, a model of hyperhomocysteinemia, on plasma and liver antioxidant status in normotensive and spontaneously hypertensive rats

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Received 3 January 2002

**Abstract**

Hyperhomocysteinemia is often associated with an increase in blood pressure. However our previous study has shown that methionine supplementation induced an increase in blood pressure in Wistar-Kyoto (WKY) rats and a decrease in blood pressure in spontaneously hypertensive rats (SHR) with significant differences in plasma homocysteine (Hcy) metabolites levels. Previously liver antioxidant status has been shown to be decreased in SHR compared to WKY rats. It has been suggested that oxidative stress may predispose to a decrease in NO bioavailability and induce the flux of Hcy through the liver transsulfuration pathway. Thus the aim of this study was 1) to investigate the effect of methionine supplementation on NO-derived metabolites in plasma and urine 2) to investigate whether abnormalities in Hcy metabolism may be responsible for the discrepancies observed between WKY rats and SHR concerning blood pressure and 3) to investigate whether a methionine-enriched diet, differently modified plasma and liver antioxidant status in WKY rats an SHR. We conclude that the increase in blood pressure in WKY rats is related to high plasma cysteine levels and is not due to a decrease in NO bioavailability and that the decrease in blood pressure in SHR is associated with high plasma GSH levels after methionine supplementation. So GSH synthesis appears to be stimulated by liver oxidative stress and GSH is redistributed into blood in SHR. So the great GSH synthesis can be rationalized as an autocorrective response that leads to a decreased blood pressure in SHR. 

*Keywords:* Homocysteine; Hypertension; Spontaneously hypertensive rats; Oxidative stress; Liver; Glutathione content

## 1. Introduction

Methionine-enrichied diet, responsible for mild hyperhomocysteinemia (HHcy), has been shown by us and others to induce development of hypertension in rats [1–3]. In humans, HHcy is generally associated with atherosclerosis and hypertension [4, 5]. Relationships between HHcy and vascular pathology have not been fully explained [5, 6]. It is well established that free radicals may contribute to the pathogenesis of essential hypertension [7–9]. Recent studies have shown that homocysteine (Hcy) increases oxidant stress in vitro [10–12]. Our group and the others have found that liver antioxidant status was decreased in various hypertensive rat models compared to normotensive controls such as genetically hypertensive (SHR) Wistar-Kyoto rats and deoxycorticosterone acetate (DOCA)-salt treated Sprague-Dawley (SD) rats [13–17]. Metabolic derivatives of Hcy such as cysteine has been shown to be even more toxic on vessels than Hcy through the induction of oxidative stress [18]. On the other hand, the metabolic derivative of methionine, glutathione reduced (GSH), is a reference antioxidant component of living organisms. We have recently shown that the systolic blood pressure of WKY rats fed for 10-weeks with a methionine-supplemented diet increased significantly, whereas systolic blood pressure was reduced in SHR. Methionine-supplemented diet induced a significant increase in plasma Hcy and methionine concentrations in both WKY and SHR, associated in WKY rats by an increase in plasma cysteine concentrations and in SHR by an increase in the GSH concentration [1].

Thus the aim of this study was 1) to investigate the effect

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0955-2863/04/$ – see front matter 
doi:10.1016/j.jnutbio.2003.09.007

of methionine supplementation on nitrates and nitrites levels (known to be nitric oxide-derived metabolites) in plasma and urine because previous findings related the development of arterial hypertension to a depletion of nitric oxide 2) to investigate whether abnormalities in Hcy metabolism may be responsible for the discrepancies observed between WKY and SHR concerning blood pressure and 3) to investigate whether a methionine-enriched diet, responsible for mild HHcy, differently modified plasma and liver antioxidant status in normotensive and spontaneously hypertensive rats.

## 2. Methods and materials

### *2.1. Animals and treatments*

Plasma and liver samples of SHR and WKY male rats fed with standard diet or methionine-enrichied diet were used from our previous study for the determination of liver, plasma copper and iron levels, hepatic and plasma antioxidant capacities, liver GSH and thiobarbituric acid reactive substance (TBARS) levels and for the evaluation of glutathione reductase (GSSG-Red), glutathione peroxidase (GSH-Px), catalase and superoxide dismutase (SOD) activities. Animals were randomized into 4 groups ($n = 12$ in each group) [1]. In summary, WKY and SHR control groups were fed for 70 days with a normal semi-synthetic diet composed per kg of diet : 200 g casein, 60 g cellulose, 400 g cornstarch, 210 g granulated sugar, 25 mL maize oil, 25 mL peanut oil, 10 g of vitamin mix, 70 g of mineral mix and containing per kg of diet : 6.50 g methionine, 10 mg vitamin $B_6$, 0.05 mg folic acid, 0.05 mg vitamin $B_{12}$. Experimental groups WKY-Met and SHR-Met, were fed for the same period with the semi-synthetic diet which was supplemented with an additional 8g/kg of D-L methionine making a total of 14.50 g methionine/kg diet (Table 1).

Twelve WKY rats and twelve SHR were used to evaluate the metabolism of homocysteine. Systolic blood pressure (SBP) was measured through carotid artery before (t = 0) and after (t = 60 min) a single intravenous administration of homocysteine (50 $\mu$mol /kg) and the dosage of homocysteine, cysteine, glutathione and methionine were performed on plasma samples.

### *2.2. Plasma and liver collection and storage.*

After 70 days of treatment, all animals were sacrificed under sodium pentobarbital (50 mg/kg.wt) anesthesia. Abdominal aorta blood was collected and placed in EDTA-vacutainer tube, immediately cooled on ice and centrifuged at 4000 rpm for 10 min at +4°C. Aliquots of plasma layer were stored at −80°C until analysis. Then, the liver was removed, weighed and washed briefly in cold physiological salt solution. The tissue was cut into approximately 1g portions, frozen in liquid nitrogen and stored at −80°C in plastic vials until use.

Table 1
Composition of the vitamin and mineral mix

| | Vitamin mix composition | | Mineral mix composition | |
|---|---|---|---|---|
| Vitamin | A | 1,980,000 UI/kg | Calcium | 100 g/kg |
| | $D_3$ | 250,000 UI/kg | Potassium | 60 g/kg |
| | $B_1$ | 2,000 mg/kg | Sodium | 40 g/kg |
| | $B_2$ | 1,500 mg/kg | Magnesium | 10 g/kg |
| | $B_3$ | 7,000 mg/kg | Iron | 3 g/kg |
| | $B_6$ | 1,000 mg/kg | Phosphorus | 77.5 g/kg |
| | $B_7$ | 15,000 mg/kg | Manganese | 0.8 g/kg |
| | $B_{12}$ | 5 mg/kg | Copper | 0.125 g/kg |
| | C | 80,000 mg/kg | Cobalt | 0.9 mg/kg |
| | E | 17,000 mg/kg | Zinc | 0.45 g/kg |
| | K3 | 40 mg/kg | Iodine | 4.9 mg/kg |
| | PP | 100 mg/kg | | |
| Choline | | 1,360 mg/kg | | |
| Folic acid | | 5 mg/kg | | |
| Biotin | | 0.3 mg/kg | | |
| P.A.B. acid | | 50 mg/kg | | |

Liver samples were thawed and homogenates were prepared (Potter homogenizer) after the addition of 4.0 mL phosphate buffer per 1g of liver. Each homogenate was rapidly frozen in liquid nitrogen and stored at −80°C. Homogenate protein contents were assayed using bicinchronic acid (BCA) as described by Smith et al. [19] before GSH content, GSH-Px, GSSG-Red, catalase, SOD activities and lipid peroxides content determination.

### *2.3. Plasma and urinary nitrite and nitrate (NOx) levels*

The dosage was assayed by using a procedure based on the Griess reaction [19]. Total NOx were expressed in $\mu$M.

### *2.4. Copper and iron determination*

Iron and copper (solutions stock standard, 1g/L) and $HNO_3$ were purchased from Prolabo (Paris, France). Iron and copper concentrations were assessed using Hitachi Z9000 electrothermal atomic absorption spectrometry (AAS). Standard solutions (up to 50 $\mu$g/L) were prepared by diluting 1 g/L iron and copper stock solutions with 1% $HNO_3$ aqueous solution. Liver homogenates were diluted 20 fold for copper determination and 600 fold for iron. For plasma determination, samples were diluted 20 fold for copper and iron. 20 $\mu$l of each preparation were injected into apparatus (AAS).

### *2.5. Plasma (PAC) and hepatic antioxidant capacities (HAC)*

The oxygen radical absorbance capacity (ORAC) of hepatic homogenate and plasma was measured according to the modified method of Cao et al. (1993). Another fluorescent protein was used : allophycocyanin (APC) instead of $\beta$-phycoerythrin [20, 21].

### 2.6. Biochemical analysis

#### 2.6.1. Determination of plasma methionine, homocysteine, cysteine and glutathione

Total Hcy, cysteine (Cys) and GSH concentrations were measured by High Pressure Liquid Chromatography (HPLC) technique with fluorimetric determination after derivation of thiols with ABD-F according to Durand et al. [22]. Total methionine concentration was measured with electrochemical detection. Briefly, 200 μl of plasma and 20 μl of 2.5 mM-N-acetylcysteine (used as internal standard for the determination of Hcy, Cys and GSH) were treated with 20 μl of 10% tri-n-butylphosphine (in dimethylformamide) for 30 min at 4°C in order to release thiols from plasma proteins and reduce them. Proteins were precipitated with 200 μl of 0.6 M-cold perchloric acid. After 10 min. of centrifugation at 4000 g, the supernatant was strained with 0.2 μM PTFE filter (Interchim, Montluçon, France) and immediately measured.

*2.6.1.1. Fluorescence detection of Hcy, Cys and GSH* 20 μl of 1.55 M-sodium hydroxide, 250 μl of 0.125 M-borate buffer (pH 8) and 30 μl of 4.6 mM-ABD-F (7-fluoro-2,1,3-benzoxadiazole-4-sulfonamide) solution (in borate buffer) were added to 100 μl of filtrated supernatant. After derivation at 50°C for 20 min, the samples were rapidly cooled and 40 μl of 1 M-HCl were added.

Samples (20 μl) were analyzed by HPLC (ESA 580 Kontron instruments 400, Strasbourg, France) equipped with a fluorescence detector (Bio-Tek, SFM 25, Kontron) set at $\lambda_{ex}$ = 385 nm and $\lambda_{em}$ = 515 nm. Eluent phase was composed of a 0.1 M-$KH_2PO_4$ buffer (pH 3.25) containing 10% acetonitrile, and the rate was fixed at 1.2 mL/min. Separation was carried out on a 250 × 4.6 mm, 5 μm diameter Nucleosil C18 analytical column (Macherel-Nagel) maintained at 35°C. Hcy, Cys and GSH concentrations were determined by the area quotient of N-acetylcysteine and Hcy, Cys and GSH peaks respectively, after standard calibration of thiols.

*2.6.1.2. Electrochemical detection of methionine* Samples were analyzed by HPLC (ESA 580, Kontron instruments 400, Strasbourg, France) associated with a Coulochem II detector (ESA, model 5200) equipped with a dual analytical cell (model 5010) and a guard cell (model 5020). For optimum detection of methionine, the electrode potentials for the guard cell, electrode 1 and electrode 2 were set at +1200mV, +450 mV and +1000 mV respectively. The mobile phase was a 0.02 M-$NaH_2PO_4$ buffer (pH 2.5) and the rate was fixed at 1.8 mL/min. Separation was carried out on a 150 × 4.6 mm, 5 μm diameter LC18 column (Sigma). 20 μl of filtrated supernatant, diluted in the mobile phase, were injected. A calibration curve of methionine was plotted in the mobile phase.

Table 2
Plasma and urinary NOx levels in SHR and WKY rats fed standard or methionine-enriched diet

| | WKY | WKY-Med | SHR | SHR-Met |
|---|---|---|---|---|
| Plasma μM | 22,1 ± 4.3 | 24,6 ± 2,3 | 27,8 ± 2.4 | 24,4 ± 2,1 |
| Urine μM/ 100g/jday | 0,41 ± 0,05 | 0,44 ± 0,06 | 0,45 ± 0,09 | 0,43 ± 0,06 |

#### 2.6.2. Determination of liver GSH, TBARS levels and GSH-Px, GSSG-Red, Catalase and SOD activities.

GSH content was determined by the procedure of Griffith [23], modified by Allen and Arthur [24]. Results were expressed as nmol/mg proteins.

*Lipid peroxidation* was estimated by the modified method of Okhawa et al. [25] based on the thiobarbituric acid reaction and using a fluorometric procedure. Malondialdehyde (MDA) standards were prepared from tetraethoxypropanone and results were calculated as pmol of thiobarbituric acid-reactive substances (TBARS) / mg proteins.

*GSH-Px activity* was measured through the rate of oxidation of NADPH in the presence of cumen hydroperoxide, GSH and GSH-Red [26]. Enzyme activity was expressed as nmol NADPH oxidized / min/ mg proteins.

*GSSG-Red activity* was measured through the rate of oxidation of NADPH in the presence of oxidized glutathione [27, 28]. Enzyme activity was expressed as nmol NADPH oxidized/min/mg proteins.

*Catalase activity* was evaluated by the analysis of the rate of hydrogen peroxide decomposition according to the method of Aebi [29]. Enzyme activity was expressed as unit U/mg proteins.

*SOD activity* was analyzed according to the method of McCord and Fridovich [30] modified by Flohe and Otting [31]. The results were expressed as U/mg proteins.

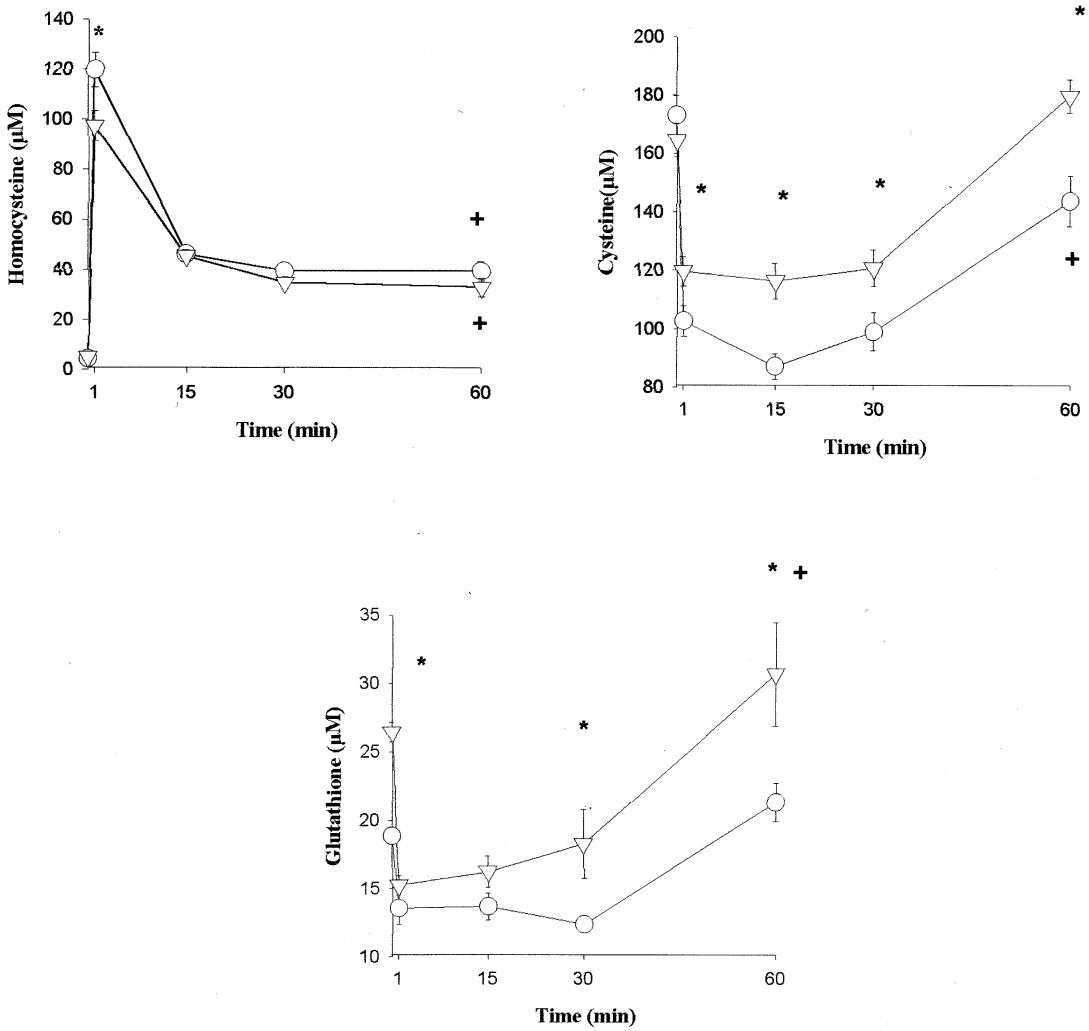
#### 2.6.3. Statistical analysis

Data were expressed as mean with standard errors (m ± SEM). For each variable, statistical analysis was performed with two-way analysis of variance, the two factors being rat strain and treatment, followed by inter-group pairwise comparisons with Tukey's post hoc test when significant differences were detected. Differences between groups were considered significant for P-values $<0.05$.

## 3. Results

### 3.1. Effect of methionine-supplemented diet on plasma and urinary nitrites and nitrates (NOx) levels

(Table 2) No change in plasma and urinary NOx levels was observed in SHR and WKY rats after methionine supplementation.




| Methionine (µM) | WKY | SHR |
|---|---|---|
| T=0 | 75.31 ± 7.61 | 76.02 ± 3.51 |
| T=60 | 47.20 ± 1.95 | 51.27 ± 3.61 |

Fig. 1. Plasma homocysteine, cysteine, glutathione and methionine levels after a single i.v administration of homocysteine (50 $\mu$mol/kg) in SHR (△) rats and WKY (○) rats * significantly different from WKY rats + significantly different from the values at t = 0 (t = 60 vs. t = 0 min)

### *3.2. Evolution of plasma homocysteine, cysteine, glutatione and methionine levels after a single i.v administration of homocysteine*

(Fig. 1) Plasma homocysteine levels were significantly higher in SHR and WKY rats after 60 min than at the beginning of the experiment (T = 0). On the contrary, plasma GSH were increased in SHR and plasma cysteine levels were high in WKY rats after 60 min. No difference in plasma homocysteine and methionine was observed between WKY and SHR, 60 min after i.v adminstration of homocysteine. But plasma cysteine and glutathione levels were significantly higher in SHR than in WKY rats.

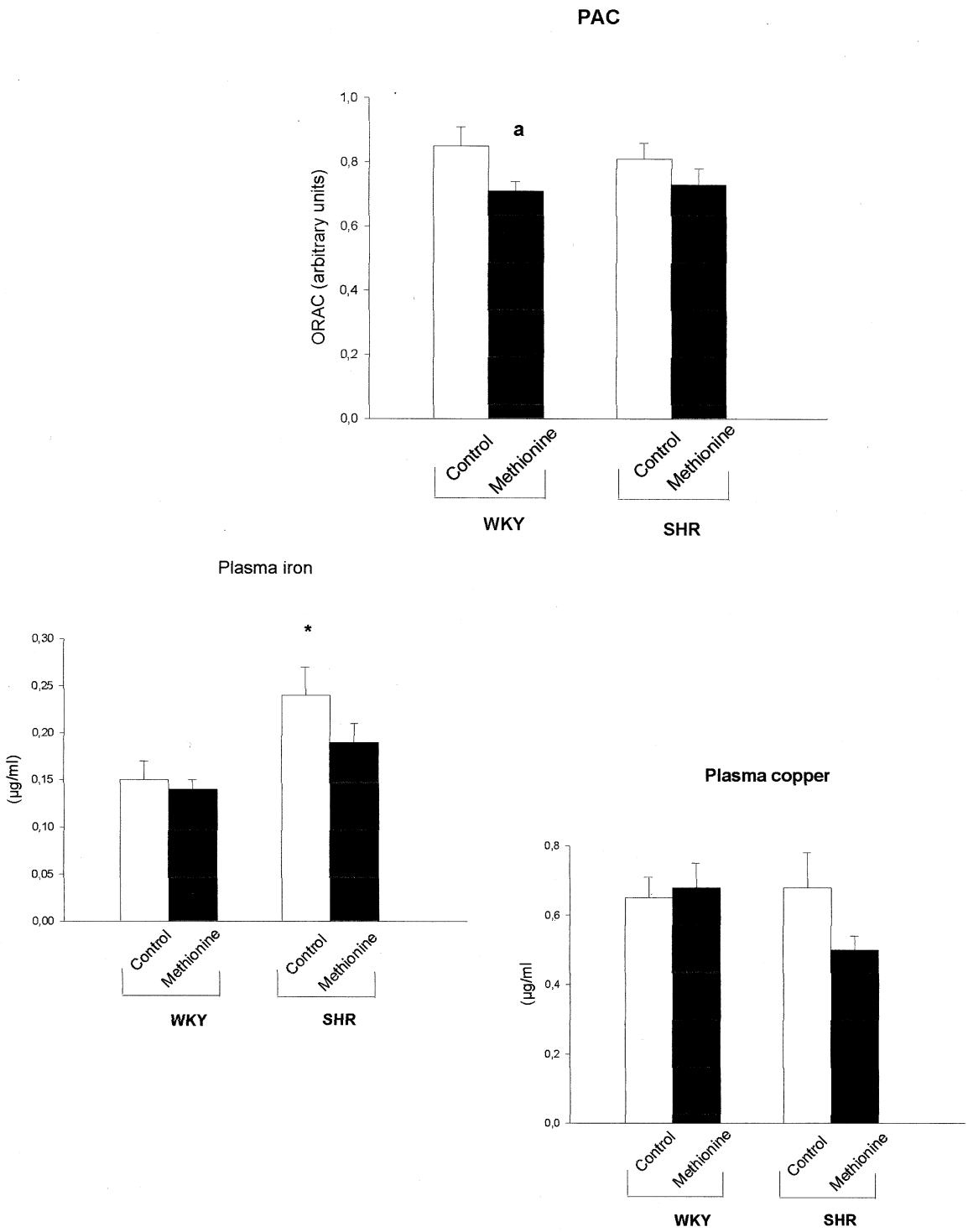



Fig. 2. Plasma antioxidant capacities (PAC), plasma iron and copper levels of WKY and SHR fed with standard or methionine (8 g/kg) enriched-diet. Values are expressed as mean with standard errors (m ± SEM). * significantly different from WKY rats. [a] significantly different from WKY fed standard diet, [b] significantly different from SHR fed standard diet.

### 3.3. Effect of methionine supplementation on plasma iron, copper levels and PAC

(Fig. 2) Plasma iron levels were significantly higher in SHR than in WKY rats. PAC was decreased in WKY whereas no change in PAC was noted in SHR after methionine supplementation.

### 3.4. Effect of methionine supplementation on liver antioxidant status

(Figs. 3 and 4) No difference was observed between WKY and SHR control rats (fed with standard diet) concerning liver TBARS levels and SOD activities. Increases in liver GSSG-Red, catalase activities and GSH, copper levels were noted in SHR control rats compared to WKY control rats. On the contrary, decreases in GSH-Px activities and iron levels were noted in SHR control groups compared to WKY control groups.

After methionine supplementation, liver TBARS levels were significantly increased in WKY and SHR groups. However this increase was more important in SHR than in WKY rats after methionine supplementation. Decreases in liver GSH levels were observed in WKY and SHR groups fed with methionine enriched diet. Decreases in GSH-Px and catalase activities were noted in WKY groups whereas no difference was observed in SHR groups concerning GSH-Px and catalase activities after methionine supplementation. Furthermore SOD and GSSG-Red activities were decreased in both SHR and WKY rats after methionine supplementation.

## 4. Discussion

The objective of the present investigation was to explore the possible metabolisms underlying the discrepancies observed in a previous study between WKY and SHR in response to a 10-week methionine supplementation in terms of blood pressure, i.e., a significant increase in systolic blood pressure of WKY rats and a reduced systolic blood pressure in SHR [1].

It has been suggested that HHcy predisposes to endothelial dysfunction through the generation of oxygen free radicals and a decrease in NO bioavailability, by a mechanism involving GSH-Px [32]. No change in plasma and urinary nitrites, nitrates levels was observed between WKY and SHR fed the standard or methionine-rich diet. In addition, as shown in our previous study, an enhanced aortic constriction to noradrenaline was observed both in WKY and SHR fed the methionine-enrichied diet [1]. These results suggest that the discrepancies between WKY and SHR in their response to methionine-supplemented diet in terms of systolic blood pressure were not related to differences in NO metabolism and bioavailability.

Numerous data obtained in various animal models including rats, have indicated that diets enriched in methionine may induce atherosclerotic lesions through increased plasma Hcy concentrations [26, 33]. Hcy increases oxidant stress in vitro through a number of mechanisms including auto-oxidation, inhibition of GSH-Px and oxidation of low density lipoprotein [10, 11], [32], [34, 35]. It is noteworthy that Hcy is a sulfur-containing amino acid that is an intermediary product in methionine metabolism. So methionine taken orally is converted to Hcy by demethylation. Once formed, Hcy can be metabolized via two pathways. The first one is a remethylation of Hcy into methionine and the second one is a reaction of transsulfuration within Hcy is transformed into cystathionine, cysteine and GSH [36]. So plasma methionine, cysteine and GSH may modulate the toxic effect of Hcy.

In our previous study, no difference in homocysteinemia was observed between SHR and WKY rats after methionine supplementation [1]. However, the analysis of plasma Hcy metabolites showed that plasma methionine and cysteine concentrations were significantly higher whereas plasma GSH concentrations were lower in WKY rats than in SHR after methionine supplementation. The increases in cysteine concentration observed in WKY rats are in accordance with previous studies reporting an increase in cystathionase activity (responsible for the formation of cysteine from cystathionine) in control rats after methionine supplementation [37]. Previously Jacob et al. have suggested that plasma total cysteine could be a risk factor for atherosclerosis in hyperlipidemic patients [38]. Indeed cysteine seems to have a higher vascular toxicity than Hcy through the induction of oxidative stress [18]. A significant decreased relaxation induced by acetylcholine was observed in aortas from Wistar rats cultured in medium containing 100 $\mu$M cysteine for 14 days [39]. Plasma cysteine levels were around 200 $\mu$M in our previous study [1]. So our results suggest a potential vascular toxicity of cysteine in WKY rats after methionine supplementation. In the present study, we measured the plasma antioxidant capacities (PAC) and we found that PAC was significantly decreased in WKY rats after methionine supplementation, while PAC was not affected in SHR after methionine supplementation. The decrease in PAC and the increase in plasma cysteine concentrations could be related to the increase in blood pressure in WKY rats, while on the other hand the no modified PAC and the increased plasma GSH concentrations could account for the decreases in blood pressure observed in SHR after a 10 week supplementation of methionine in diet.

Plasma iron levels were higher in SHR than in WKY rats. These results suggest a difference in trace element regulation between SHR and their respective normotensive WKY rats. It has been shown that iron in excess can act as an inductor of lipid peroxidation and be responsible for deleterious effects on vascular functions in rats [40]. However no difference in PAC were observed between SHR and WKY rats despite of high plasma iron levels. But plasma GSH concentrations were higher in SHR than in WKY rats

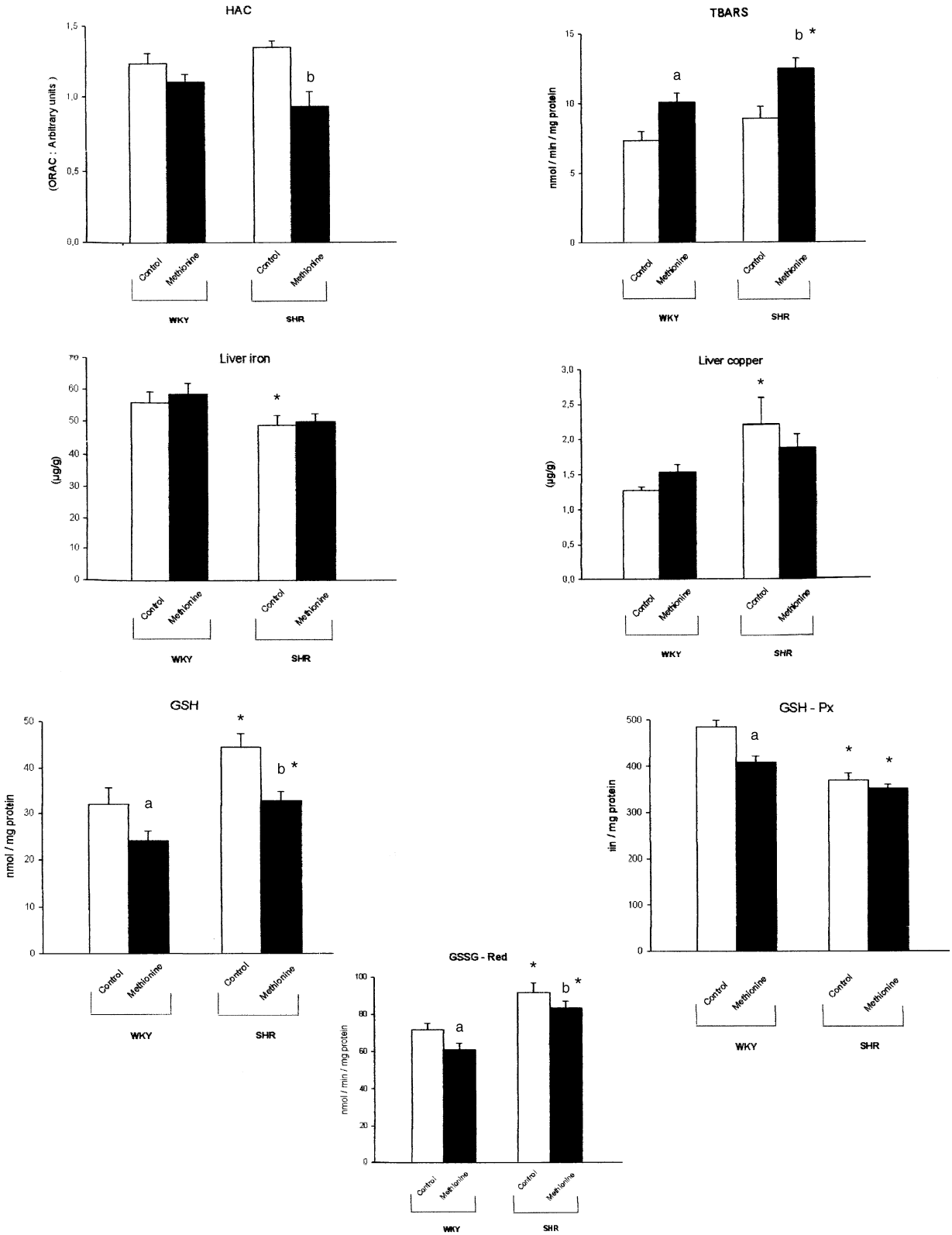



Fig. 3. Hepatic antioxidant capacities (HAC), liver TBARS, iron, copper and GSH levels, GSH-Px and GSSG-Red activities of normotensive (WKY) and spontaneously hypertensive (SHR) rats fed with standard or methionine (8 g/kg) enriched-diet. Values are expressed as mean with standard errors (m ± SEM). * significantly different from WKY rats. [a] significantly different from WKY fed standard diet, [b] significantly different from SHR fed standard diet.

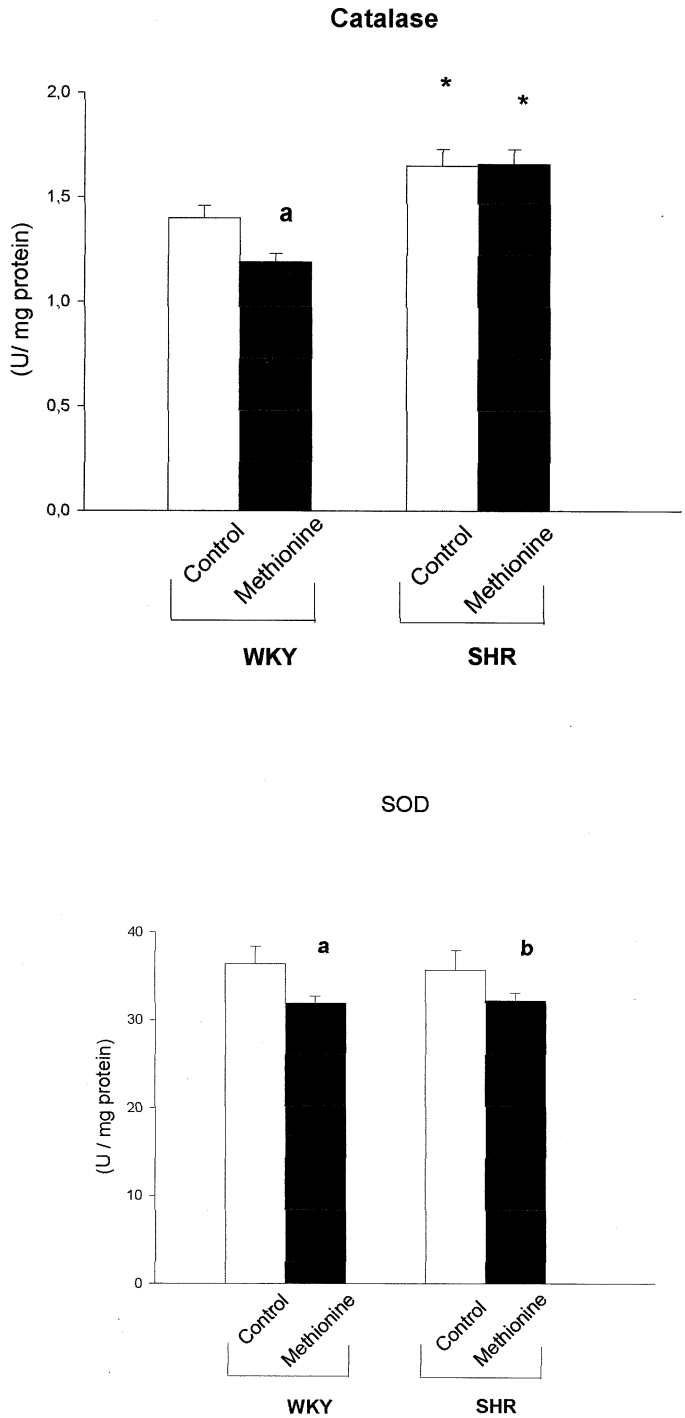



Fig. 4. Liver catalase and superoxide dismutase (SOD) activities in normotensive (WKY) and spontaneously hypertensive (SHR) rats fed with standard or methionine (8 g/kg) enriched-diet. Values are expressed as mean with standard errors (m ± SEM). * significantly different from WKY rats. [a] significantly different from WKY fed standard diet, [b] significantly different from SHR fed standard diet.

and GSH is known to be a powerful antioxidant [41]. These results suggest that a reaction between GSH overload and iron induced lipid peroxidation products might be produced in extracellular compartment explaining the apparent no change in PAC in SHR compared to WKY rats.

After methionine supplementation, plasma GSH concentrations were further increased in SHR compared to WKY rats. Akpaffiong and Taylor showed that the endothelium-dependant impaired relaxation of SHR aortic rings to acetylcholine was improved by prior in vivo administration of GSH. On the contrary, acetylcholine-induced relaxations of aortic rings from WKY was not affected by prior GSH administration [42].

Taken all together, our results strengthen the hypothesis that methionine supplementation, by inducing GSH synthe-

sis in SHR is responsible for the observed decrease in blood pressure while high plasma cysteine levels were induced in WKY rats are responsible for the observed increases in blood pressure.

To further explore homocysteine metabolism in SHR and WKY rats, a single i.v.administration of Hcy (50 $\mu$mol / kg) was performed. Plasma GSH levels were also increased in SHR and plasma cysteine levels were high in WKY rats 60 min after i.v administration of Hcy. High plasma levels of both cysteine and GSH were observed in SHR compared to WKY rats at the end of the experiment. The increases in GSH observed in the 10-week methionine loaded SHR suggest, that in these rats, Hcy is mostly metabolized into cysteine which than preferencially converted into GSH, while the high plasma methionine levels observed in the 10-week methionine loaded WKY rats suggests the existence of a remethylation pathway of Hcy into methionine in these rats. These results suggest that differences in the pathways of Hcy metabolism between WKY and SHR explain increased cysteine and GSH respectively after the 10-week methionine load in their diet.

The liver plays a central role in the turnover of total body GSH [43]. Moreover the transsulfuration of Hcy to cystathionine is present in the liver of rats [33]. Mosharov et al. showed that oxidative stress induced the flux of Hcy through the transsulfuration pathway in human liver cells by stimulation of cystathionine $\beta$-synthase [36]. The consequence of this would be an increase in cystathionine levels from Hcy that could lead to an increase in the concentrations of the downstream liver metabolites, particularly GSH.

As we have noticed previously, liver antioxidant status is decreased in SHR compared to normotensive WKY rats. Our results showed that liver GSH-Px and GSSG-Red activities were significantly lower and higher respectively in SHR than in WKY rats. These results are in accordance with Binda et al. [13]. Catalase activity was higher in SHR than in WKY rats. These results are also in accordance with others studies [16, 17], [44]. In our study, liver antioxidant status was decreased in SHR and liver GSH levels were high in SHR compared to WKY rats. These results may thus suggest a regulation of the transsulfuration pathway in the liver of SHR under oxidative stress conditions. Moreover oxidative stress may inhibit the remethylation pathway of Hcy into methionine [36]. This may explain the lower plasma methionine levels in SHR than in WKY rats after methionine supplementation because liver TBARS levels are high in SHR compared to WKY rats after methionine supplementation.

Liver copper and iron levels in SHR were higher and lower respectively than in WKY rats. It is well known that high levels of copper may act as an inductor of lipid peroxidation. In this study no change in HAC and TBARS levels was observed between SHR and WKY rats. Toxic effects of copper can be reversed by the presence of high levels of GSH in the liver of SHR.

Previous experiments support the evidence that hypertension is associated with liver oxidative stress [13 to 17]. After methionine supplementation, **GSSG-Red** and catalase activities were decreased in WKY rats but no change in the activities of these enzyme was observed in SHR. These results show that alterations in liver antioxidant enzymes were greater in WKY rats than in SHR and are associated with an increase of blood pressure after methionine supplementation.

Liver GSH content was higher in SHR than in WKY rats after methionine supplementation and may contribute to a redistribution of GSH into systemic circulation. Hepatocyte GSH levels are much greater than the circulating extracellular concentrations and the liver accounts for over 90% of GSH inflow into the systemic circulation [43]. In this case, GSH acts directly as a free radical scavenger. It is probable that methionine also exerts its beneficial effect through GSH production in others tissues such as blood vessels inducing decreases in blood pressure observed in SHR. Further experiments are needed to explore the remethylation pathway and the level of the activity of cystathionine $\beta$-synthase (catabolizing Hcy in cystathionine) in SHR compared to WKY rats in order to confirm that Hcy is converted in cysteine and finally in GSH in genetically hypertensive Wistar-Kyoto rats.

In conclusion, the present work shows that the increase in blood pressure in WKY rats is related to high plasma cysteine levels and the decrease in blood pressure in SHR is associated with high plasma GSH levels after methionine supplementation. This study provides evidence that GSH synthesis is stimulated by liver oxidative stress in SHR and may contribute to a redistribution of GSH into systemic circulation. So the great GSH synthesis can be rationalized as an autocorrective response that leads to a decreased blood pressure in SHR.

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