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Biochemical and Biophysical Research Communications 355 (2007) 221–227
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Effects of catechin on homocysteine metabolism in hyperhomocysteinemic mice

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Received 25 January 2007
Available online 2 February 2007

Abstract

We have recently focused on the interaction between hyperhomocysteinemia, defined by high plasma homocysteine levels, and paraoxonase-1 expression and found a reduced activity of paraoxonase-1 associated with a reduced gene expression in the liver of cystathionine beta synthase (CBS) deficient mice, a murine model of hyperhomocysteinemia. As it has been demonstrated that polyphenolic compounds could modulate the expression level of the paraoxonase-1 gene *in vitro*, we have investigated the possible effect of flavonoid supplementation on the impaired paraoxonase-1 gene expression and activity induced by hyperhomocysteinemia and have evaluated the link with homocysteine metabolism. High-methionine diet significantly increased serum homocysteine levels, decreased hepatic CBS activity, and down-regulated paraoxonase-1 mRNA and its activity. However, chronic administration of catechin but not quercetin significantly reduced plasma homocysteine levels, attenuated the reduction of the hepatic CBS activity, and restored the decreased paraoxonase-1 gene expression and activity induced by chronic hyperhomocysteinemia. These data suggest that catechin could act on the homocysteine levels by increasing the rate of catabolism of homocysteine.

Keywords: Homocysteine; Cystathionine beta synthase; Mice; Liver; Polyphenols; Catechin; Quercetin

An inborn error of metabolism, hyperhomocysteinemia due to cystathionine beta synthase (CBS) deficiency, results in elevated levels of homocysteine (Hcy) in plasma [1]. Hcy is a thiol-containing amino acid produced during methionine (an essential amino acid supplied by dietary proteins) metabolism via the adenosylated compounds *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH) (Fig. 1). Once Hcy is formed, it may be recycled to methionine after remethylation by two different pathways. The first one involves methionine synthase (MS), an enzyme that uses 5-methyltetrahydrofolate as the methyl donor, which is generated by 5,10-methylene tetrahydrofolate reductase (MTHFR) [2]. The second pathway, which occurs in the liver and the kidney, involves the enzyme betaine-homocysteine methyltransferase (BHMT). Hcy may also undergo condensation with serine to form cystathionine, which is catalyzed by CBS, the first enzyme involved in the transsulfuration pathway. Cystathionine is subsequently hydrolyzed to form cysteine (Cys) and

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doi:10.1016/j.bbrc.2007.01.142

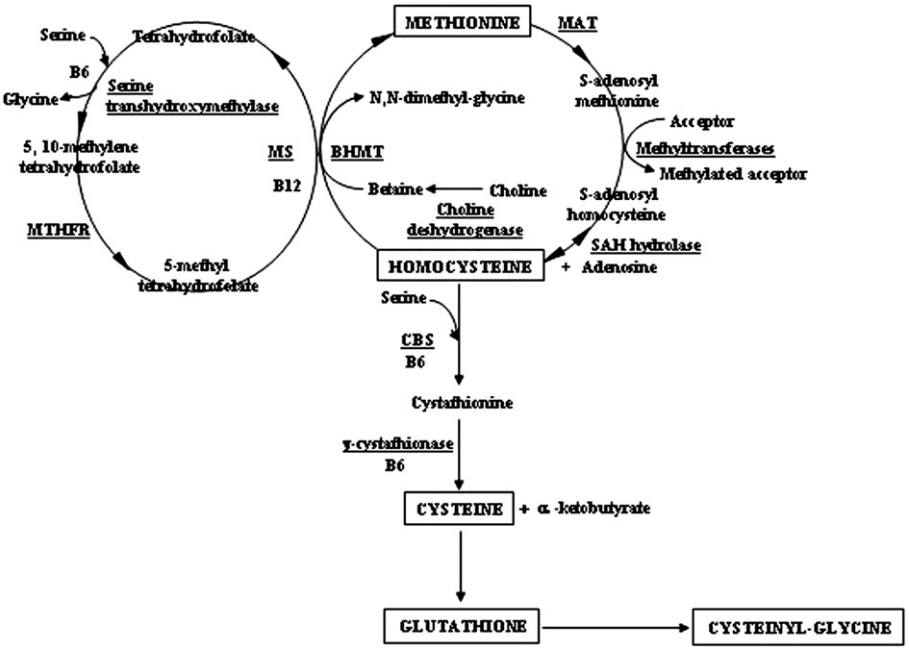

Fig. 1. The homocysteine metabolism. BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine beta synthase; MAT, methionine adenosyl transferase; MS, methionine synthase; MTHFR, 5,10-methylene tetrahydrofolate reductase; SAH hydrolase, *S*-adenosyl homocysteine hydrolase.

Cys, in turn, can be incorporated into protein or used to synthesize the antioxidant glutathione (GSH).

Elevated plasma Hcy level is now recognized as an important risk factor for cardiovascular diseases and atherosclerosis [3]. The pathogenesis of atherosclerosis is complex and involves multiple genetic and environmental factors. Among the determinants which predispose to premature thromboembolic and atherothrombotic events, the status in the antioxidant enzyme paraoxonase-1 (PON1) is considered as a risk factor for atherosclerotic vascular disease. PON1 is synthesized in the liver and secreted into the serum as a high-density lipoprotein (HDL) associated protein which plays a major role in the protective role of HDL against coronary artery disease [4]. Moreover, PON1 is a thiolactonase which hydrolyses homocysteine thiolactone, which is formed as a result of editing reactions of some aminoacyl-tRNA synthetases, to Hcy [5]. We have recently focused on the interaction between hyperhomocysteinemia and PON1 expression, and found a reduced activity of PON1 associated with a reduction of gene expression in liver of a murine model of chronic hyperhomocysteinemia induced by the combination of genetic and dietary approaches, heterozygous *Cbs*-deficient mice fed a methionine-enriched diet [6–8]. As PON1 has been shown previously to exhibit antioxidant activity and to be associated with a decreased risk of cardiovascular diseases, pharmacological modulation of PON1 activity and/or gene expression could constitute a useful approach for the prevention of cardiovascular diseases linked to hyperhomocysteinemia. Among dietary factors, some phenolic flavonoids quercetin and catechin increase serum PON1 activity in mice [9], in agreement with their antioxidant properties. More recently, Gouedard et al. showed that treatment of HuH7 hepatoma cell line by dietary polyphenols increased PON1 mRNA levels [10]. Taken together, in addition to their antioxidant properties, polyphenols could also modulate the expression level of the PON1 gene itself.

We have previously demonstrated by comparison of thiol compounds between humans and mice that heterozygous *Cbs*-deficient mice fed a methionine-enriched diet present a plasma thiol compounds profile more closely related to human one than that of wild-type mice fed a methionine-enriched diet [11]. Therefore, we aimed to study the effects of quercetin and catechin on the impaired PON1 gene expression and activity induced by hyperhomocysteinemia and to evaluate the consequences on Hcy metabolism in heterozygous *Cbs*-deficient mice fed a methionine-enriched diet.

Materials and methods

Chemicals. L-Methionine, (+)-catechin, quercetin, propargylglycine, phenyl acetate, pyridoxal phosphate, L-serine, DL-Hcy, SAM, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), were obtained from Sigma (Sigma–Aldrich, France).

Mice and experimental protocol. Animal care was conducted in accordance with internal guidelines of the French Agriculture and Forestry Ministry for animal handling. Mice were housed in a controlled environment with unlimited access to food and water on 12-h light/dark cycle. We made every effort to minimize suffering and the number of animal used. Mice heterozygous for targeted disruption of the *Cbs* gene

($Cbs^{+/-}$) [8] were generously donated by Dr. N. Maeda (Department of Pathology, University of North Carolina, Chapel Hill, NC, USA). $Cbs^{+/-}$ mice, on a C57BL/6 background were produced by mating male $Cbs^{+/-}$ mice with female wild-type C57BL/6 ($Cbs^{+/+}$) mice. Mice were fed on a standard laboratory diet (A03, Usine d'Alimentation Rationnelle, Epinay sur Orge, France) ad libitum, which has an approximative content of 21% proteins, a methionine content of 4288 mg/kg, a folic acid content of 0.5 mg/kg, and a vitamin B12 content of 0.02 mg/kg. Tail biopsies were performed on mice at 4 weeks of age and polymerase chain reaction was used for genotyping of the targeted CBS allele [8]. $Cbs^{+/-}$ mice, aged six months were used.

Mice from the same litter were divided into four groups and maintained for 3 months on the following diets before the experiments: (1) control diet (control) consisting of a standard A03 rodent diet (Usine d'Alimentation Rationnelle, Epinay sur Orge, France), (2) high-methionine diet (Met), consisting of a control diet supplemented with 0.5% L-methionine in drinking water for 3 months, (3) high-methionine diet with 0.001% catechin or quercetin for the last month (Met/Cat or Met/Quer), and (4) control diet with 0.001% catechin or quercetin for the last month (Cat or Quer). The daily methionine intake of the mice fed on the standard diet is 21 mg of methionine, and 36 mg of methionine fed the high-methionine diet. The daily quercetin or catechin intake was 30–50 μg. Dietary supplementation did not affect the growth or food consumption of the mice during the experimental feeding period.

Preparation of serum samples, tissue collection, and plasma total Hcy assays. At the time of sacrifice, blood samples were collected into tubes containing an 1/10 volume of 3.8% sodium citrate, placed on ice immediately, and plasma was isolated by centrifugation at 2500*g* for 15 min at 4 °C. Liver was harvested, snap-frozen, and stored at −80 °C until use. Plasma total Hcy (tHcy), defined as the total concentration of Hcy after quantitative reductive cleavage of all disulfide bonds, total Cys, total GSH and total cysteinyl-glycine (CysGly) were assayed by using the fluorimetric high-performance liquid chromatography method described by Fortin and Genest [12].

RNA extraction and determination of mRNA levels. Total RNA was prepared from livers from mice by the guanidinium thiocyanate procedure. The quantity and purity of the RNA was assessed by measuring absorbance at 260 and 280 nm. Reverse transcription was carried out on 2 μg total RNA as described by the manufacturer (Ambion, UK). The mRNA levels were assessed by real-time quantitative reverse transcription-polymerase chain reaction (Q-PCR). cDNA (0.2 μl) was diluted with PCR mix (Light Cycler FastStart DNA Master SYBR Green I Kit, Roche Diagnostics) containing a final concentration of 3 mM $MgCl_2$ and 0.5 μM of primers in a final volume of 20 μl. The primers were designed with Primer 3 software. The primer pairs were selected to yield a single amplicon based on dissociation curves. Primer sequences specific for mouse PON1 transcripts are TCCAGGCTTACTGGGATCGAAA for left primer and CCTCGTGGGACTGGTGTTGG for right primer. The mouse superoxide dismutase-1 (SOD1) mRNA was used as an endogenous control [6,7]. Primer sequences specific for mouse SOD1 transcripts are TGGGGACAATACACAAGGCTGT for left primer and TTTCCA CCTTTGCCCAAGTCA for right primer. Q-PCR was performed on total RNA isolated from liver of individual mice in a Lightcycler system (Roche Diagnostics). The thermal cycler parameters were as follows: hold for 8 min at 95 °C for one cycle followed by amplification of cDNA for 40 cycles with melting for 5 s at 95 °C, annealing for 5 s at 65 °C and extension for 10 s at 72 °C. Each reaction was performed in triplicate. ΔΔCp analysis of the results allows to assess the ratio of the target mRNA versus mRNA [13].

Determination of PON1 activity. PON1 activity assay was performed with liver samples corresponding of 300 μg of total proteins. PON1 activity toward phenyl acetate was quantified spectrophotometrically using 20 mM Tris–HCl, pH 8, with 1 mM $CaCl_2$ and 10 mM phenyl acetate. The reaction was monitored for 1 min at room temperature by measuring the appearance of phenol at 270 nm with the use of continuously and automated recording spectrophotometer. All values are corrected for non-enzymatic hydrolysis.

Determination of CBS activity. CBS activity assay was performed with liver samples corresponding to 400 μg of total proteins, as previously described [14], with 1 mM propargylglycine, 0.2 mM pyridoxal phosphate, 100 mM serine, and Hcy, 0.4 mM SAM, using DTNB (Ellman's reagent) based-assay.

Data analysis. Statistical analysis was done with one-way ANOVA followed by Student's unpaired *t*-test using Statview software. The results are expressed as means ± SEM. Data were considered significant when $p < 0.05$.

Results

Effect of quercetin or catechin administration on the PON1 gene expression and activity in liver of hyperhomocysteinemic mice

Since there was no difference in hepatic PON1 expression and activity between heterozygous *Cbs*-deficient mice and wild-type mice receiving a control diet or a methionine-enriched diet [6,7], the heterozygous *Cbs*-deficient mice receiving control diet are used as a control group. Moreover, the combination of genetic and dietary approaches allow us to discriminate between the effects of hyperhomocysteinemia and those due to the loss of CBS expression by genetic deletion. To determine the effect of diet supplemented with quercetin or catechin on *Pon1* mRNA expression in liver of hyperhomocysteinemic mice, total RNA was isolated from liver from the five groups of mice and mRNA expression of *Pon1* was assayed using Q-PCR (Fig. 2A and B). The methionine-alone group, fed a methionine-enriched diet for 3 months (Met), showed a 15% reduction of *Pon1* mRNA level as compared with the group of mice fed the control diet (control) (Fig. 2A and B). Such a diet led to an intermediate hyperhomocysteinemia in mice (∼40 μM; Table 1). This decrease was moderate but statistically significant ($p < 0.0026$) (Fig. 2A and B). Mice fed the control diet (control) or the high-methionine diet (Met) were fed for the last month with catechin or quercetin. Catechin (Met/Cat, Fig. 2B) but not quercetin (Met/Querc, Fig. 2A) administration for 4 weeks significantly up-regulated the *Pon1* mRNA expression by 15%, as compared with the methionine-alone group ($p < 0.0001$) (Met; Fig. 2B). Moreover, catechin (Cat) administration alone did not regulated the *Pon1* mRNA expression as compared with the control group (control; Fig. 2B). These results show that catechin, but not quercetin, counteracts Hcy-induced impairment of *Pon1* mRNA expression in liver of hyperhomocysteinemic mice. Then activity of PON1 was also examined in liver extracts of mice fed the high-methionine diet with or without catechin supplementation. Commensurate with the difference in hepatic mRNA *Pon1* abundance, the mean hepatic activity of PON1 in $Cbs^{+/-}$ mice fed the high-methionine diet (Met) was approximately 20% lower than that in $Cbs^{+/-}$ mice fed the control diet (control, Fig. 2C). Even if catechin supplementation (Cat, Fig. 2C) did not share statistical difference with the control diet (control, Fig. 2C), catechin administration significantly augmented the hepatic activity of PON1 in the mice on the methionine-enriched

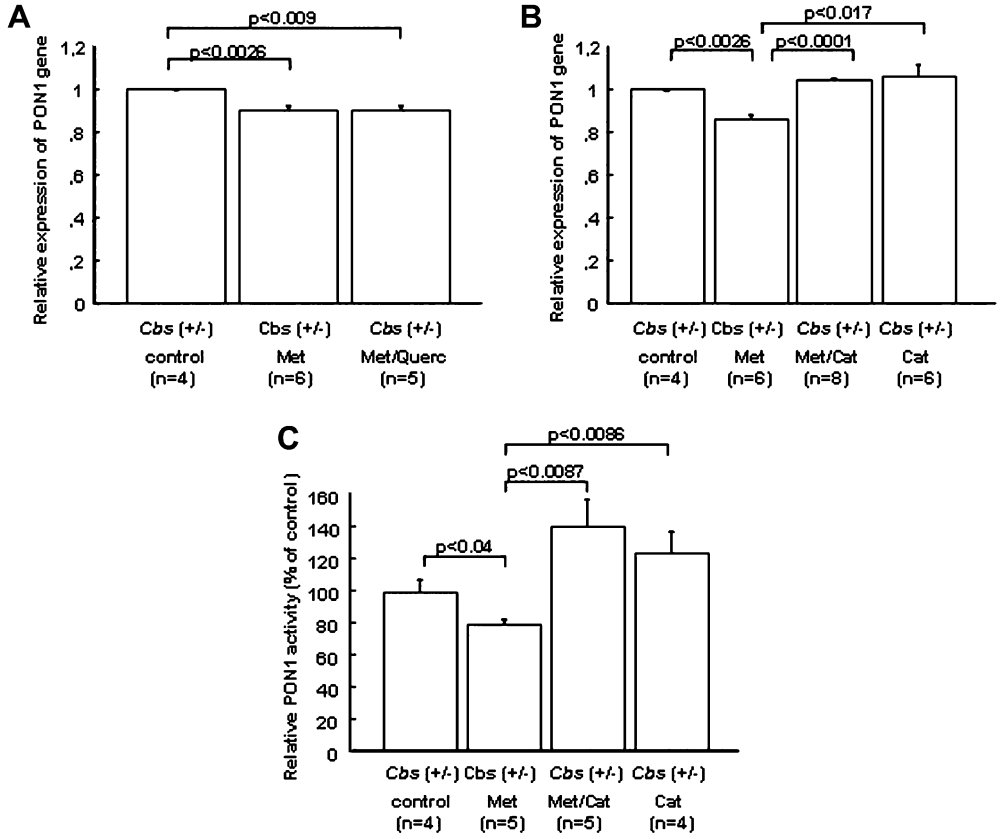

Fig. 2. Comparison of relative expression of PON1 gene based upon real-time quantitative reverse transcription-polymerase chain reaction (Q-PCR) (A,B) and PON1 activity data (C) obtained from heterozygous *Cbs*-deficient ($Cbs^{+/-}$) mice fed a control diet (control) supplemented with catechin (Cat) or a high-methionine diet (Met) supplemented with quercetin (Met/Querc) or catechin (Met/Cat). Q-PCR values (A,B) and PON1 activity values (C) are means ± SEM of *n* (number of mice) mice normalized to the mean of $Cbs^{+/-}$ mice fed a control diet. Statistical analysis was done with one-way ANOVA followed by Student's unpaired *t*-tests.

diet (Met/Cat, Fig. 2C). Taken together, these results show that catechin not only restore the decreased *Pon1* gene expression but also the altered activity of PON1 induced by chronic hyperhomocysteinemia.

Effect of quercetin or catechin administration on thiol compounds in hyperhomocysteinemic mice

We have previously demonstrated that Hcy appears to be one of the determinants of PON1 activity in serum [15]. Moreover, the fact that Hcy is itself a product of PON1 activity [5] suggests an autoregulatory mechanism whereby increased Hcy concentration lead to the retroinhibition of the PON1 gene expression [6,7]. Therefore, in order to determine thiol compounds metabolism in hyperhomocysteinemic mice fed the diet supplemented with quercetin or catechin, serum was analyzed for tHcy, Cys, CysGly and GSH, reflecting cellular and mainly liver production. As expected, plasma tHcy concentrations of the $Cbs^{+/-}$ mice fed methionine-enriched diet

Table 1
Plasma thiol compounds levels in $Cbs^{+/-}$ mice fed control diet (control) supplemented with catechin (Cat) or a high-methionine diet (Met) supplemented with catechin (Met/Cat) or quercetin (Met/Quer)

Diet (*n*, number of mice)	tHcy (μM) (lower value–higher value)	Cys (μM)	GSH (μM)	CysGly (μM)
Control ($n = 7$)	8.7 ± 0.8 (6.5–12.4)	168.5 ± 12.4	84.5 ± 13.5	2.57 ± 0.28
Met ($n = 7$)	40.4 ± 5[*] (28.2–63.4)	176.7 ± 6.8	137.8 ± 22.1	3.3 ± 0.26
Cat ($n = 6$)	7 ± 0.8[§§,$$] (4.8–9.7)	171.3 ± 15.3	75.6 ± 13.3[§]	2.58 ± 0.25
Met/Cat ($n = 8$)	22.4 ± 2.2[*,§§] (15.6–32.4)	158.5 ± 6.9	90.2 ± 9.7[§]	2.76 ± 0.34
Met/Quer ($n = 5$)	43.1 ± 6.3 (30.2–64.4)	148.2 ± 5.3	116.6 ± 29.7	2.74 ± 0.44

Statistical analysis was done with one-way ANOVA followed by Student's unpaired *t*-tests.
[*] $p < 0.0001$ (versus control diet).
[§] $p < 0.05$ (versus Met diet).
[§§] $p < 0.004$ (versus Met diet).
[$$] $p < 0.0001$ (versus Met/Cat diet).

(Met; Table 1) were 4.6-fold higher than those of the mice fed the control diet (control, Table 1). With regard to other thiols, the hyperhomocysteinemic diet did not modify the levels of Cys and his byproducts, CysGly and GSH.

Mice fed the methionine-enriched diet supplemented with quercetin (Met/Quer, Table 1) did not showed difference in plasma thiol compounds levels compared to mice fed the methionine-enriched diet (Met, Table 1). Catechin administration alone (Cat, Table 1) did not reduce significantly tHcy levels compared to mice fed the control diet (control, Table 1). However, tHcy was 1.8-fold lower in the plasma of mice fed the methionine-enriched diet supplemented with catechin (Met/Cat, Table 1) compared to mice fed the methionine-enriched diet (Met, Table 1), but the Hcy levels remained elevated as compared with mice on the control diet (control, Table 1). Even if the difference in GSH levels between mice fed the methionine-enriched diet (Met, Table 1) and mice fed the control diet (control, Table 1) were not statistically different, GSH levels were 1.5-fold lower in plasma of mice fed the methionine-enriched diet supplemented with catechin (Met/Cat, Table 1) compared to mice fed the methionine-enriched diet (Met, Table 1). Our results showed that catechin administration could help to alleviate tHcy elevations caused by methionine-enriched diet.

Effect of catechin administration on CBS activity in liver of hyperhomocysteinemic mice

It has been shown that plasma Hcy levels reflect mainly liver production, where the majority of dietary methionine is metabolized [16,17]. As CBS is a key enzyme of Hcy metabolism [18] and his activity is critical in lowering serum tHcy after a methionine load, we have assayed the CBS activity in liver samples of the four groups of mice. Mice fed the methionine-enriched diet (Met, Fig. 3A) showed a significant decrease of CBS activity ($p < 0.0003$) (Fig. 3A) and a significant increase in plasma tHcy level (Table 1) when compared with mice fed a control diet (control, Fig. 3A), suggesting that the low activity of CBS might be associated, at least in part, with the hyperhomocysteinemia in mice fed the high methionine-diet. Even if catechin administration alone (Cat, Fig. 3A) did not influence significantly hepatic CBS activity compared to mice fed the control diet (control, Fig. 3A), catechin administration augmented the hepatic activity of CBS ($p < 0.0274$) (Fig. 3A) and diminished the plasma tHcy level (Table 1) in the mice on the methionine-enriched diet (Met/Cat, Fig. 3A). However, the CBS activity remained statistically decreased ($p < 0.0437$) and the plasma tHcy levels remained statistically elevated ($p < 0.0001$) as compared with mice on the control diet (control, Fig. 3A). We also found that catechin administration alone did not influence CBS activity in liver of wild-type mice fed a control diet (Fig. 3B), which suggests that catechin administration has no direct effect on normal CBS enzyme.

Discussion

Elevated plasma Hcy level is well-recognized as an important vascular risk factor and atherosclerosis in the coronary, cerebrovascular and peripheral arterial circulation, even if the degree of hyperhomocysteinemia is moderate [3]. Moreover, high methionine intake has also been reported to accelerate atherosclerosis [19]. Animal models of chronic hyperhomocysteinemia induced by the combination of genetic and dietary approaches, heterozygous *Cbs*-

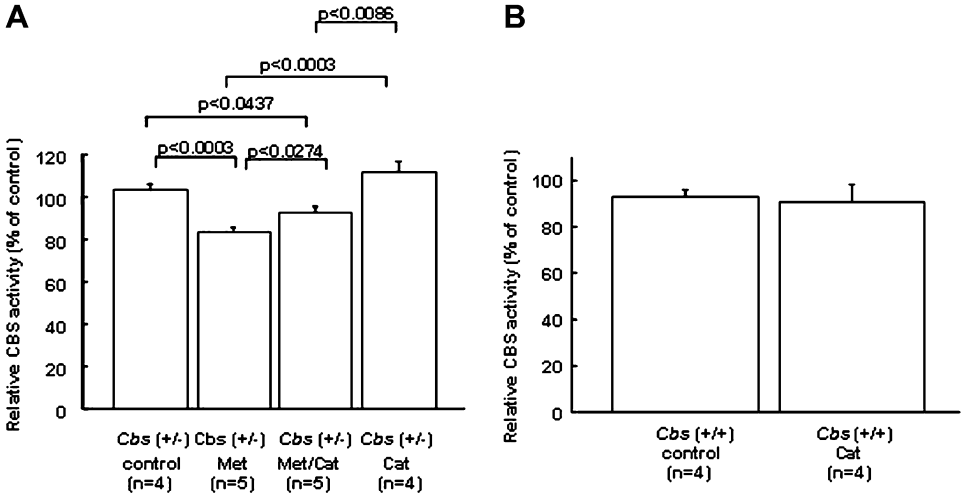

Fig. 3. Comparison of hepatic CBS activity obtained from heterozygous *Cbs*-deficient ($Cbs^{+/-}$) mice (A) fed a control diet (control) supplemented with catechin (Cat) or a high-methionine diet (Met) supplemented with catechin (Met/Cat) and wild-type ($Cbs^{+/+}$) mice (B) fed a control diet (control) supplemented with catechin (Cat). CBS activity assay was performed on extracts from liver of individual mice. CBS activity values are means ± SEM of *n* (number of mice) mice normalized to the mean of $Cbs^{+/-}$ mice fed a control diet (A) or to the mean of $Cbs^{+/+}$ mice fed a control diet (B). Statistical analysis was done with one-way ANOVA followed by Student's unpaired *t*-tests.

deficient mice fed a methionine-enriched diet, show that a mild to intermediate increase in Hcy alone is sufficient to induce endothelial dysfunction [20], which plays a crucial role in the pathogenesis of atherothrombotic vascular diseases, both as a target and as a mediator of the disease process. As we have recently found a reduction of gene expression of the anti-atherosclerotic enzyme PON1 in liver of heterozygous *Cbs*-deficient mice fed a methionine-enriched diet [6,7], we decided to study the effects of polyphenolic components on PON1 gene expression and activity, and Hcy metabolism in this model of hyperhomocysteinemic mice.

We confirmed previous data [6,7] and showed that a chronically giving methionine in drinking water not only decreased the *Pon1* mRNA levels in liver of mice but also the hepatic activity, and increased plasma tHcy levels, which are associated with premature atherosclerosis. The liver is the major site for the metabolism of Hcy. Because CBS is the key enzyme of Hcy catabolism, it may be speculated that its reduced activity accounts for the enhanced hyperhomocysteinemia observed when mice were fed a methionine-enriched diet. Then we have measured the CBS activity in the liver where it is most abundantly expressed and active [18] and found a significant decrease of CBS activity in liver of mice fed the methionine-enriched diet. However, we showed that a daily administration of catechin, but not quercetin, stimulated the activity of CBS in high-methionine diet-fed mice, and partly reduced the increase of Hcy-induced by methionine. The Hcy levels, although significantly reduced, remained elevated as compared with the control diet group. It is not surprising because these mice were continuously on a high-methionine diet. Murthy et al. have found a similar action on CBS activity by rosiglitazone, a ligand of the peroxisome proliferator-activated receptor γ, in liver of rats fed a high-methionine diet [21]. The important role of CBS in the regulation of plasma tHcy levels has also been supported by the results obtained from transgenic mice. It has been demonstrated that elevating CBS activity by overexpression of CBS decreased plasma tHcy by 45% in transgenic mice [22]. It might be expected that elevating CBS activity would raise the levels of other serum thiols because they all contain Cys, which is downstream of CBS. GSH is the most abundant low-molecular weight thiol and plays a key role in the cellular defense against oxidative stress. Whereas GSH export from cells may confer antioxidant protection, export of Hcy may prevent injury in the cells themselves. Here we have shown that the decrease in plasma tHcy levels induced by methionine-enriched diet supplemented with catechin was accompanied by a decrease in GSH levels without significant variation into Cys and CysGly levels. The γ-cystathionase activity might be rate limiting, resulting in a lowering of tHcy but without any increase of Cys.

Our results suggest that the reduction in serum tHcy levels was caused, in part, by increased catabolism. However, when we compared CBS activity from heterozygous *Cbs*-deficient mice or wild-type mice fed a control diet supplemented with catechin, we did not found differences from mice fed a control diet. Hcy levels are regulated not only by folate bioavailability but also by SAM and its metabolite SAH. Dayal et al. [20] have found an elevation of plasma tHcy levels but also a decreased hepatic SAM/SAH ratio in heterozygous *Cbs*-deficient mice fed a methionine-enriched diet, which suggests an altered SAM-dependent methylation. Liver constitutes a crucial site for polyphenol metabolism since this tissue possesses conjugative enzymes like UDPGT, sulfotransferase and catechol-*O*-methyltransferase (COMT) [23]. As catechin constitutes particularly good substrate for hepatic COMT which uses SAM as methyl donor [24], the modulation of certain Hcy-metabolizing enzymes could reflect the catechin metabolism in liver of hyperhomocysteinemic mice.

It has been suggested that high dose of dietary polyphenols from black tee or coffee can increase the cellular production of Hcy, through increased methylation reactions [25]. Such methylation reactions transfer a methyl group from SAM to polyphenols and thereby produce Hcy [25,26]. However, regular ingestion of black tea did not alter mean tHcy concentrations [26]. The daily catechin or quercetin intake used here is 1.2–2 mg/kg/day, which has been shown to reduced progression of atherosclerosis in apolipoprotein E-deficient mice [27]. Differences in polyphenol metabolism could affect plasma tHcy after consumption of a polyphenol-rich diet. In this sense, some flavonoids are not only substrates but also potent inhibitors of liver cytosolic COMT [28]. However, although catechin and quercetin have a similar chemical structure, it has been shown that they display different properties towards human mammary COMT, quercetin being three times more potent than catechin at inhibiting this enzyme [29]. Taken together, these results could explain the difference between catechin and quercetin on hyperhomocysteinemia.

Here, we also showed that a daily administration of catechin counteracts Hcy-induced impairment of PON1 gene expression and activity in liver of hyperhomocysteinemic mice. Previous results have shown that catechin increases serum PON1 activity in apolipoprotein E-deficient mice [9] due to their antioxidant properties, associated with reduced progression of atherosclerosis [27]. As PON1 has been shown previously to exhibit antioxidant activity and to be associated with a decreased risk of cardiovascular diseases [4], pharmacological modulation of PON1 activity and gene expression by dietary approach can constitute a useful approach for the prevention of cardiovascular diseases linked to hyperhomocysteinemia.

In summary, the major finding in this study is that catechin supplementation in hyperhomocysteinemic mice can attenuate an increase of the plasma tHcy levels induced by a methionine-enriched diet. Lowering tHcy through improved nutrition or other means might offer preventive or therapeutic benefits. In this sense, as folate, vitamin

B12 and vitamin B6 are important cofactors in the metabolic pathways of Hcy, our study provides important evidence for therapeutic use of catechin, especially in diseases resistant to interventions aiming to reduce elevated plasma Hcy level by B-complex vitamin therapy.

Acknowledgments

This work was supported by an European Union Grant and by Onivins (Office National Interprofessionnel des Vins), Ministère de l'Agriculture, programme Vin et Santé, Pathologie et biologie vasculaires. Work in Fernando Rodrigues-Lima/Jean-Marie Dupret laboratory is supported by Association pour la Recherche sur le Cancer (ARC), Association Française contre les Myopathies (AFM) and Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (AFSSET). We thank Dr N. Maeda (Department of Pathology, University of North Carolina, Chapel Hill, NC) for providing heterozygous *Cbs* mice. We thank A. Djemat and C. Noll for technical assistance.

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