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EFFECT OF QUERCETIN ON PARAOXONASE 1 ACTIVITY – STUDIES IN CULTURED CELLS, MICE AND HUMANS

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There is increasing evidence that the HDL-associated enzyme paraoxonase 1 (PON1) may have a protective function in the atherosclerotic process. An enhancement of PON1 activity by dietary factors including flavonoids is therefore of interest. Quercetin, a flavonol frequently present in fruits and vegetables has been shown to induce PON1 in cultured liver cells, but the *in vivo* efficacy of a dietary quercetin supplementation has yet not been evaluated. To this end, we fed laboratory mice quercetin-enriched diets with quercetin concentrations ranging from 0.05 to 2 mg/g diet for 6 weeks and determined the expression of the hepatic PON1 gene and its protein levels. Since we could establish a moderate but significant induction of PON1 mRNA levels by dietary quercetin in mice, we aimed to proof whether healthy human volunteers, given graded supplementary quercetin (50, 100 or 150 mg/day) for two weeks, would respond with likewise enhanced plasma paraoxonase activities. However, PON1 activity towards phenylacetate and paraoxon was not changed following quercetin supplementation in humans. Differences between mice and humans regarding the PON1 inducing activity of quercetin may be related to differences in quercetin metabolism. In mice, unlike in humans, a large proportion of quercetin is methylated to isorhamnetin which exhibits, according to our reporter gene data in cultured liver cells, a potent PON1 inducing activity.

Key words: *quercetin, isorhamnetin, flavonoid metabolism, paraoxonase 1, atherosclerosis*

INTRODUCTION

Paraoxonase 1 (PON1; EC 3.1.8.1) is a calcium-dependent serum esterase which is closely associated with the high-density lipoprotein complex (HDL) (1). The enzyme has initially been characterized by its ability to hydrolyse organophosphates such as paraoxon, but was later shown to have also arylesterase and lactonase activity and hydrolyses a number of exogenous and endogenous compounds. An anti-atherogenic role for PON1 has been suggested from several studies since PON1 is able to protect low density lipoprotein (LDL) particles against oxidation (1, 2), preserves HDL function *via* inhibition of HDL oxidation (3) and hydrolyses homocysteine thiolactone thus preventing N-homocysteinylation (4, 5). PON1 knockout mice are highly susceptible to develop atherosclerosis compared to wildtype control mice (6). Transgenic mice overexpressing PON1 display decreased atherosclerotic lesions (7). Low serum levels of PON1 have therefore been associated with an increased prevalence of atherosclerosis and have been suggested as an independent risk factor for cardiovascular disease (CVD) (8).

Several polymorphisms in the promotor and coding region of the human PON1 gene are known to date and determine the transcriptional level as well as the catalytic activity of PON1 (9). Serum activity of PON1 towards organophosphates varies greatly between individuals which is mainly caused by the glutamine (Q allele)-arginine (R allele) substitution at position 192. Individuals with the Q-type represent the lower activity mode, whereas the QR heterozygotes and R homozygotes account for intermediate and higher activity groups towards the substrate paraoxon, respectively (10). The substitution of leucine (L) by methionine (M) at position 55 (M/L55 polymorphism), in contrast, contributes significantly to the variation in serum PON1 concentration (11).

However, the PON status (or phenotype) of an individual is not only determined by genetic factors. Lifestyle, nutritional and environmental factors have been reported as influencing parameters affecting serum PON1 activity levels (12, 13). Therefore, PON1 is considered a promising target for both nutritional and pharmacological intervention against CVD (9, 14). Quercetin, a flavonoid which is ubiquitously distributed in edible plant foods (16-17), may exhibit free radical scavenging activity and has been shown to reduce LDL oxidation (15, 18, 19). Epidemiological data suggest an inverse relation between flavonoid intake and the risk for CVD (20). Although there is some evidence from cell culture studies that quercetin induces the expression of PON1 (21), *in vivo*-studies investigating the influence of a dietary quercetin supplementation on hepatic expression and activity of PON1 are missing. Therefore, studies were conducted in laboratory mice as well as in healthy human volunteers which were supplemented with increasing doses of

quercetin and changes in paraoxonase expression and activity monitored. Furthermore, a reporter gene assay in cultured hepatocytes was conducted to compare the PON1 inducing activity of quercetin with its methylated metabolite isorhamnetin.

MATERIALS AND METHODS

Mouse study and diets

To examine whether dietary quercetin supplementation results in an increase in paraoxonase 1 expression, 60 female C57BL/6 mice aged 6-7 weeks and a mean starting weight of 16.6±0.79 g (Harlan Teklad, Borchen, Germany) were divided into 6 groups of 10 animals each and fed quercetin-enriched diets for 6 weeks. A semi-synthetic standard diet (ssniff, Soest, Germany) based on corn starch (43%), casein (24%), and 10% corn oil was supplemented with increasing concentrations of quercetin dihydrate (>98% purity; Roth, Karlsruhe, Germany) ranging from 0.05 to 2 mg/g diet. The control group received the unsupplemented standard diet only. Diets were prepared weekly and stored at -20°C until use. The animals were kept in macrolon cages under standard conditions (21-25°C, with a 12 h day-night cycle) according to the German Regulations of Animal Welfare with permission of the responsible authority. Diets and water were provided for ad libitum intake and live weight was recorded weekly. At the end of the dietary intervention period, mice were anaesthetised and decapitated. Blood was collected in EDTA tubes, centrifuged to generate plasma (8000 g, 4 min, 4°C) and stored at -80°C. Liver was removed and stored immediately at -80°C.

Human study

To investigate the effects of dietary quercetin supplementation at different quercetin dosages on plasma PON1 activity in humans, a controlled intervention study was conducted in 35 healthy normal-weight volunteers (17 female, 18 male). The study was initially designed to examine the bioavailability of quercetin (for details see (22)). Baseline characteristics of the subjects are presented in *Table 1*. The study protocol was approved by the ethical committee of the Medical Faculty of the Christian-Albrechts-University of Kiel, Germany and was in accordance with the Helsinki Declaration. Briefly, the study was conducted in a double-blinded parallel design and consisted of a 2-week wash-out period followed by the 2-week supplementation period. During the wash-out period, all subjects ingested a quercetin-low diet to limit the influence of dietary quercetin on the results of the study. After the wash-out period, subjects were randomly assigned to take 50, 100, or 150 mg/d (Q50-Q150) quercetin (aglycone) for 2 weeks. Subjects were instructed to take a total of 6 quercetin capsules per day, 2 capsules with each principal meal. Quercetin dosages were selected to represent ~5- (50 mg/d), 10- (100 mg/d), or 15-fold (150 mg/d) of the estimated daily dietary quercetin intake in Germany of ~10 mg. Fasting venous blood samples were obtained at the end of the 2-week wash-out period (d 0, identified as the study baseline) and the end of the supplementation period (d 14). Blood was drawn into tubes containing lithium heparin or no additives (Sarstedt, Numbrecht, Germany). Plasma and serum were collected after centrifugation at 2000xg; 15 min at 4°C. After aliquotation in gas-tight cryovials, plasma and serum were immediately frozen and stored at -75°C until analysis.

Quercetin analysis in plasma

Plasma quercetin and isorhamnetin levels were measured according to the method of (23). Briefly, plasma samples were acidified, treated with β-glucuronidase/sulfatase type H-2 (Sigma, Deisenhofen Germany) and centrifuged after acetone extraction. After evaporation of the resulting supernatant, the remainings were resolved in methanol/aqua bidest., injected into HPLC (Jasco FP 920 fluorescence detector; Gross-Umstadt, Germany) and detected at excitation 422 nm and emission 485 nm. Calibration curves for quercetin and isorhamnetin were obtained by the addition of these flavonols from methanolic stock solutions to native pig plasma with rhamnetin serving as an internal standard.

RNA isolation and real time PCR

RNA was isolated from liver samples (20-30 mg) using TRIsure lysis reagent (Bioline, Luckenwalde, Germany) and RNA quantified photometrically (Spectrophotometer DU800, Beckman Coulter, Krefeld, Germany). Quantitative real time PCR was performed as one step procedure (SensiMix™ One-step Kit; Quantace, Berlin, Germany) with SybrGreen detection using the Rotorgene 6000 cycler (Corbett Life Science, Sydney, Australia). Quantitation was done by use of a standard curve. Primers were designed by standard tools (Spidey, Primer3, NCBI Blast) and purchased from MWG (Ebersberg, Germany). A 178 bp fragment of the murine PON1 gene (Gene ID 18979), specific for this isoform (no homology to PON2 and PON3 mRNA) was amplified using forward primer 5´-CAGCCTGTCCATCTGTCTCA-3´ and reversed primer 5´-CACCCGTCTCGATTCCTTTA-3´ and normalized to the mRNA levels of the housekeeping gene GAPDH (Gene ID 14433; 131 bp fragment; primer forward 5´-CCGCATCTTCTTGTGCAGT-3´ and reversed primer 5´-GGCAACAATCTCCACTTTGC-3´).

Western blotting

Liver cell protein was extracted from tissue samples (30 mg) using RIPA buffer (50 mmol/l Tris-HCl, 150 mmol/l NaCl, 0.5% deoxycholate, 0.1% SDS, and 1% NP-40; pH 7.4 with Protease-inhibitor-cocktail, 1:100; Sigma, Saint Louis, USA) by incubation on ice for 30 min and subsequent centrifugation at 12000 g (4°C, 30 min). Protein concentration was determined in the supernantants by the BCA Assay (Pierce, Illinois, USA). 40 µg protein was separated on a 12% SDS/polyacrylamide gel and transferred onto an immunoblot polyvinylidenfluoride membrane. The membrane was blocked with 3% non-fat dried milk in Tris-buffered saline, pH 7.4, with 0.05% Tween-20 (TBS/T) for 2 hrs and probed with polyclonal rabbit anti-PON1 antibody (1:1000; abcam, Cambridge, UK) at 4°C overnight. Then, the membranes were incubated with a goat anti-rabbit IgG secondary antibody (1:4000) conjugated with horseradish peroxidase (BioRad, Munich, Germany) for 45 min. Specific bands were visualized by enhanced chemiluminescence (ECL) reagent on a ChemiDoc system and quantitated densitometrically by using the program Quantity One® (all from BioRad). The membranes were stripped (strip buffer: 8 g glycine, 2.5 ml HCl, 1 l H_2O) and subsequently incubated with rabbit polyclonal antibody against β-actin which was used as loading control (1:800, Santa Cruz Biotechnology, Heidelberg, Germany) and proceeded as described above. The predicted sizes for PON1 and β-actin are 40 and 42 kDa, respectively, which were checked by the use of molecular weight markers.

Paraoxonase activity measurement

Paraoxonase and arylesterase activities were measured in human serum samples with paraoxon and phenylacetate as substrates according to (24) and (25). Hydrolysis rate of paraoxon (diethyl-*p*-nitrophenyl phosphate; Supelco) was measured by

monitoring the increase of absorbance at 412 nm using 100 mmol/l Tris–HCl buffer (pH 8.0) with 1 mmol/l paraoxon, 2 mmol/l $CaCl_2$ and 1 M NaCl. One unit of PON1 activity is defined as 1 nmol of 4-nitrophenol formed per minute under the above assay conditions (molar absorptivity coefficient 17,600 M^{-1} cm^{-1}). Phenylacetate was used as a substrate to measure the arylesterase activity (Arylesterase Kit, Zeptometrix, New York, USA). Enzymatic activity was calculated from the molar absorptivity coefficient of the produced phenol (1310 M^{-1} cm^{-1}) with one unit of arylesterase activity defined as 1 µmol phenylacetate hydrolyzed/min and expressed as kU/L serum. Blanks were simultaneously run to correct for spontaneous substrate breakdown.

Cell culture

PON1 induction capacity of quercetin, isorhamnetin and resveratrol (used as a positive control) was evaluated in cultured hepatocytes. Huh7 liver hepatoma cells of human origin had been stably transfected with a reporter plasmid containing 1009bp [-1013, -4] of the PON1 gene 5´-region cloned into the firefly luciferase reporter vector pGL3 basic (Promega) as described previously (26). Stable clones originated from X. Coumoul/R. Barouki, INSERM, France. Cells were cultivated in Dulbecco`s modified Eagle`s medium with 10% heat inactivated fetal calf serum, 100 U/ml streptomycin and 100 mg/ml penicillin (all from PAA, Coelbe, Germany). Cells were seeded at an initial density of 200000 cells per well (24well plate) and incubated with 25 µmol/l quercetin, isorhamnetin or resveratrol (Sigma, Deisenhofen, Germany) for 48 h as recently described (21). Then, the cells were washed with PBS, lysed and subjected to luciferase activity measurement (Luciferase assay system; Promega, Madison, WI, USA) by luminescence reading (Infinite 200 reader; Tecan, Crailsheim, Germany) and normalized to total cell protein.

Statistical analysis

Statistical analysis was performed using SPSS version 15.0 (Munich, Germany). Data were analysed for normality of distribution (Kolmogorow-Smirnov and Shapiro-Wilk test) and equality of variance (Levene test) prior to one-way ANOVA followed by Dunnett test (mouse study) to compare the dose-dependent quercetin effects with the control group. If not normally distributed, data were logarithmized for ANOVA. Data are expressed as means with their standard errors and significance was accepted at $p<0.05$. For human data, between-group-comparisons were analyzed by Kruskall-Wallis H test und Mann-Whitney U-test. Within-group comparisons were done using the Wilcoxon matched-pairs signed-ranks test. Results are expressed as mean±standard deviation or for skewed human data as median and 25./75. percentiles. Spearman's correlation test was used to analyze relationships between different parameters. All tests were 2-tailed, and a $p<0.05$ level of significance was used to assess statistical significance.

RESULTS

Mouse study - quercetin levels in plasma samples

Feeding laboratory mice with the quercetin enriched diets for a period of six weeks did not change feed intake and live weight gain. Final body weights ranged from 19.1-20.1 g with a mean±SD of 19.5±1.6 for all animals and did not differ between the dietary treatment groups. Plasma levels of quercetin and its methylated derivative isorhamnetin, however, were significantly influenced by the dietary quercetin supply (each $p<0.001$ in ANOVA). As shown in *Fig. 1A*, plasma levels increased dose-dependently in response to the dietary quercetin supplementation. Dietary quercetin ranged from 0.05-2 mg/g diet and resulted in plasma levels of quercetin and isorhamnetin of 0.33 and 0.17 up to 2.31 and 2.12 µmol/l, respectively. In mice fed the control diets, quercetin was detectable at low concentrations (0.12 µmol/l) whereas its metabolite isorhamnetin could only be detected in traces (0.02 µmol/l plasma). Furthermore, low amounts of kaempferol could be determined in all samples and did not differ among the dietary groups.

Induction of hepatic PON1 by quercetin in mice

Hepatic mRNA levels of murine PON1 were determined in response to the dietary quercetin supplementation. *Fig. 1B* shows an increase in hepatic PON1 mRNA levels in the quercetin fed mice and PON1 mRNA levels were significantly higher as compared to controls with a maximal 2 fold induction in the highest supplementation group (2 mg/g diet). Further statistical calculation revealed a significantly positive correlation of plasma quercetin and isorhamnetin levels with hepatic PON1 induction (r=0.688 and 0.721, respectively; each with $p<0.01$; Spearman). Murine hepatic PON2 and PON3 were not significantly affected by the quercetin supplementation (data not shown). Dietary quercetin supplementation resulted in a moderate (50%) increase of hepatic PON1 protein levels as determined by Western blotting (*Fig. 2*).

Human study

Two-week supplementation of the diet with 50, 100, or 150 mg/d quercetin (Q50, Q100, or Q150, respectively)

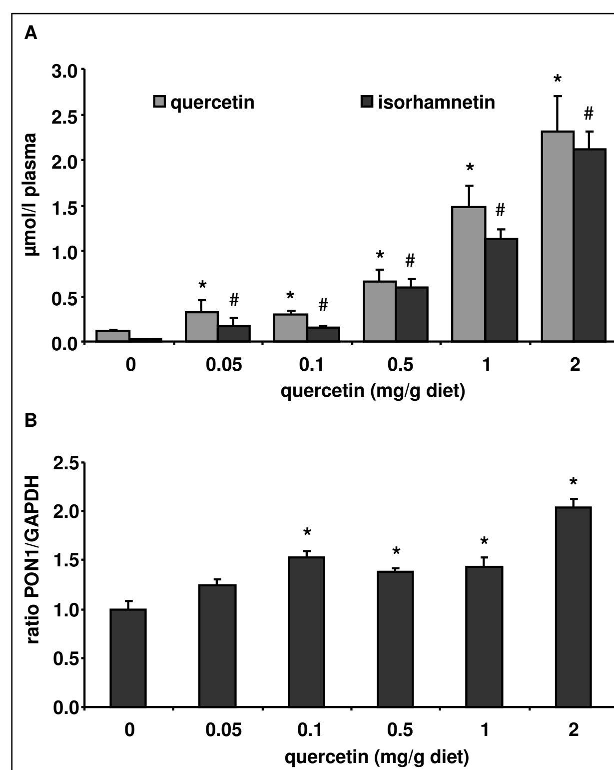

Fig. 1. Plasma quercetin and isorhamnetin concentrations (A) and hepatic mRNA levels of PON1 (B) in C57BL/6 mice fed quercetin supplemented diets for a period of 6 weeks. Data are mean with SEM. * and # significantly differ from control group (p<0,05; Dunnett t-test). Plasma quercetin and isorhamnetin data were ln-transformed before ANOVA.

Table 1. Characteristics of the study subjects at baseline (adapted from (19))

Variable	Total group (n=35; 17 female, 18 male)
Age (years)	26.2 ± 3.7
Body height (m)	1.75 ± 0.11
Body weight (kg)	68.1 ± 12.6
Body-Mass-Index (kg/m^2)	22.1 ± 2.2
Blood pressure (mmHg) Systolic Diastolic	 122.6 ± 8.5 76.0 ± 4.8
Plasma glucose (mmol/l)	4.6 ± 0.4
Serum total cholesterol (mmol/l)	4.52 ± 0.94
Serum LDL cholesterol (mmol/l)	2.29 ± 0.65
Serum HDL cholesterol (mmol/l)	1.73 ± 0.45
Serum triacylglycerols (mmol/l)	1.06 (0.78/1.26)

Values are means±SD or medians (25./75. percentile)

Table 2. Plasma paraoxonase 1 (PON) activity in healthy men and women supplemented for 2 weeks with 50, 100, or 150 mg/d quercetin

Variable	Time	Q50 (n=11)	Q100 (n=12)	Q150 (n=12)
PON1 activity (kU/L) (substrate phenylacetate)	Baseline End	125.7 (111.0/153.2) 127.5 (109.5/154.0)	108.8 (102.0/122.7) 114.9 (100.0/127.9)	117.7 (100.5/150.5) 126.5 (102.4/147.6)
PON1 activity (U/L) (substrate paraoxon)	Baseline End	60.0 (49.4/112.2) 58.3 (51.0/114.2)	108.3 (49.4/149.3) 103.6 (48.9/139.0)	87.7 (51.9/156.2) 87.1 (50.4/165.4)

Values are median (25./75. percentiles)
Intra-group comparisons: no significant differences
Inter-group comparisons: no significant differences

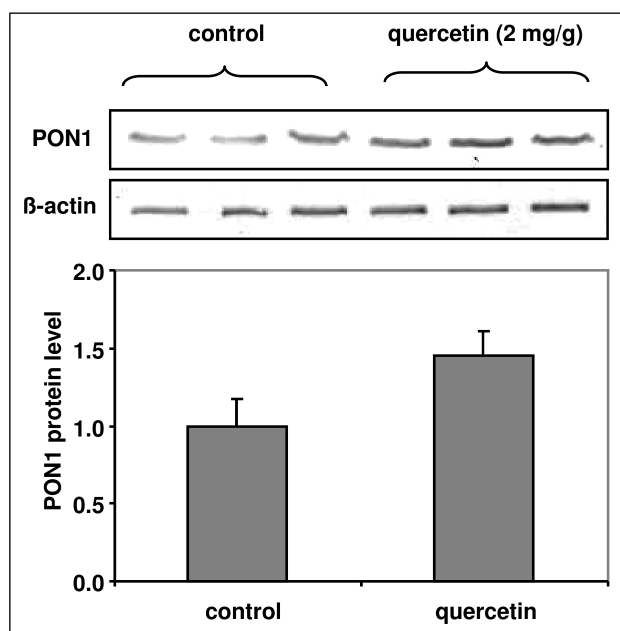

Fig. 2. Paraoxonase 1 protein levels in the liver of C57BL/6 mice fed quercetin supplemented diets for 6 weeks. Shown is a representative Western blot with three animals of the control and the highest quercetin supplementation group (2 mg quercetin/g diet). PON1 was normalized to β-actin levels and results given as mean with SEM.

significantly increased median plasma quercetin concentrations from 52.5 to 145 nmol/l (Q50), from 44.9 to 217 nmol/l (Q100), and Dose-Respons 380 nmol/l (Q150, $p<0.01$ for all). Plasma isorhamnetin concentrations were very low and ranged between 1.1 to 4.0 nmol/l at baseline and 9.2 nmol/l (Q50), 15.7 nmol/l (Q100) and 23.3 nmol/l (Q150) after supplementation (for detailed results see (22)). Quercetin supplementation did not significantly affect plasma PON1 activities towards paraoxonase and phenylacetate (*Table 2*). There were no significant associations between plasma PON1 activity levels and HDL cholesterol concentrations (data not shown) and also no significant correlations between plasma PON1 activity towards the substrates phenylacetate and paraoxon and plasma quercetin concentrations. Individual paraoxonase and phenylacetate activities showed a high variability (VK_{inter} at baseline for PON1 paraoxonase activity = 73%, for PON1 phenylacetate activity = 22%). No significant gender-specific differences in plasma PON1 activities were observed.

PON1 reporter gene assay in hepatocytes

To compare the PON1 inducing activity of quercetin and isorhamnetin, Huh7 liver cells were incubated with 25 µmol/l quercetin and isorhamnetin for a period of 48 h. As shown in *Fig. 3*, isorhamnetin induced a 2.7 fold increase in PON1 promotor activity as compared to controls whereas incubation with its parent compound quercetin increased PON1 promotor activity by 1.4 fold as compared to control. Resveratrol which was used as a positive control (25 µmol/l) induced PON1 promotor activity by 3.4 fold in comparison to the solvent treated controls.

DISCUSSION

In the present study we demonstrate that a dietary supplementation with the flavonol quercetin up-regulates PON1

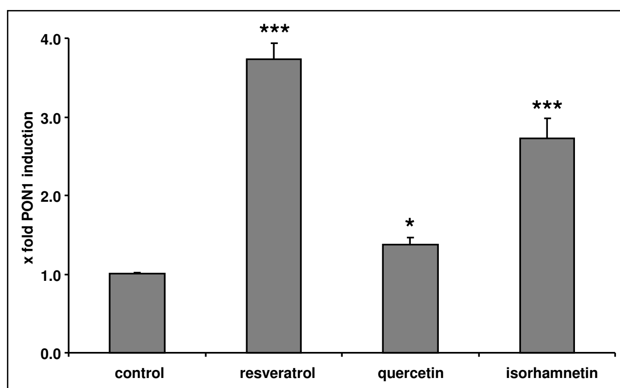

Fig. 3. Effect of quercetin and isorhamnetin on the activity of the PON1 promotor. Huh7 stably transfected clones which express the luciferase gene under the control of the 1009bp promotor of the PON1 gene, were incubated with 25 µmol/l quercetin or isorhamnetin for 48 h. Resveratrol (25 µmol/l) was used as control. Data are mean with SEM of triplicate samples from three independent cell passages. * and ** indicate significant differences from solvent control treatment (* $p<0.05$; *** $p<0.001$; t-test).

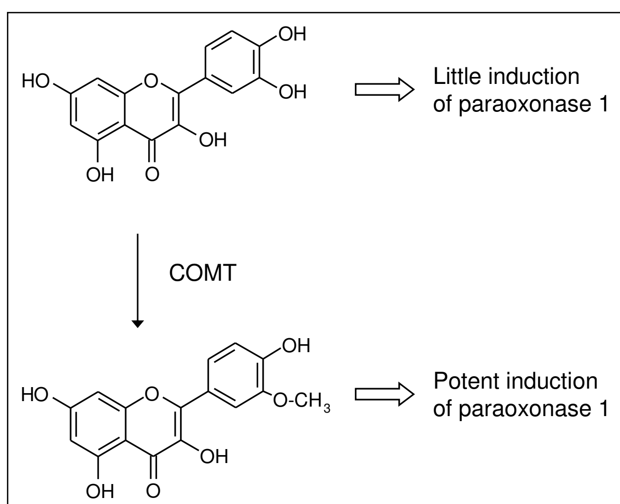

Fig. 4. Biotransformation of quercetin to isorhamnetin by catechol-O-methyl transferase.

expression in the liver of laboratory mice. Although there is some evidence that polyphenols may induce PON1 in cultured human hepatoma cells (Huh7) (21, 27), data regarding PON1 induction *in vivo* are sparse. Since PON1 is mainly synthesized in the liver and secreted into the circulation (28), an induction of hepatic PON1 may at least partly be reflected by an enhancement of PON activity in plasma (6). Moreover, our data show that hepatic PON1 induction positively correlates with plasma levels of quercetin and isorhamnetin suggesting a dose-dependent relationship between dietary quercetin and PON1 gene expression in rodents. Feeding mice with as low as 0.1 mg quercetin per g diet, which resulted in plasma levels of 0.3 and 0.2 µmol/l for quercetin and isorhamnetin, respectively, was accompanied by a significant 1.5 fold up-regulation of hepatic PON1 mRNA levels, as compared to the control group. A further increase of the dietary quercetin levels to a maximum of 2 mg per g diet did even further induce hepatic PON1 mRNA levels up to twofold with corresponding plasma levels of 2.3 µmol/l for quercetin and 2.1 µmol/l for isorhamnetin, respectively. The dietary quercetin concentrations as used in the present study (0.05-2 mg/g diet) correspond to 10-400 mg quercetin per kg body weight, which is many times higher than quercetin concentrations which can normally be achieved due to flavonoid-rich diets in man (29). However, quercetin is also available in a purified form and commercially available quercetin supplements often contain quercetin concentrations in a range between 500-1000 mg/capsule (30).

The underlying molecular mechanisms by which quercetin induces PON1 gene expression have yet not been fully elucidated. Cell culture data indicate that quercetin affects Ahr (Aryl hydrocarbon receptor) and SP1 transcription factor activity thereby driving PON1 gene expression (21, 31).

In the mouse study differences in PON1 mRNA levels due to dietary quercetin were not fully reflected on the protein level. In fact only a 50% increase of hepatic PON1 protein levels in quercetin as compared to control animals was evident indicating potential posttranscriptional regulation of the PON1 gene. Overall current data suggest that dietary quercetin is a rather weak inductor of PON1 in mouse liver *in vivo*.

Nevertheless, following the assumption, that quercetin supplementation would lead to increased hepatic PON1 mRNA levels, we supplemented (healthy) human volunteers with quercetin at three different dosages (50, 100 and 150 mg/day) for two weeks after an initial two week wash-out phase where the volunteers were given a flavonoid-poor diet (22). However, as our data indicate, PON1 activity, as measured with two different substrates, was not changed in response to the quercetin supplementation, although maximal mean quercetin plasma concentrations of 0.38 µmol/l (0.33, 0.64; 25th, 75th percentile) were achieved in the highest quercetin group (150 mg/day) which is a 570% increase compared to baseline quercetin levels for this particular group. However, the highest plasma quercetin concentration measured in the mouse study was eight fold higher as compared to the highest plasma quercetin concentration measured in the human study. The matter of fact that PON1 was changed in mice but not in humans may be related to differences in the quercetin concentrations administered and resulting plasma concentrations, differences in the duration of the experimental trials (6 *vs*. 2 weeks) and differences in the biomarkers monitored (PON1 mRNA and protein *vs*. PON activity levels). In this context it needs to be taken into account that in the mouse study PON1 mRNA and protein levels were determined in the liver whereas in humans only PON1 activity in plasma could be evaluated. PON activity measurement in mouse plasma was not possible due to the limited amount of plasma which was entirely used for the HPLC analysis of quercetin and isorhamnetin.

Unlike in other studies in humans where dietary vitamin E (32) and pomegranate juice (33, 34) resulted in an enhancement of plasma PON1 activity, in the present study quercetin did not elevate PON1 activity in humans. It should be considered that in our study young healthy and lean volunteers (with BMI 22.1±2.2) were recruited exhibiting physiological cholesterol and triglyceride levels. Possibly, PON activity in these volunteers is already at a relatively high level thus a further induction of PON activity due to quercetin was not possible.

Another explanation why dietary quercetin induced PON1 in mice but not in humans may be related to the metabolism of quercetin. While in mice a large proportion of quercetin was methylated to isorhamnetin (up to 48%), only low amounts of isorhamnetin could be detected in the human plasma samples. Interestingly, our reporter gene data indicate that isorhamnetin is more potent than quercetin in inducing PON1 activity. Thus, differences between mice and humans regarding the PON1 inducing activity of quercetin seem to be at least partly determined by the capacity of the liver to convert quercetin into its methylated metabolite isorhamnetin. Thus in mice quercetin

may serve as a "prodrug" which is converted into isorhamnetin in the liver which in turn induces PON1 as summarized in *Fig. 4*. Studies are warranted to test the hypothesis whether an inhibition of the hepatic catechol-ortho-methyl transferase (COMT; which converts quercetin into isorhamnetin) may be accompanied by a decrease in the PON1 inducing activity of quercetin.

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Conflict of interests: None declared.

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