Eur J Nutr 41 : 237 – 243 (2002)
DOI 10.1007/s00394-002-0389-8

ORIGINAL CONTRIBUTION

Achim Bub
Stephan Barth
Bernhard Watzl
Karlis Briviba
Birgit Maria Herbert
Petra Maria Lührmann
Monika Neuhäuser-Berthold
Gerhard Rechkemmer

Paraoxonase 1 Q192R (PON1–192) polymorphism is associated with reduced lipid peroxidation in R-allele-carrier but not in QQ homozygous elderly subjects on a tomato-rich diet

Summary *Background* The oxidative modification of LDL is considered to play a central role in the pathogenesis of atherosclerosis and coronary heart disease (CHD). Paraoxonase (PON1) protects LDL from oxidation and may therefore retard the developement of atherosclerosis. The PON1–192 polymorphism is associated with diminished PON1 concentrations and an increased risk for CHD in RR-allele subjects. *Aim of the study* To investigate the effect of tomato juice consumption on PON1 activity and other parameters related to oxidative stress in healthy elderly subjects. Furthermore, the PON1–192 genotype has been determined in the volunteers in order to see whether possible treatment effects are related to the PON1–192 polymorphism. *Methods* Fifty elderly subjects were randomly assigned to control (mineral water) or intervention group (tomato juice). Subjects of the tomato juice group consumed daily 330 mL tomato juice for 8 weeks. Antioxidant status was measured as LDL oxidation, plasma malondialdehyde, ferric reducing ability of plasma (FRAP) and PON1 activity. The PON1–192 polymorphism was determined by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR). Plasma carotenoids were analyzed by HPLC. *Results* Tomato juice consumption reduced LDL-oxidation and improved antioxidant status in R-allele carriers, but not in the QQ genotype group. PON1 activity increased irrespective of the genotype in both, control and intervention group. *Conclusions* The changes in antioxidant status after tomato juice consumption seem to depend on the PON1–192 genotype. Healthy elderly, carrying the R-allele, could specificly reduce their higher cardiovascular risk by changing dietary habits.

Key words tomato juice – lycopene – antioxidant – lipid peroxidation – paraoxonase – elderly

Received: 9 October 2002
Accepted: 28 October 2002

Achim Bub (✉) · S. Barth · B. Watzl · K. Briviba · G. Rechkemmer
Federal Research Centre for Nutrition
Institute of Nutritional Physiology
Haid-und-Neu-Str. 9
76131 Karlsruhe, Germany
Tel.: +49-721/6625-411
Fax: +49-721/6625-404
E-Mail: achim.bub@bfe.uni-karlsruhe.de

B. M. Herbert · P. M. Lührmann · M. Neuhäuser-Berthold
Institute of Nutritional Sciences
Justus-Liebig-University
Giessen, Germany

List of abbrevitations

CHD coronary heart disease; *dNTP* deoxynucleoside triphosphate; *FRAP* ferric reducing ability of plasma; *PBMC* peripheral blood mononuclear cells; *PCR* polymerase chain reaction; *PON* paraoxonase; *Q* glutamine; *R* arginine; *RFLP* restriction fragment length polymorphism; *TBARS* thiobarbituric acid reactive substances.

Introduction

The oxidative modification of LDL is considered to play a central role in the pathogenesis of atherosclerosis and coronary heart disease (CHD) [1]. Paraoxonase 1 (PON1) is an HDL associated enzyme that belongs to the paraoxonase gene family (PON1, PON2, PON3) and protects LDL from oxidation [2–4]. PON1 may therefore reduce the development of atherosclerosis [5, 6]. An activity polymorphism for PON1 has been shown to be an amino acid substitution glutamine(Q)/arginine(R) at position 192 [7, 8]. The QQ-genotype results in an isoen-

zyme showing a low activity towards paraoxon (an organophosphate), the isoenzyme corresponding to the RR-genotype exhibits a high activity towards paraoxon. For the PON1 substrate phenylacetate, substrate activity has been shown not to be affected by the PON1–192 genotype [5]. With respect to the PON1–192 polymorphism recent findings indicate that diminished PON1 concentrations and an increased prevalence of the RR-allel is present in populations at increased risk for CHD [9].

Dietary factors such as alcohol [10], pomegranate juice [11] and dietary fat [12–15] have been reported to modulate PON1 activity and may thus have an impact on CHD. It has also been shown by Kleemola et al. [16] that a high vegetable intake is associated with lower PON1 activity and that PON1 activity is negatively correlated with the intake of β-carotene as revealed by a 3-day food record. Data on other carotenoids, e. g. lycopene, were not presented in this study.

Tomatoes and tomato products are the major source for lycopene in the human diet. Tomato consumption resulting in high adipose tissue concentration of lycopene is associated with a reduced risk for myocardial infarction [17], while a low serum lycopene concentration is correlated with an increased incidence of acute coronary events and stroke [18]. Whether lycopene intake and lycopene plasma concentration would affect PON1 activity has not been investigated so far. Therefore, we studied the effect of tomato juice consumption on PON1 activity and other parameters related to oxidative stress in healthy elderly subjects. Furthermore, the PON1–192 genotype has been determined in the volunteers in order to see whether possible treatment effects are related to a distinct PON1–192 genotype.

Material and methods

Subjects

Subjects were participants of the Longitudinal Study in an Aging Population of Giessen (GISELA), Germany, in which the nutritional and health status of free-living elderly people is investigated in yearly intervals. Subjects had to be at least 60 years of age, physically mobile, and available around Giessen for the long term. The GISELA study was initiated in 1994 and in 1997 453 elderly people were enrolled in this investigation. Out of the 453 subjects included in the survey, selection for the participants of the tomato study was based on the following exclusion criteria: smoking, alcohol consumption > 50 g/d, food allergies, cancer, coronary heart disease, chronic inflammatory diseases (rheumatoid arthritis, Crohn disease, colitis ulcerosa), diabetes mellitus, asthma, prescription medication or nonsteroidal anti-inflammatory drugs on a regular basis, vitamin or mineral supplements for the last 3 months, corticosteroid treatment, intake of immunostimulators for the last 4 weeks. Fifty-three elderly subjects (33 females, 20 males) were enrolled after screening. The study was approved by the Ethical Committee of the Department of Medicine, Justus-Liebig-University Giessen and all participants gave their consent in writing.

Study design

Subjects were randomly assigned to control (mineral water) or intervention group (tomato juice). Subjects of the tomato juice group consumed daily 330 mL tomato juice (providing 47.1 mg lycopene and 1.7 mg β-carotene; Schoenenberger, Magstadt, Germany) for a period of 8 weeks. Instead of tomato juice subjects of the control group consumed the same volume as mineral water. Both groups were instructed to drink the tomato juice or water with their main meal. Since the current intake of lycopene in Germany is around 1 mg/d [19] and the tomato juice provided about 40 times more lycopene, subjects were allowed to continue with their regular diet throughout the study period including tomato products in the control group. During the entire intervention period, subjects were asked to protocol any intercurrent diseases or medicine use. Blood samples were drawn after an overnight fast between 7 am and 10:30 am at the Institute of Nutritional Sciences, University of Giessen.

Analytical methods

The "ferric reducing ability of plasma" (FRAP) was used to determine antioxidant activity in plasma [20]. The assay is based on the principle that electron-donating antioxidants can be described as reductants. In this context, the reducing ability of plasma may be refered to as antioxidant activity. The *ex vivo* oxidation of isolated LDL was performed by using a modified method of Esterbauer et al. [21]. The LDL oxidation process was followed by recording the conjugated diene absorption at 234 nm. From the resulting curve, the time interval (minutes) between the intersept of the linear last square slope of the curve with the initial-absorbance axis is defined as the "lag time" and is a measure of the susceptibility of LDL to oxidation. Both methods have been described earlier [22]. Plasma malondialdehyde was measured as thiobarbituric acid reactive substances (TBARS) by HPLC with fluorescence detection [23, 24]. Carotenoids in plasma were measured by reversed-phase HPLC [25]. Serum triacylglycerol, cholesterol and HDL-cholesterol were determined by enzymatic kits (Roche Mannheim, Germany). LDL-cholesterol was calculated by using the "Friedewald"-formula. Plasma pro-

tein thiols were measured with a spectrophotometric method using dithionitrobenzene, while glutathione served as standard [26].

Arylesterase activity of paraoxonase 1

PON1 activity was determined spectrophotometrically using phenylacetate as substrate as described by Gan et al. [27] with minor modifications. The assay mixture contained 5 mmol/L of phenylacetate and 0.9 mmol/L $CaCl_2$ in 20 mmol/L Tris-HCl, pH 8, at 25 °C. The reaction was recorded at 270 nm. Nonenzymatic hydrolysis of phenylacetate was substracted from the total rate of hydrolysis. Results are expressed as U/mL. The E_{270} for the reaction is 1310 mol x $L^{-1}\,cm^{-1}$ and 1 unit of arylesterase activity is equal to 1 micromole of phenylacetate hydrolyzed per milliliter per minute.

Determination of the paraoxonase 192 genotype

Peripheral blood mononuclear cells (PBMC) were isolated from blood (K-EDTA) by density gradient centrifugation using Histopaque 1077 (Sigma, Deisenhofen, Germany) as described earlier [28]. Genomic DNA was extracted from isolated PBMC using the GenomicPrep Blood DNA Isolation Kit (Amersham, Freiburg, Germany) according to the manufacturers directions. The quantity and quality of prepared genomic DNA were measured at 260/280 nm in a UV/VIS spectrophotometer (Lamda Bio 20, Perkin Elmer, Wellesley, MA, USA). The determination of the paraoxonase 192 (PON1–192) genotype followed a previously published protocol [8] with some minor modifications: The 99 bp target region, which encompasses the polymorphic region of the human PON1 gene, was amplified by polymerase chain reaction (PCR) using specific sense 5′ > TAT TGT TGC TGT GGG ACC TGA G < 3′ (nt 58–79; GenBank Acc. HSPON1EX6) and antisense 5′ > CAC GCT AAA CCC AAA TAC ATC TC (nt 134–156; Acc. HSPON1EX6) primers. The 50 µL reaction mixture contained 1 µg of genomic DNA, 10 nmol of each dNTP, 5 µL of 10x reaction buffer (100 mM KCl, 100 mM $(NH_4)_2SO_4$, 200 mM Tris/HCl pH 8.8, 20 mM $MgSO_4$, 1 % Triton X-100), 200 ng of each primer and 0.5 µL VentR DNA polymerase (2 U/µL, New England Biolab, Schwalbach, Germany). After denaturation of DNA at 95 °C for 3 min, the reaction mixture was subjected to 40 PCR cycles (Thermocycler PTC 200; MJ Research Inc., Waltham MA, USA), each cycle comprising denaturation at 94 °C for 60 sec, primer annealing at 55 °C for 30 sec and extension at 72 °C for 60 sec with a final extension time at 72 °C for 5 min. Efficiacy of PCR amplification was checked after electrophoretic separation of a 20 µL aliquot in 3 % agarose and UV-visualization of the amplification product by ethidium bromide staining. Subsequently, a 25 µL aliquot of the PCR product was digested with 5.0 U *Alw1* restriction endonuclease (2 U/µL, New England Biolabs) for 2 h at 37 °C, and the digested products were separated by agarose gelelectrophoresis using 5 % MS500 MoSieve agarose (PeqLab, Erlangen, Germany) in 1x TBE buffer, stained with ethidium bromide and visualized using computer based image analysis (FluorS Imager; Biorad, München, Germany). The 192 Q/R transition creates a unique *Alw1* site in the PCR amplicon. DNA samples homozygous for the 192 Q allele present the original undigested 99 bp PCR product, those homozygous for the 192 R allele present two restriction fragments 66 bp and 33 bp and those heterozygous show the original product of 99 bp plus the restriction fragments of 66 bp and 33 bp. Each genotype was read by two independent observers.

Statistical analysis

Results are given as means ± standard deviation (SD). Baseline characteristics for the PON1 genotype groups (QQ, QR, RR) were analyzed by ANOVA. For comparison of the treatment groups (tomato juice or mineral water), baseline data versus post-treatment data within groups were analyzed by using Students paired t-test or Wilcoxon's rank test for data that were not normally distributed. Normal distribution of the data was analyzed by using the variance ratio test (F-test). Differences between treatment groups were analyzed by using Student's t-test for independent samples (or the Mann-Whitney U test for data that were not normally distributed) on mean pre- to post-intervention differences. Since the RR-genotype was present in 5 subjects only, we merged the QR and RR groups resulting in a R-allele-carrier group. For further analysis the QQ-genotype group was compared with the R-allele-carrier group. Statistical significance was accepted at the $p < 0.05$ level. All statistical calculations were performed with the StatView computer software program (SAS Institute 1998, Cary, NC, USA).

Results

Fifty subjects completed the study; 3 subjects were excluded from the study because they received a drug treatment during the intervention period. No other subjects reported any intercurrent diseases during the study. Subject characteristics are summarized in Table 1.

PON1–192 genotyping revealed 20 subjects carrying the QQ-genotype, 25 QR-genotypes, and 5 RR-genotypes respectively (Table 1). A representative agarose gel image showing the DNA fragments after the *Alw1* digestion is given in Fig. 1. At week 0, there were no differences among the genotype groups with respect to subject characteristics, lipid status (Table 1), LDL-oxida-

European Journal of Nutrition, Vol. 41, Number 6 (2002)

Table 1 Baseline characteristics of study participants[1]

		Control	Tomato	QQ	QR	RR
n	50	21	29	20	25	5
Sex (F, M)	32, 18	13, 8	19, 10	11, 9	17, 8	4, 1
Age (y)	70±6	70±5	70±6	70±5	70±6	70±3
Body mass index (kg/m^2)	27±3	27±3	26±3	26±3	26±2	30±3*
Triacylglycerol (mg/dl)	101±40	91±28	109±45	97±41	107±41	93±27
Cholesterol (mg/dl)	223±33	220±36	225±32	219±29	219±29	254±55
LDL-Cholesterol (mg/dl)	135±31	132±33	138±31	130±29	134±29	161±47
HDL-Cholesterol (mg/dl)	63±13	65±12	62±14	64±14	61±13	69±6

[1] values are means ± SD; *: $p < 0.05$ for between group differences (QQ, QR, RR)

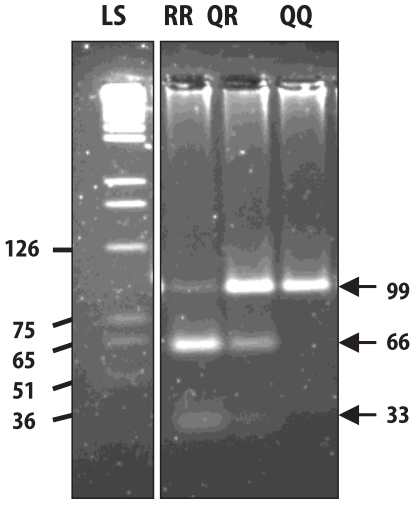

Fig. 1 Determination of the paraoxonase1–192 polymorphism. Representative agarose gel image showing the DNA fragments after the *Alw1* digestion showing the 3 PON1-192 genotypes. *RR* homozygous mutant; *QR* heterozygote; *QQ* homozygous wildtype. Right panel: 33, 66, 99 indicate the size (base pairs) of the digested DNA fragments. Left panel: standard DNA size markers (length standard: LS)

tion, antioxidant status, and PON activity (data not shown). Only BMI was higher in the RR-genotype group at baseline ($p < 0.05$), which resulted from one male with a BMI of 34.

There were no differences in any of the measured parameters between the tomato group and controls at week 0. Tomato juice consumption reduced LDL-oxidation and improved antioxidant status. Lag time ($p < 0.05$) and FRAP ($p < 0.05$) were increased in the tomato group but not in controls (Table 2). In both groups, PON activity was higher after 8 weeks ($p < 0.05$). TBARS, plasma thiols and serum lipids (data not shown) did not change in the tomato and control group, respectively.

In order to see whether the PON1–192 genotype is related to the changes in LDL-oxidation and antioxidant status after tomato juice consumption, we evaluated these parameters in the tomato group with respect to the frequency of the QQ-genotype and the R-allele-carriers (Table 3). Tomato juice consumption increased lag time ($p < 0.05$) and FRAP ($p < 0.05$) and reduced TBARS ($p < 0.05$) in R-allele-carriers. In the QQ-genotype group however, there were no changes regarding LDL-oxidation and antioxidant status. The observed increase in PON activity seems not to be a result of the tomato juice intervention, since PON activity had also increased in the control group (Table 2). In the tomato group, plasma thiols and serum lipids (data not shown) were not different between QQ-genotype and the R-allele-carriers.

The increase in plasma carotenoid concentrations after tomato juice consumption could contribute to the improved antioxidant status and LDL-oxidation. Plasma carotenoid concentrations of the control and tomato

Table 2 Effect of tomato juice consumption on lipid peroxidation and antioxidant status in healthy elderly[1]

	Week 0		Week 8	
	Control	Tomato	Control	Tomato
Lag time (min)	83±12	86±14	82±11	93±14*, [a]
TBARS (µmol/L)	0.70±0.2	0.68±0.2	0.73±0.3	0.66±0.3
PON (U/mL)	102±28	101±29	119±25*	113±32*
FRAP (µmol/L)	887±122	901±224	901±122	948±172*
Thiol groups (µmol/L)	267±53	310±104	291±57	319±113

[1] values are means ± SD; n = 21 (control) or n = 29 (tomato); *TBARS* thiobarbituric acid reactive substances; *PON* paraoxonase-arylesterase activity; *FRAP* ferric reducing ability of plasma; [a] $p < 0.05$ for between group differences (control vs. tomato juice) and * $p < 0.05$ for within group differences (pre-post comparison) after tomato juice consumption

Table 3 Effect of tomato juice consumption and paraoxonase (PON) Q192R polymorphism on lipid peroxidation and antioxidant status in healthy elderly[1]

	Week 0		Week 8	
	QQ	QR/RR	QQ	QR/RR
Lag time (min)	88±19	84±11	94±13	93±15*
TBARS (μmol/L)	0.69±0.2	0.67±0.2	0.72±0.3	0.62±0.2*
PON (U/mL)	99±35	103±26	114±38*	112±28*
FRAP (μmol/L)	990±246	838±190	1009±202	905±138*
Thiols (μmol/L)	319±83	303±119	310±78	326±134

[1] values are means ± SD; n = 12 (QQ) or n = 17 (QR/RR); *TBARS* thiobarbituric acid reactive substances; *PON* paraoxonase-arylesterase activity; *FRAP* ferric reducing ability of plasma; * $p < 0.05$ for within group differences (pre-post comparison) after tomato juice consumption

group have already been published [28]. Table 4 shows the plasma carotenoid concentrations of the tomato group with respect to the PON1 genotype. In both groups, the QQ-genotype and the R-allele-carriers, lycopene and β-carotene increased after tomato juice consumption, while plasma α-carotene, lutein, and β-cryptoxanthin did not change. There were no significant differences in plasma carotenoids between the genotype groups before and after tomato juice consumption. However, there was a tendency for total-lycopene to be higher in the R-allele-carriers ($p = 0.11$).

Discussion

The aim of our study was to investigate the effect of tomato juice consumption on PON1 activity and other parameters related to oxidative stress in healthy elderly subjects. Tomato juice consumption for 8 weeks reduced LDL-oxidation in healthy elderly. This is in line with previous studies showing that in healthy volunteers tomato products protected LDL from oxidation [22, 29]. Additionally, the antioxidant status as measured by the FRAP-assay increased after tomato juice consumption (providing 47.1 mg lycopene/d). Lee et al. [30] also reported an increased antioxidant activity (FRAP) after consumption of tomato products (46 mg lycopene/d) for one week in young volunteers. In contrast, Pellegrini et al. [31] found no effect of tomato puree consumption (7 mg lycopene/d) for two weeks on total antioxidant capacity (TRAP). The type of tomato product (juice, soup, puree) and/or the amount of lycopene supplied may account for the observed differences in antioxidant activity. Lycopene, the major carotenoid in tomatoes, exhibits antioxidant activities in different in vitro systems [32–35] and may be responsible for the observed antioxidant effects. Furthermore, tomatoes contain considerable amounts of polyphenolics and phenolic acids [36, 37], which could also contribute to these effects.

In order to see whether the PON1–192 genotype has an impact on LDL-oxidation and antioxidant status after tomato juice consumption, we analysed the tomato group with respect to the QQ-genotype and the R-allele-carriers. Within the tomato group we found that only in R-allele-carriers tomato juice intervention significantly reduced LDL-oxidation and TBARS, respectively, and increased antioxidant activity (FRAP). This is the first report, that a dietary intervention in humans has an impact on the antioxidant status depending on a distinct PON1–192 genotype. Other groups investigated the ef-

Table 4 Plasma carotenoid concentrations and PON1 Q192R polymorphism in healthy elderly after tomato juice consumption[1]

	Week 0		Week 8	
	QQ	QR/RR	QQ	QR/RR
	(μmol/L)			
Lutein	0.29±0.19	0.34±0.20	0.31±0.19	0.33±0.18
β-Cryptoxanthin	0.17±0.11	0.19±0.16	0.15±0.08	0.17±0.14
total-Lycopene	0.32±0.31	0.22±0.13	0.91±0.34*	1.06±0.39*,[a]
all-trans-Lycopene	0.13±0.05	0.13±0.09	0.48±0.19*	0.59±0.22*
cis-Lycopene	0.19±0.29	0.09±0.05	0.43±0.16*	0.47±0.18*
α-Carotene	0.17±0.11	0.20±0.15	0.20±0.11	0.21±0.08
all-trans-β-Carotene	0.68±0.27	0.79±0.50	0.97±0.23*	1.20±0.59*
cis-β-Carotene	0.05±0.02	0.05±0.02	0.08±0.05*	0.08±0.03*

[1] values are means ± SD; n = 12 (QQ) or n = 17 (QR/RR); [a] $p = 0.11$ for between group differences (QQ vs. QR/RR) and * $p < 0.05$ for within group differences (pre-post comparison) after tomato juice consumption

fect of pomegranate juice [11], alcoholic beverages [10] and used-cooking fat [13] on arylesterase activity of PON1 in healthy volunteers. While pomegranate juice and alcoholic beverages increased PON activity, used-cooking fat decreased it. Additionally, pomegranate juice reduced LDL oxidation while the used-cooking fat increased LDL oxidation. The authors concluded that the changes in PON activity due to the dietary intervention may reduce the developement of CHD, since low serum PON activity has been reported to be associated with an increased risk for coronary artery disease [5]. Unfortunately, in these dietary intervention studies the PON1–192 polymorphism had not been determined, which would have allowed to see whether the changes in PON activity and LDL oxidation depend on the PON1–192 genotype. In our study, PON activity increased in the control and in the tomato group with no differences regarding the PON1–192 genotype. There were no significant changes in serum HDL concentrations which could account for these changes in PON activity. We suppose, that the observed increase in PON-arylesterase activity may represent a "seasonal" effect during the 8 weeks lasting study period, which we can not explain sofar. Therefore, tomato juice consumption improved antioxidant status in healthy elderly independent of PON activity. However, arylesterase activity of PON1 may not be the adequate method to determine the LDL-protecting properties of PON, since the phenylacetate substrate activity is not affected by the PON1–192 genotype [5]. The enzyme activity related polymorphism for PON1 at position 192 results in an isoenzyme showing a low activity towards paraoxon (QQ-genotype) and an isoenzyme exhibiting a high activity towards paraoxon (RR-genotype), while the lipid hydroperoxide hydrolyzing activity, which is important for the LDL-protecting activity, is low for this isoenzyme [5]. A method for directly measuring the lipid hydroperoxide hydrolyzing activity of PON in human serum is not available so far. Therefore, we prefered phenylacetate as a substrate instead of the toxic paraoxon for PON activity measurements.

The antioxidant status (LDL oxidation, TBARS, FRAP) improved significantly after tomato juice only in the R-allele-carriers, although tomato juice consumption increased plasma carotenoid concentrations in the QQ-genotype group and the R-allele-carriers. Assuming that lycopene from tomato juice is responsible for the improved antioxidant status, differences in plasma lycopene concentrations between the genotype groups could account for the observed differences in antioxidant status. The net increase in total lycopene was higher in R-allele-carriers as compared to the QQ-genotype (QQ: $0.60 \pm 0.38\mu M$ vs. QR/RR: $0.85 \pm 0.39\mu M$). However, these results were not statistically significant ($p = 0.11$). Differences in carotenoid bioavailability among the PON1–192 genotypes, which we can not explain with this study, could contribute to differences in the antioxidant status. We did not assess the concentrations of other antioxidants from tomatoes in the plasma of the study participants and therefore can not discuss their role in improving LDL oxidation and antioxidant activity after tomato juice consumption in healthy elderly.

Recently, Senti et al. [38] investigated the involvement of the PON1–192 polymorphism in the different responses of plasma lipids to physical activity. They found that men with the R-allele need to be physically active to achieve a favorable lipoprotein profile which is similar to that observed in QQ homozygous men. Comparable results related to oleic acid intake have been presented by Tomás et al. [15]. They found that high oleic acid intake was associated with increased HDL cholesterol and PON1 activity only in QR and RR genotypes (R-allele carrier), respectively. From these and from our results we may speculate, that R-allele carrier, who are at a higher risk for CHD as compared to the QQ-genotye, could specifically reduce their risk by changing dietary and life style (exercise) habits.

In conclusion, our data show that tomato juice consumption for 8 weeks improves antioxidant status (LDL oxidation, TBARS, FRAP) in healthy elderly. These changes seem to depend on the PON1–192 polymorphism, since the improvement of the antioxidant status is present in R-allele carriers (QR/RR) only, but not in the QQ wildtype. However, antioxidant status in this study is not related to PON-arylesterase activity. The reason for the differences in the relative increase in plasma lycopene between R-allele carriers and the QQ wildtype has to be investigated in future studies.

Acknowledgements The authors thank M. Broßart, T. Gadau, M. Giorgi-Kotterba, D. Haase and U. Stadler-Prayle for their excellent technical assistance and the elderly subjects for taking part in this study. Tomato juice was provided by Schoenenberger Pflanzensäfte GmbH, Magstadt, Germany, which is gratefully acknowledged.

References

1. Diaz MN, Frei B, Vita JA, Keaney JF (1997) Mechanisms of disease: Antioxidants and atherosclerotic heart disease. N Engl J Med 337:408–416
2. Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN (1991) Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. Atherosclerosis 86:193–199
3. Mackness MI, Durrington PN, Mackness B (2000) How high-density lipoprotein protects against the effects of lipid peroxidation. Curr Opin Lipidol 11:383–388

4. Aviram M, Billecke S, Sorenson R, Bisgaier CL, Newton R, Rosenblat M, Erogul J, Hsu C, Dunlop C, LaDu B (1998) Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities. Arterioscler Thromb Vasc Biol 18: 1617–1624
5. Mackness MI, Mackness B, Durrington PN, Fogelman AM, Berliner JXLA, Navab M, Shih D, Fonarow GC (1998) Paraoxonase and coronary heart disease. Curr Opin Lipidol 9:319–324
6. Aviram M (1999) Does paraoxonase play a role in susceptibility to cardiovascular disease? Mol Med Today 5:381–386
7. Adkins S, Gan KN, Mody M, La Du BN (1993) Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. Am J Hum Genet 52:598–608
8. Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE (1993) The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 3:73–76
9. Mackness B, Mackness MI, Durrington PN, Arrol S, Evans AE, McMaster D, Ferrières J, Ruidavets JB, Williams NR, Howard AN (2000) Paraoxonase activity in two healthy populations with differing rates of coronary heart disease. Eur J Clin Invest 30:4–10
10. vanderGaag MS, vanTol A, Scheek LM, James RW, Urgert R, Schaafsma G, Hendriks HFJ (1999) Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomized intervention study in middle-aged men. Atherosclerosis 147: 405–410
11. Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Colemann R, Hayek T, Presser D, Fuhrman B (2000) Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr 71: 1062–1076
12. Mackness N, Bouiller A, Hennuyer N, Mackness B, Hall M, Tailleux A, Duriez P, Delfly B, Durrington P, Fruchart JC, Duverger N, Caillaud JM, Castro G (2000) Paraoxonase activity is reduced by a pro-atherosclerotic diet in rabbits. Biochem Biophys Res Commun 269: 232–236
13. Sutherland WHF, Walker RJ, DeJong SA, vanRijn AM, Phillips V, Walker HL (1999) Reduced postprandial serum paraoxonase activity after a meal rich in used cooking fat. Arterioscler Thromb Vasc Biol 19:1340–1347
14. Kudchodkar BJ, Lacko AG, Dory L, Fungwe TV (2000) Dietary fat modulates serum paraoxonase 1 activity in rats. J Nutr 130:2427–2433
15. Tomás M, Sentí M, Elosua R, Vila J, Sala J, Masià R, Marrugat J (2001) Interaction between the Gln-Arg 192 variants of the paraoxonase gene and oleic acid intake as a determinant of high-density lipoprotein cholesterol and paraoxonase activity. Eur J Pharmacol 432: 121–128
16. Kleemola P, Freese R, Jauhiainen M, Pahlman R, Alftha G, Mutanen M (2002) Dietary determinants of serum paraoxonase activity in healthy humans. Atherosclerosis 160:425–432
17. Kohlmeier L, Kark JD, Gomez Gracia E, Martin BC, Steck SE, Kardinaal AFM, Ringstad J, Thamm M, Masaev V, Riemersma R, MartinMoreno JM, Huttunen JK, Kok FJ (1997) Lycopene and myocardial infarction risk in the EURAMIC Study. Am J Epidemiol 146: 618–626
18. Rissanen TH, Voutilainen S, Nyysönen K, Lakka TA, Sivenius J, Salonen R, Kaplan GA, Salonen JT (2001) Low serum lycopene concentration is associated with an excess incidence of acute coronary events and stroke: the Kuopio Ischaemic Heart Disease Risk Factor Study. Br J Nutr 85:749–754
19. Pelz R, Schmidt-Faber B, Heseker H (1998) Carotenoid intake in the German National Food Consumption Survey. Z Ernährungswiss 37:319–327
20. Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal Biochem 239:70–76
21. Esterbauer H, Striegl G, Puhl H, Rotheneder M (1989) Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Rad Res Comms 6:67–75
22. Bub A, Watzl B, Abrahamse L, Delincée H, Adam S, Wever J, Müller H, Rechkemmer G (2000) Moderate intervention with carotenoid-rich vegetable products reduces lipid peroxidation in men. J Nutr 130:2200–2206
23. Lepage G, Munoz G, Champagne J, Roy CC (1991) Preparative steps for the accurate measurement of malondialdehyde by high-performance liquid chromatography. Anal Biochem 197: 277–283
24. Burkart V, Liu H, Bellmann K, Wissing D, Jäättelä M, Cavallo MG, Pozilli P, Briviba K, Kolb H (2000) Natural resistance of human beta cells toward nitric oxide is mediated by heat shock protein 70. J Biol Chem 275:19521–19528
25. Müller H, Bub A, Watzl B, Rechkemmer G (1999) Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. Eur J Nutr 38:35–44
26. Motchnik PA, Frei B, Ames BN (1994) Measurement of antioxidants in human blood plasma. Methods Enzymol 234: 269–279
27. Gan KN, Smolen A, Eckerson HW, La Du BN (1991) Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities. Drug Metab Dispos 19: 100–106
28. Watzl B, Bub A, Blockhaus M, Herbert BM, Lührmann PM, Neuhäuser-Berthold M, Rechkemmer G (2000) Prolonged tomato juice consumption has no effect on cell-mediated immunity of well-nourished elderly men and women. J Nutr 130:1719–1723
29. Agarwal S, Rao AV (1998) Tomato lycopene and low density lipoprotein oxidation: A human dietary intervention study. Lipids 33:981–984
30. Lee A, Thurnham DI, Chopra M (2000) Consumption of tomato products with olive oil, but not sunflower oil increases the antioxidant activity of plasma. Free Radical Biol Med 29:1051–1055
31. Pellegrini N, Riso P, Porrini M (2000) Tomato consumption does not affect the total antioxidant capacity of plasma. Nutrition 16:268–271
32. DiMascio P, Kaiser S, Sies H, Conn PF, Schalch W, Truscott TG (1989) Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538
33. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice Evans CA (1996) Antioxidant activities of carotenes and xanthophylls. FEBS Lett 384:240–242
34. Oshima S, Ojima F, Sakamoto H, Ishiguro Y, Terao J (1996) Supplementation with carotenoids inhibits singlet oxygen- mediated oxidation of human plasma low-density lipoprotein. J Agric Food Chem 44:2306–2309
35. Stahl W, Junghans A, deBoer B, Driomina ES, Briviba K, Sies H (1998) Carotenoid mixtures protect multilamelar liposomes against oxidative damage: synergistic effects of lycopene and lutein. FEBS Letters 427:305–308
36. Beecher GR (1998) Nutrient content of tomatoes and tomato products. Proc Soc Exp Biol Med 218:98–100
37. Paganga G, Miller NE, RiceEvans CA (1999) The polyphenolic content of fruit and vegetable abd their antioxidant activities. What does a serving constitute? Free Radical Res 30:153–162
38. Sentí M, Aubó C, Elosua R, Sala J, Tomás M, Marrugat J (2000) Effect of physical activity on lipid levels in a population-based sample of men with and without the Arg192 variant of the human paraoxonase gene. Genet Epidemiol 18: 276–286