The age-related paraoxonase 1 response is altered by long-term caloric restriction in male and female rats

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Abstract Caloric restriction (CR) has been shown to attenuate age-related oxidative damage and to improve major atherosclerotic risk factors. Paraoxonase 1 (PON1), an enzyme specifically associated with HDL containing apolipoproteins A-I and J, has been reported to prevent the proatherosclerotic effects of oxidized LDL. The aim of this study was to evaluate whether modulation of PON1 activity is part of the underlying CR mechanisms that attenuate the age-associated negative effects. Experimental groups were 1 year old rats of both genders subjected to 40% CR for 1 year and two ad libitum-fed groups, also including rats of both genders, euthanized at 6 months or 2 years. Aging impaired the serum lipid profile and increased lipid peroxidation, PON1 activities, and the content of both PON1 and apolipoprotein J in HDL, which suggests an HDL subfraction redistribution to protect LDL more effectively from oxidation. The CR-associated improved lipid profile and the decreased lipid peroxide levels would lead to the decreased arylesterase activity seen in old CR animals, suggesting that PON1 modulation is not an integral part of the main antioxidant mechanisms of CR but rather that CR would determine a more youthful and less oxidative situation in which the protection of LDL would be less necessary.—Thomàs-Moyà, E., M. Gianotti, A. M. Proenza, and I. Lladó. **The age-related paraoxonase 1 response is altered by long-term caloric restriction in male and female rats. *J. Lipid Res.* 2006. 47: 2042–2048.**

Supplementary key words aging • gender • apolipoprotein A-I • apolipoprotein J • lipid peroxidation

Aging is considered a gradual, time-dependent accumulation of irreversible molecular damage leading to a lower functionality and resistance or adaptability to stress, in which diet is one of the most important extrinsic factors involved (1). Although the mechanisms underlying the aging process are poorly understood, the oxidatively altered forms of DNA, proteins, and lipids detected in the cells of aged organisms support the idea of an increased oxidative status of aged animals as one of the factors responsible for the described functional deterioration (2). Regarding lipid peroxidation, a main promoter of atherosclerosis development (3), both serum levels and susceptibility to oxidation of LDL have been described to increase with aging (4, 5), thus justifying the greater atherosclerotic and cardiovascular risk at advanced ages (6).

Caloric restriction (CR) is a dietary intervention that has been described to delay the onset or slow the progression of most age-related diseases and to extend maximum life span in rodents and nonhuman primates, leading to a more youthful physiology at advanced ages (7, 8). Among its beneficial effects, CR improves major atherosclerotic risk factors in humans and rodents (9, 10) as well as reducing the accrual of oxidative damage of macromolecules (11), rendering lower levels of oxidized LDL and reducing the age-related increased sensitivity of endothelial cells to these modified lipoproteins (12).

There is ample evidence linking paraoxonase 1 (PON1), an enzyme specifically associated with HDL containing apolipoprotein A-I (apoA-I) and apoJ (13, 14), with the prevention of atherosclerosis (15). PON1 is first synthesized in the liver and subsequently released into the blood (16), where it has been shown to protect HDL and LDL against oxidation (17, 18). Even though the biochemical activity that mediates the antiatherogenic role of PON1 and its biological substrates has not been definitely established (19), a higher serum PON1 activity has been related to a lower risk of cardiovascular disease (15, 20).

Apart from the expression of PON1 in the liver, its release into the blood represents a key step in the modulation of its circulating concentration and activity. In this sense, the proposed mechanism by which PON1 would be released has been suggested to involve scavenger receptor class B type I (SR-BI), because this HDL receptor allows the transient association of HDL with the hepatocyte membrane without internalization or destruction of these lipoproteins (21). Once in the blood, apoA-I and

Manuscript received 15 May 2006 and in revised form 29 June 2006.

Published, JLR Papers in Press, June 30, 2006.
DOI 10.1194/jlr.M600215-JLR200

Abbreviations: apoA-I, apolipoprotein A-I; CR, caloric restriction; PON1, paraoxonase 1; SR-BI, scavenger receptor class B type I; TBARS, thiobarbituric acid-reactive substances.

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apoJ stabilize PON1 function and its association with HDL (13, 14).

Serum PON1 activity and concentration have also been shown to be modulated by lifestyle and dietary factors such as short-term CR (22, 23). Taking into consideration the protective role of both PON1 and CR against atherosclerosis development, the question raised would be whether changes in PON1 activity are part of the underlying CR mechanisms that cause the attenuation of the negative effects of aging, at least with regard to atherosclerosis risk factors. To answer this question, we first investigated serum PON1 response to the age-associated oxidative status and the main factors involved. Second, we evaluated serum PON1 response to the antiaging effects of 1 year of 40% CR in old rats.

MATERIALS AND METHODS

Materials

Oligonucleotide primer sequences, Lightcycler-FastStart DNA Master SYBR Green I for real-time PCR, and Tripure® isolation reagent were purchased from Roche Diagnostics (Basel, Switzerland). RT-PCR chemicals were from Applied Biosystems. Rabbit polyclonal antibody to human PON1 was kindly provided by Drs. M. Mackness and B. Mackness (24). Rabbit polyclonal antibodies to human apoA-I (No. 178422) and rat SR-BI (NB 400-104G2) were supplied by Calbiochem (San Diego, CA) and Novus Biological, respectively. Goat polyclonal antibody to rat apoJ was purchased from Santa Cruz Biotechnology (No. sc-13747; Santa Cruz, CA). Chemiluminescence kit (ECL) for immunoblot development was purchased from Amersham (Little Chalfont, UK).

Kits for the measurement of serum lipid profiles were purchased from Linear Chemicals SL (Barcelona, Spain): HDL-cholesterol direct (No. 1133505), LDL-cholesterol (No. 1142005), total cholesterol MR (No. 1118005), and triglyceride MR (No. 1155005). Substrates for the measurement of PON1 activities (diethyl *p*-nitrophenyl phosphate and phenylacetate) were from Sigma-Aldrich (St. Louis, MO). Routine chemicals used were supplied by Pronadisa (Madrid, Spain), Panreac (Barcelona, Spain), Sigma-Aldrich, and Roche Diagnostics.

Animals and experimental protocol

Animal experiments were performed in accordance with the general guidelines approved by our institutional ethics committee and European Union (86/609/EEC) regulations. One year old rats (Charles River, Barcelona, Spain), six males and six females, were subjected to 40% energy restriction for 1 year (old CR rats). The same number of age-matched rats was always fed ad libitum with a pelleted standard diet (old rats) until the moment of euthanasia. The amount of food offered to old CR rats was updated weekly according to the intake of old rats. Additionally, another group of ad libitum-fed rats was included in the study and euthanized at 6 months of age (young rats). The rats, which had free access to water, were housed individually in wire-bottom cages to prevent coprophagia at 22°C with a 12 h light/dark cycle.

The energy content of the diet supplied to the experimental groups (A04; Panlab, Barcelona, Spain) was 3,000 kcal/kg. The composition of the diet was 60.5% carbohydrates, 15.4% protein, 2.9% fat, 3.9% fiber, 5.3% minerals, and 12% humidity.

Sample collection and measurement of serum lipid profile and thiobarbituric acid-reactive substances

Animals were euthanized by decapitation at the beginning of the light cycle. Livers were immediately removed, frozen in liquid nitrogen, and stored at −70°C until use for total RNA isolation and tissue homogenization. Serum samples were also stored at −70°C until analysis. HDL-cholesterol levels were measured using an enzymatic homogeneous assay. LDL and total cholesterol and triglyceride levels were measured using spectrophotometric assay kits. Serum levels of thiobarbituric acid-reactive substances (TBARS), as an index of serum lipid peroxides, were measured as described previously (25).

Measurement of serum PON1 activities

PON1 activities were assayed in a microtiter plate spectrophotometer (Bio-Tek© Instruments) by measuring the rate of hydrolysis of phenylacetate (arylesterase activity) and paraoxon (paraoxonase activity) as described previously (26).

Analysis of PON1 mRNA levels by real-time RT-PCR

Total cellular RNA was isolated from liver samples using Tripure® isolation reagent according to the manufacturer's protocol. One microgram of total RNA was reverse-transcribed to cDNA and subsequently diluted 1:10 and frozen at −70°C until PCR was carried out.

Real-time PCR was performed using SYBR Green detection technology in a Lightcycler Rapid Thermal Cycler (Roche Diagnostics) as described previously (22).

Real-time PCR efficiencies were calculated on average efficiencies from each sample, which were calculated using the following formula: $e = (F/F_o)^{1/(n - no)}$, where F and F_o indicate fluorescence values belonging to the linear segment of each PCR quantification curve and n and n_o represent their corresponding crossing points. PON1 and 18S ribosomal mRNA (used as a housekeeper) real-time PCR efficiencies when aging effects were measured were 1.85 and 1.75, respectively. When CR effects were determined, real-time PCR efficiencies were 1.82 and 1.88 for PON1 and 18S ribosomal mRNA, respectively.

Western blot analysis of serum PON1, apoA-I, apoJ, and liver SR-BI protein levels

Equal amounts of serum protein (30 μg) were fractionated on 15% SDS-PAGE gels and electrotransferred onto a nitrocellulose filter. Ponceau S staining was performed systematically to check for correct loading and electrophoretic transfer. Rabbit polyclonal antibodies against human apoA-I, PON1, and SR-BI and a goat polyclonal antibody against rat apoJ were used as primary antibodies. Anti-rabbit and anti-goat IgG-alkaline phosphatase antibodies were used as secondary antibodies. Development of immunoblots was performed using an enhanced chemiluminescence kit. Bands on films were quantified by photodensitometric analysis (Kodak 1D Image Analysis Software). The apparent molecular masses of PON1 (43 kDa), apoA-I (28 kDa), apoJ (50 kDa), and SR-BI (80 kDa) were estimated using protein molecular mass standards.

Statistical analysis

All data are expressed as mean values ± SEM of six animals per group. Statistical analysis was performed using a statistical software package (SPSS 13.0 for Windows, Inc.). Statistical differences between young and old rats were analyzed by two-way ANOVA to assess the effects of aging and gender. The effects of CR and gender were assessed in the same way but between old and old CR rats. Student's *t*-test, as a post hoc comparison, was performed when an

TABLE 1. Effects of aging, CR, and gender on body weight, serum lipid profile, and TBARS

Variable	Gender	Young	Old	Old CR	Anova I	Anova II
Body weight (g)	Male	482 ± 14	686 ± 38	442 ± 9	A	G, CR
	Female	261 ± 8	396 ± 16	243 ± 21		
Triglyceride (mmol/l)	Male	2.65 ± 0.25	3.76 ± 0.07	1.46 ± 0.10[a]	G, A	G, CR, G*CR
	Female	1.80 ± 0.41	2.21 ± 0.18[b]	1.36 ± 0.15[a]		
Total cholesterol (mmol/l)	Male	1.24 ± 0.09	1.93 ± 0.15[c]	1.18 ± 0.06[a]	G, A, G*A	G, CR, G*CR
	Female	1.13 ± 0.07	1.27 ± 0.15[b]	1.01 ± 0.09		
HDL-cholesterol (mmol/l)	Male	0.49 ± 0.09	0.43 ± 0.06	0.33 ± 0.02[a]	A, G*A	G*CR
	Female	0.70 ± 0.06[b]	0.33 ± 0.02[c]	0.45 ± 0.04[a,b]		
LDL-cholesterol (mmol/l)	Male	0.46 ± 0.03	1.38 ± 0.26	1.22 ± 0.18	A	NS
	Female	0.37 ± 0.05	1.40 ± 0.28	1.09 ± 0.14		
LDL/HDL-cholesterol ratio	Male	1.12 ± 0.21	3.24 ± 0.40	4.46 ± 0.42	A	G*CR
	Female	0.55 ± 0.10	4.19 ± 0.69	2.40 ± 0.20[a,b]		
TBARS (μmol/l)	Male	2.94 ± 0.38	3.31 ± 0.34	2.70 ± 0.16	NS	CR
	Female	3.44 ± 0.34	3.62 ± 0.39	2.84 ± 0.17		

Anova I, two-way ANOVA to assess the effects of aging and gender between young and old rats; Anova II, two-way ANOVA to assess the effects of CR and gender between old and old CR rats; CR, caloric restriction; TBARS, thiobarbituric acid-reactive substances. Values are expressed as means ± SEM of six animals per group. For ANOVA ($P < 0.05$), G indicates gender effect, A indicates aging effect, and CR indicates CR effect. G*A and G*CR represent the corresponding interactive effects.

[a] $P < 0.05$, old CR versus old, by *t*-test.

[b] $P < 0.05$, female versus male, by *t*-test.

[c] $P < 0.05$, old versus young, by *t*-test.

interactive effect of aging and gender or CR and gender was shown. $P < 0.05$ was considered statistically significant.

Statistical PCR data analysis was performed using the Relative Expression Software Tool (REST 2005 BETA V1.9.9) (27). Differences in mRNA levels between groups were analyzed by the Pair Wise Fixed Reallocation Randomization Test© (28), a proper model to avoid the normal distribution of data.

RESULTS

Effects of aging, CR, and gender on body weight, serum lipid profile, and serum lipid peroxide levels (TBARS)

In both genders, body weight increased with aging and decreased after 1 year of 40% CR, reaching that of the younger rats (**Table 1**). Moreover, aging led to a worsened lipid profile, rendering not only higher triglyceride and total cholesterol levels but also trebling the LDL/HDL-cholesterol ratio. Thus, aging made female rats lose the more advantageous serum lipid profile that they showed at early ages. Serum lipid peroxide levels showed a tendency to increase with aging, although they did not reach statistical significance.

Long-term CR strongly attenuated the effects of aging on male and female rats by reducing triglyceride and total cholesterol levels, with old CR rats reaching similar levels as young animals. HDL-cholesterol levels were improved by CR only in female rats, whereas LDL-cholesterol levels were not modified in either males or females. As a result, the LDL/HDL-cholesterol ratio was reduced only in old CR females. Long-term CR induced a 20% reduction in serum lipid peroxides in both male and female rats.

Effects of aging, CR, and gender on serum PON1 activities

Aging strongly increased serum arylesterase activity in both genders and serum paraoxonase activity only in male rats (**Fig. 1**). The age-associated increase of both activities was higher in male rats than in females, such that aging inverted the activity profile observed in younger animals, with old male rats reaching higher serum PON1 activities than old females.

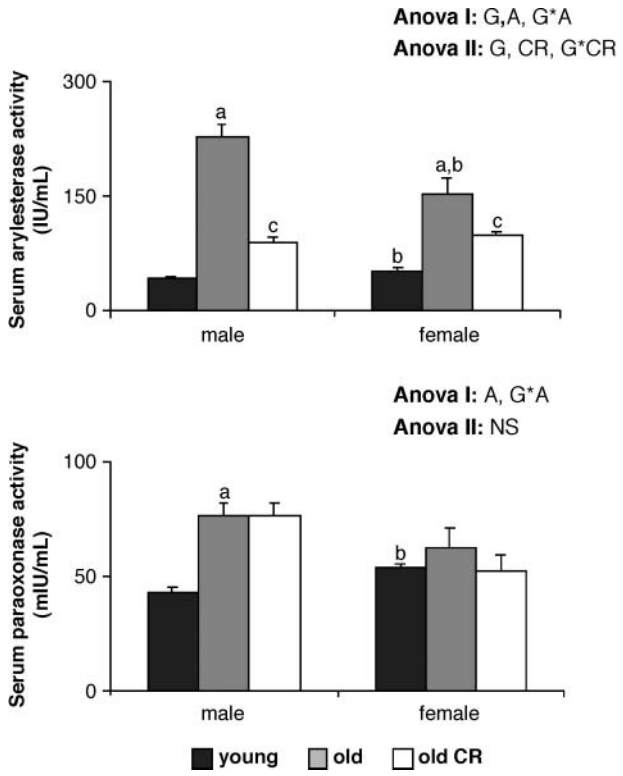

Fig. 1. Effects of aging, caloric restriction (CR), and gender on serum paraoxonase and arylesterase activities. Data represent means ± SEM of six animals. For ANOVA ($P < 0.05$): G indicates gender effect, A indicates aging effect, and CR indicates CR effect. G*A and G*CR represent the corresponding interactive effects. $P < 0.05$ by *t*-test for old versus young (a), female versus male (b), and old CR versus old (c).

TABLE 2. Effects of aging, CR, and gender on liver PON1 mRNA levels

Variable	Young	Old	Old Cr
PON1			
Male			
Expression	3.19[a]	1	2.23
REST error	1.72–5.96		1.48–3.68
Female			
Expression	1.38	2.2	1.84
REST error	0.5–3.25	1.06–4.02	1.27–2.96
18S ribosomal mRNA			
Male			
Expression	1.16	1	1.18
REST error	1.89–1.65		0.93–1.60
Female			
Expression	1.25	1.14	1.04
REST error	1.05–1.50	0.94–1.33	0.85–1.38

PON1, paraoxonase 1; REST, Relative Expression Software Tool. mRNA levels of old male rats were set as 1. 18S ribosomal mRNA expression was used as a housekeeper. Statistical differences between experimental groups were assessed by the Pair Wise Fixed Reallocation Randomization Test© ($P < 0.05$). Statistical comparisons were performed between old versus young rats and old CR versus old rats. Gender statistical comparisons were also performed in each experimental group.

[a] Statistical differences were only detected between old and young male rats.

Long-term CR decreased serum arylesterase activity in male and female rats, reaching similar levels in both genders. No effect of CR was observed on serum paraoxonase activity.

Effects of aging, CR, and gender on liver PON1 mRNA levels

Aging greatly decreased male rat liver PON1 mRNA levels (**Table 2**), whereas it did not modify those of females. No differences were detected in any of the other comparisons performed.

Effects of aging, CR, and gender on serum levels of PON1, apoA-I, and apoJ and liver content of SR-BI

Serum PON1 levels, which were lower in young male rats compared with young females, increased with aging only in the former ($P < 0.001$) (**Table 3**). Thus, and paralleling the changes observed in arylesterase activity, aging inverted the profile observed in younger animals, with old male rats reaching higher serum PON1 levels than old females. ApoA-I levels, which were significantly lower in young male rats compared with young females, decreased with aging only in the latter. ApoJ levels, which also tended to be higher in young female rats compared with young males, perfectly dovetailed with changes of arylesterase activity, increasing with aging in both genders. Long-term CR reduced PON1 levels of male rats ($P = 0.009$), whereas no changes were observed in females. Neither were apoA-I and apoJ levels modified by CR.

Liver SR-BI levels were not modified by aging, CR, or gender. The PON1/HDL-cholesterol and apoJ/HDL-cholesterol ratios, indicative of the PON1 and apoJ contents in the total HDL population, increased with aging in both genders (**Table 4**).

DISCUSSION

Aging is associated with increased PON1 activities as a consequence of a higher content of PON1 molecules in the blood, which probably corresponds to the need to respond to the negative effects of senescence. Long-term CR attenuates the aging effects and reduces serum PON1 activities, suggesting that PON1 may not be included among the main protective mechanisms triggered by CR.

The results presented in this work show an age-associated worsened serum lipid profile in both genders, leading to an increased atherogenic risk, as reflected by the higher LDL/HDL-cholesterol ratio in old animals. In addition, aging attenuated the gender differences observed between male and female rats at early ages, with old females presenting a more unfavorable state compared with their younger counterparts and having lost their advantageous profile compared with males. At early ages, these gender differences have been attributed, in humans and rats, to the effects of female sex hormones, the levels of which are reduced with aging (29, 30).

TABLE 3. Effects of aging, CR, and gender on serum levels of PON1, apoA-I, and apoJ and liver content of SR-BI

Variable	Gender	Young	Old	Old CR	Anova I	Anova II
PON1	Male	100 ± 20	294 ± 32[a]	177 ± 15[b]	A, G*A	G*CR
	Female	156 ± 5[c]	195 ± 21[c]	207 ± 20		
ApoA-I	Male	100 ± 30	132 ± 21	161 ± 23	G, A, G*A	NS
	Female	373 ± 74[c]	155 ± 26[a]	152 ± 33		
ApoJ	Male	100 ± 23	239 ± 30	253 ± 21	A	NS
	Female	154 ± 30	261 ± 22	267 ± 26		
SR-BI	Male	100 ± 13	80 ± 13	75 ± 8	NS	NS
	Female	79 ± 4	75 ± 15	93 ± 15		

ApoA-I, apolipoprotein A-I; SR-BI, scavenger receptor class B type I. Anova I and Anova II are described in Table 1. Protein levels were measured by immunoblot analysis and expressed as arbitrary units. Levels of control male rats were set as 100%. Values are expressed as means ± SEM of six animals per group. For ANOVA ($P < 0.05$), G indicates gender effect, A indicates aging effect, and CR indicates CR effect. G*A and G*CR represent the corresponding interactive effects.

[a] $P < 0.05$, old versus young, by *t*-test.

[b] $P < 0.05$, old CR versus old, by *t*-test.

[c] $P < 0.05$, female versus male, by *t*-test.

TABLE 4. Effects of aging, CR, and gender on PON1, apoA-I, and apoJ content in HDL

Variable	Gender	Young	Old	Old CR	Anova I	Anova II
PON1/HDL-cholesterol [(AU/mmol) $\times 10^{-14}$]	Male	1.65 ± 0.59	6.67 ± 1.10	5.45 ± 1.22	A	NS
	Female	1.38 ± 0.32	5.27 ± 0.86	3.93 ± 0.59		
ApoA-I/HDL-cholesterol [(AU/mmol) $\times 10^{-14}$]	Male	2.83 ± 1.08	3.63 ± 0.95	6.08 ± 0.99	NS	NS
	Female	3.08 ± 0.86	4.72 ± 0.59	3.41 ± 0.74		
ApoJ/HDL-cholesterol [(AU/mmol) $\times 10^{-14}$]	Male	4.61 ± 1.57	13.6 ± 2.90	25.2 ± 6.03	A	NS
	Female	3.89 ± 0.59	17.5 ± 1.97	11.3 ± 1.51		

AU, arbitrary units. Anova I and Anova II are described in Table 1. Values are expressed as means ± SEM of six animals per group. For ANOVA ($P < 0.05$), A indicates aging effect.

In agreement with previous studies (31), young female rats showed higher PON1 activities than young males. However, this more advantageous antioxidant protection shown by female rats seems to be lost in old females, and the gender-related differences in PON1 activities observed at early ages were inverted with aging. Moreover, serum lipid peroxide levels showed a tendency to increase with aging and correlated positively with LDL-cholesterol levels ($r = 0.533$, $P = 0.019$), in accordance with the higher susceptibility of LDL to oxidation with age, as has been reported in previous studies (4, 5). Therefore, and because the antiatherogenic properties of PON1 have been shown to come from its ability to reduce LDL oxidation (17, 18, 32), we assumed that the increased PON1 activities and levels with aging could be indicative of the activation of a response mechanism designed to respond to the adverse effects of senescence, leading to the observed attenuation of lipid oxidative damage. The strong correlations found between PON1 activities and LDL-cholesterol levels ($r = 0.865$, $P < 0.001$ and $r = 0.628$, $P = 0.002$ for arylesterase and paraoxonase activities, respectively) further support this idea.

In contrast to our results, a loss of PON1 activity with aging has been described in humans (33–35). Although the influence of genotype on human serum PON1 activity cannot be ruled out, interspecies differences could explain the different antiaging mechanisms involving PON1 that act in both rats and humans.

Although the liver is the main source of serum PON1 (16), the age-related changes observed in its enzymatic activities cannot be attributed to higher liver expression at the transcriptional level. Furthermore, the lack of differences in liver SR-BI levels suggest that aging did not affect the SR-BI-mediated PON1 release to the blood. Serum levels of apoA-I, the main protein component of HDL (36), showed a similar profile to those of HDL-cholesterol, thus reflecting the age-associated changes of the total HDL population. However, HDL represent a highly heterogeneous population (37), and PON1 specifically associates with those HDLs also containing apoJ (13). Aging strongly increased apoJ levels, perfectly dovetailing with both PON1 activities and levels. In addition, the PON1/HDL-cholesterol and apoJ/HDL-cholesterol ratios in old rats further suggest an age-associated increase in the levels of the HDL subfraction responsible for PON1 transport in the blood. This is in agreement with the previously described age-dependent increased levels of the HDL_3 subfraction (38, 39), the one with which PON1 associates and in which the greater inhibition of LDL oxidation has been detected (40, 41). Thus, both male and female old rats would respond to the age-related increased oxidative damage by means of a redistribution of HDL subfractions, leading to a higher number of PON1 molecules in the blood to bring about a more effective protection of LDL from oxidation. In addition, the lack of gender differences in the age-related response of PON1 reflects the loss of the more favorable blood profile with aging of females with respect to males.

The antiaging effects of CR are reflected in the improved serum lipid profiles of both male and female old rats subjected to long-term CR compared with ad libitum-fed rats of the same age. However, the LDL/HDL-cholesterol ratio decreased only in old CR females, indicating that CR allowed female rats a better improvement of their situation compared with males. The strong correlation found in old and old CR rats between LDL-cholesterol and lipid peroxide levels ($r = 0.641$, $P = 0.002$), together with the CR-associated decrease of TBARS levels, suggest that oxidation of these lipoproteins was probably attenuated by CR, although LDL-cholesterol levels were not restored. Thus, the lower arylesterase activity observed in old CR animals could be explained by this lower risk of LDL oxidation. It is worth noting that paraoxonase and arylesterase activities did not follow the same pattern in response to CR. Because no other serum enzyme has been described to hydrolyze paraoxon, this apparent discrepancy could be attributable to differences in the specific regulation of both activities, such that some factors related to CR could cause an increase in paraoxonase activity, thus demonstrating that, despite decreased serum PON1 levels, paraoxonase activity was not altered by CR. Nevertheless, arylesterase activity is more closely related to the levels of PON1 protein (23); thus, this activity would be more indicative of the status of the PON1 enzyme. In this sense, both serum arylesterase activity and PON1 levels decreased with CR in both genders.

Because no effect of CR was observed on liver PON1 expression and SR-BI-mediated release to the blood or on PON1 and apoJ content of HDL, the lower arylesterase activity shown by old CR rats cannot be attributed to changes of PON1 liver transcription or to HDL redistribution; therefore, other processes underlying CR must be involved.

The results presented in this work are in agreement with those obtained in a previous study performed in 8 week old rats subjected to 40% CR over 14 weeks (22), in which

we reported a CR-associated PON1 activity reduction in a less oxidative environment. In this work, aging influenced the PON1 response to CR by neutralizing the gender-dependent differences described previously in young rats (22), which were not detected in this study. Young CR females showed a greater reduction of serum PON1 activity and levels compared with their age-matched males, thus reflecting the need of females to save energy to promote their own survival and that of the species (22). In this sense, the age-related decrease of female sexual hormone levels entails, as well as the loss of the reproductive capacity of females, an equalized atherogenic risk between genders.

In summary, PON1 modulation by aging entails an HDL redistribution leading to a higher number of HDL particles responsible for PON1 transport in the blood, representing the activation of a protection mechanism to respond to the greater oxidative status of old animals. Our results also suggest that PON1 modulation is not an integral part of the main mechanisms by which CR exerts its age-related oxidative risk attenuation; rather, CR would determine a more youthful and less oxidative situation in which the protection of LDL would be less necessary.

The authors thank Drs. M. I. Mackness and B. Mackness from the University of Manchester (Department of Medicine) for kindly providing the rabbit anti-human PON1 antibody. This work was supported by the Fondo de Investigaciones Sanitarias of the Spanish Government (Grants PI042294 and PI042377). E.T-M. was funded by a grant for a research project of the Instituto de Salud Carlos III (Grant G03/140).

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