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Caloric Restrictions Affect Some Factors Involved in Age-Related Hypercholesterolemia

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Abstract Ageing has been defined as a progressive decrease in physiological capacity and a reduced ability to respond to environmental stresses. It has been observed that diet-restricted animals show a minor morbidity in age-related disease. Among these age-related diseases, hypercholesterolemia is the most recurring one and it is often associated with cardiac failure. Several studies have been published indicating age-dependent changes in circulating levels of cholesterol in both humans and in rodents; recently changes have also been reported in the proteins involved in cholesterol homeostasis, that is, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), Insig-induced gene (Insig) protein, SREBP cleavage activating protein (SCAP), sterol regulatory element binding protein (SREBP), and low density lipoprotein receptor (LDLr). Most age-related modifications of biochemical parameters are normalized or very improved in food-restricted animals, so the aim of this work is to examine whether or not alterations of the factors involved in cholesterol homeostasis which occur during ageing could be counteracted by caloric restriction (CR). The data show that the diet restrictions used attenuate the age-related effects on the factors involved in the synthesis and the degradation rate of HMG-CoAR; in spite of this, CRs have a good effect on the age-related hypercholesterolemia whose reduction seems to depend both on the correct membrane LDLr localization and on the proper restored HMG-CoAR activity. J. Cell. Biochem. 101: 235–243, 2007.

Key words: ageing; caloric restriction; cholesterol; HMG-CoA reductase; Insig; LDLr; SREBPs; rat liver

Ageing has been defined as the deteriorative changes which occur during the adult period of life underlying an increased vulnerability to challenges and a decreased ability of the organism to survive [Masoro, 2005]. Ageing is dependent on multiple factors, including genetics, hormone and growth factor signaling [Geesaman, 2006], body weight, fat content [Kloting and Bluher, 2005], and environmental features [Briones, 2006]. Ageing is further characterized by an accumulation of advanced glycation endoproduct (AGEs) [Yamagishi et al., 2006], determined by a local concentration of glucose, and by insulin resistance [Kloting and Bluher, 2005]. Another factor that is deeply involved in the ageing processes is the increased amount of reactive oxygen species (ROS) [Droge, 2003].

It has been known since 70 years that restricting the food intake of laboratory rats extends their average and maximum life span. In addition, such life extension has been observed over the years in many other species, including mice, hamsters, dogs, fish, invertebrate animals, and yeast [Masoro, 2005]. The underlying biological mechanism responsible for the life extension is still unknown, although

Abbreviations used: AGEs, advanced glycation endoproduct; AMPK, AMP-activated kinase; CR, caloric restriction; ER, endoplasmic reticulum; HMG-CoAR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; Insig, insulin induced gene; IRS, insulin receptor substrate; LDLr, low density lipoprotein receptor; PP2A, protein phosphatase 2A; ROS, reactive oxygen species; SCAP, SREBP cleavage activating protein; SREBPs, sterol regulatory element binding proteins.

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many hypotheses have been proposed. Beside life extension, diet-restricted animals, show a minor morbidity in age-related disease. Among these, hypercholesterolemia is the most recurring and it is often associated with cardiac failure. In fact, even if cholesterol plays a very important role in cell metabolism and structure, excessive amount of cholesterol in cells can destroy membrane functions or result in atherosclerotic damage of blood vessels [Small and Shipley, 1974].

Liver is the principal site of cholesterol homeostasis [Dietschy et al., 1993], carried out through many mechanisms comprehending: biosynthesis via 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR, E.C. 1.1.1.34) activity, uptake through lipoprotein receptors, release in the blood, esterification, degradation, and conversion in bile acids [Weber et al., 2004].

Transcriptional regulation of HMG-CoAR and low density lipoprotein receptor (LDLr) depends on sterol regulatory element binding proteins (SREBPs) family whose activation depends in turn on two recently described proteins, Insig 1 and 2 (insulin-induced gene 1 and 2).

SREBPs belong to the large family of basic helix-loop-helix-leucine zipper (bHLH-Zip) transcription factors. Synthesized on the membrane of the endoplasmic reticulum (ER), the SREBPs move by vesicles to the Golgi complex, where they are processed sequentially by two proteases [Brown and Goldstein, 1999; Goldstein et al., 2002]. These cleavages release a cytosolic transcription factor that enters the nucleus and activates genes which produce many proteins, including HMG-CoAR and LDLr [Horton et al., 2002]. Three members of SREBP family, SREBP-1a, -1c, and -2, have been identified [Hua et al., 1993; Yokoyama et al., 1993]. The activity of SREBPs in liver is controlled at two levels: transcriptional and post-transcriptional.

Post-transcriptionally, SREBP is regulated mainly by sterols which inhibit the proteolytic processing of the membrane-bound SREBP precursors [Brown and Goldstein, 1999]. This control is mediated by two proteins: SREBP cleavage activating protein (SCAP) and Insig [Janowski, 2002; Yabe et al., 2002; Yang et al., 2002]. SCAP, a protein of ER, forms tight complexes with newly synthesized SREBPs, and is responsible for the transport of SREBPs to the Golgi apparatus.

Insig-1 (whose transcription is insulin-dependent) and Insig-2 (that is constitutively expressed) regulate lipid synthesis by binding in a sterol-dependent fashion to SCAP and to HMG-CoAR, controlling in such ways HMG-CoAR transcription and degradation. Sterol-stimulated binding of Insigs to SCAP leads to ER retention of the SCAP/SREBP complex, preventing the proteolytic generation of the transcriptionally active nSREBPs [Horton et al., 2002]; on the other hand, sterol-stimulated binding of Insigs to HMG-CoAR leads to its ubiquitination and proteasomal degradation [Sever et al., 2003a,b].

Many experimental data reported age-dependent changes in circulating levels of cholesterol both in humans [Ceda et al., 1998; Brizzi et al., 2003] and other animal models [Marino et al., 2002; Pallottini et al., 2006]. Our previous results showed that in old (24 months) "ad libitum" (AL) fed rats, the hepatic HMG-CoAR is fully activated, its "in vitro" degradation rate is lowered and its protein content unchanged; moreover, cholesterol synthesis is increased, cholesterol content is not changed in the liver but higher in the blood [Marino et al., 1998, 2002; Pallottini et al., 2003, 2004]. Later, a decrease of Insigs levels, an increase of nSREBP-2 but a decreased HMG-CoAR mRNA, has also been observed [Pallottini et al., 2006] and these data could explain the unchanged enzyme protein levels. In fact, the observable, reduced HMG-CoAR degradation rate is balanced by the decreased mRNA level. Moreover, a diminution of LDLr on liver cell membrane has been observed, which, together with the increased HMG-CoAR activation state, represents a good explanation of the hypercholesterolemia detectable during ageing [Pallottini et al., 2006].

Caloric restriction (CR) is the most potent environmental variable that has been shown to increase longevity and to prevent many age-related disease in various organisms [Masoro, 2000]. Most age-related modifications of biochemical parameters result normalized or less changed in food-restricted animals (i.e., AGEs, cholesterolemia, glicemia) [Cefalu et al., 2000; Marino et al., 2002; Teillet et al., 2002].

Our previous data reported that anti-aging CR protects against isoprenoid changes in blood and liver [Marino et al., 1998, 2002], this being consistent with the physiological HMG-CoAR activation state. On the contrary, CR does not

prevent the lowering of the observable, HMG-CoAR degradation rate in aged AL fed rats [Pallottini et al., 2004].

In this study, we examine whether or not alterations of factors involved in cholesterol homeostasis (Insig, SCAP, SREBPs, LDLr) which occur during ageing could be counteracted by CR. We, indeed, analyze these factors in the liver of 24-month-old male rats maintained at two different CR, intermittent feeding (IF) and 40% reduced feeding (DR), using as controls 3-month and 24-month-aged AL fed rats.

MATERIALS AND METHODS

Materials

All materials used were obtained from commercial sources and were of the highest quality available. All materials with no specified source are obtained from Sigma-Aldrich (Milan, Italy).

Animals

Male Sprague Dawley rats were used. Animals were randomly divided into four different experimental groups by the age of 3 months. One group was used as the young-adult control. The three left groups were maintained till age 24 months and: (1) fed daily AL with a standard laboratory diet (Harlan autoclavable Teklad diet, Harlan-Italy, S. Pietro in Natisone, Italy, containing: 12% water, 18.4% crude protein, 5.5% crude fiber, 5.6% crude ash), (2) fed AL with the standard diet every other day (IF), and (3) fed with a 40% reduced calorie intake (DR). Animals had free access to water. Food was withdrawn 16 h before experimentation. Rats on CR diet were sacrificed on the day of fasting.

The animals were anesthetized with intraperitoneal injection of pentobarbital (50 mg/kg body weight); 5 ml blood were taken from the inferior vena cava and liver was quickly excised and immediately frozen into liquid nitrogen and stored at −80°C. The Official Italian Regulation N. 116/92 for the care and use of laboratory animals was followed.

Western Blot Analysis

Insig-1, Insig-2, SREBP-1 (C-terminus), and SREBP-2 (C-terminus) analysis. The Western blot analysis of Insig-1, Insig-2, SREBP-1 (C-terminus), SREBP-2 (C-terminus) were performed on microsomes prepared as described by Bruscalupi et al. [1985], then solubilized in 0.125 M Tris-HCl (pH 6.8) containing 10% SDS, 1 mM phenylmethylsulphonyl fluoride (Sample Buffer), and boiled for 2 min. Protein concentration was determined by the method of Lowry et al. [1951]. Twenty micrograms of protein from solubilized microsomes were resolved by 12% (for Insigs) and 7% (for SREBPs) SDS–PAGE at 100 V for 60 min. The proteins were subsequently transferred electrophoretically onto nitrocellulose for 80 min at 100 V. The nitrocellulose was treated with 3% bovine serum albumin in 138 mM NaCl, 27 mM KCl, 25 mM Tris-HCl, 0.05% Tween 20 (pH 6.8) (Block Buffer), and probed at 4°C overnight with Insig-1 and -2 antibody (Southwestern Medical Center, University of Texas, Dallas, TX, USA), SREBP-1 and -2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

For protein detection, secondary anti-rabbit antibodies for SREBP-1, anti-mouse for Insig-1 and -2, anti-goat for SREBP-2 (Santa Cruz Biotechnology) were used. Bound antibodies were visualized using enhanced chemoluminescence detection (ECL, Amersham-Pharmacia, Little Chalfont, UK).

Insulin receptor substrate analysis. Western blot analysis of insulin receptor substrate (IRS) was performed on total liver lysate obtained as follows: 100 mg of liver tissue were solubilized by sonication in Sample Buffer in presence of 50 mM NaF to avoid dephosphorylation, and boiled for 2 min. Protein concentration was determined using the method by Lowry et al. [1951]. Twenty micrograms of proteins from solubilized tissue were resolved by 7% SDS–PAGE at 100 V for 60 min, and subsequently transferred electrophoretically as described above by probing at 4°C overnight with anti-P-IRS1/2 (Santa Cruz Biotechnology) and then stripped and probed with anti-actin (Santa Cruz Biotechnology). For protein detection secondary anti-rabbit antibodies for IRS, and secondary anti-mouse for actin, were used. Bound antibodies were visualized as described above.

Nuclear SREBP-1 and nuclear SREBP-2 analysis. The Western blot analyses of nSREBP-1, nSREBP-2 were performed on nuclei prepared as follows: 500 mg of liver were homogenized in sucrose 0.25 M (10% w/v), and then centrifuged 10 min at 1,000*g*. The pellet was washed three times at the same speed in sucrose solution to obtain nuclear fraction. Nuclei were solubilized in Sample Buffer, and

boiled for 2 min. Twenty micrograms of protein from solubilized nuclei were resolved by 10% SDS–PAGE at 100 V for 60 min. The proteins were subsequently transferred electrophoretically as above described. The nitrocellulose was treated with Block Buffer, probed at 4°C overnight with SREBP-1 (N-terminus) and -2 (N-terminus) (Santa Cruz Biotechnology).

For protein detection, secondary anti-rabbit antibodies for SREBP-1, anti-mouse for SREBP-2 were used. Bound antibodies were visualized as described above.

LDLr and Caveolin-1 analysis. The Western blot analysis of LDLr was performed on total membrane obtained as follows: 500 mg liver tissue were homogenized in 0.01 M Tris-HCl, 0.001 M $CaCl_2$, 0.15 M NaCl, 0.001 M PMSF pH 7.5. Homogenate was centrifuged for 10 min at 500*g*, the supernatant centrifuged again 10 min at 500*g*. The supernatant was centrifuged at 100,000*g* for 30 min and the pellet was resuspended and centrifuged again at 100,000*g* for 20 min. The pellet was resuspended, aliquots were transferred in 1.5 ml Eppendorf tubes and centrifuged for 3 min at 14,000 rpm. The pellets were resuspended in Sample Buffer for the protein determination by Lowry et al. [1951] method and for 7% SDS–PAGE. The proteins separation and blot were performed as described above. LDLr and Caveolin-1 detection were made probing the nitrocellulose with polyclonal antibodies (Santa Cruz Biotechnology). For LDLr detection we used secondary anti-goat antibodies (Santa Cruz Biotechnology), and for Caveolin-1 detection anti-rabbit (Santa Cruz Biotechnology). Bound antibodies were visualized as described above.

Plasma Cholesterol Content Assay

Blood was collected on EDTA (0.4 mg/ml blood). Plasma was separated by centrifugation. The analysis of total and lipoprotein-linked plasma cholesterol (Chol) was performed using the diagnostics kits for Total-Chol, High Density Lipoprotein-Chol (HDL-Chol), and LDL-Chol (Sigma Chemical Co.).

Normalization of Protein Measured

The normalization of the analyzed protein content has been performed only where it was possible to use anti-actin antibody, (i.e., in the total lysate).

Statistical Analysis

All the statistical analysis was performed using Student's *t*-test by GraphPad Instat3.

RESULTS

As it is well known that CR prevents most of the common features of ageing, in the present work we have studied, the effect of two different CR on some proteins involved in cholesterol homeostasis in aged animal liver.

First the levels of Insigs (Fig. 1), two proteins deeply involved both in synthesis and in degradation of the HMG-CoAR, the key enzyme of cholesterol biosynthetic pathway were analyzed. Panel A shows that the age-dependent decrease of Insig-1 is only partially prevented by CR; on the contrary Insig-2 level remains

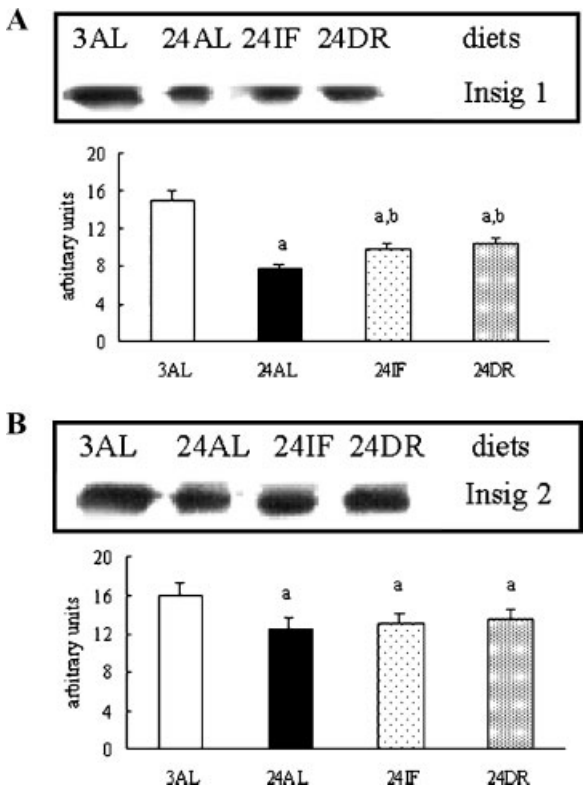

Fig. 1. Insig-1 and Insig-2 protein levels in 3- and 24-month-old AL or IF or DR fed rat liver. Western blots showing Insig-1 (**Panel A**) and Insig-2 (**Panel B**) levels 3-month and 24-month-old AL or IF or DR rat liver microsomes. Proteins from 20 μg solubilized liver microsomes were resolved by SDS–PAGE, and subsequently transferred electrophoretically onto nitrocellulose and probed with anti-Insig-1 and -Insig-2 polyclonal antibodies. For details see the text. Panels show a typical Western blotting and the densitometric analysis obtained from four different experiments. a: $P < 0.001$ as from a Student's *t*-test with respect to 3-month-old AL. b: $P < 0.001$ as from a Student's *t*-test with respect to 24-month-old AL.

unchanged with respect to the aged AL fed animal (Panel B).

Insig-1 is an insulin-induced protein, so in order to study the mechanism underlying the modification that occurs in the CR animals, the sensitivity to this hormone has been checked by measuring the IRS activation. The results show that CR does not completely prevent the declined activation provoked by ageing (Fig. 2).

Since SREBPs are the transcription factors able to transactivate HMG-CoAR gene, LDLr gene and other genes involved in cholesterol homeostasis and are also regulated by Insigs, in AL and CR old rats, SREBPs levels were analyzed. In Figure 3 the reported levels of SREBP-1 show that the age-related decrease of its microsomal content have been partially restored in CR animals (Panel A), but the nuclear active portion have remained unchanged (Panel B). As regards SREBP-2 the picture is different (Fig. 4); in fact the microsomal level has not changed with respect to AL-aged animals (Panel A) while the age-related increase of its nuclear fraction has only been partially prevented by CR (Panel B).

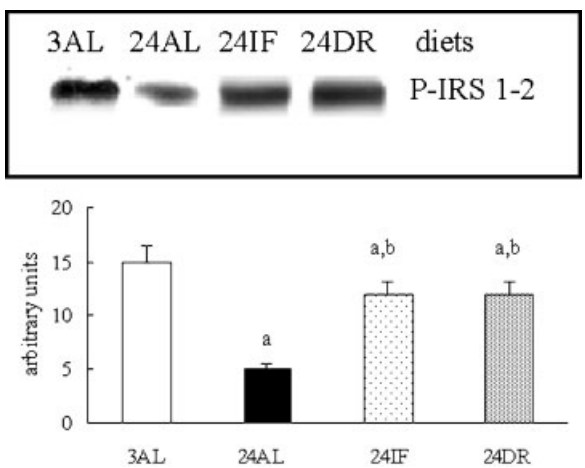

Fig. 2. P-IRS 1–2 in 3- and 24-month-old AL or IF or DR fed rat liver. The figure represents on the top a typical Western blotting, on the bottom the ratio between the densitometric analysis of the P-IRS 1–2 levels and actin obtained from four different experiments. The experiments were performed on 3-month and 24-month-AL or IF or DR fed rat liver total lysates. Proteins from 20 μg total liver lysate were resolved by SDS–PAGE, subsequently transferred electrophoretically onto nitrocellulose and then probed with anti-P-IRS 1–2 antibody then stripped and probed with anti-actin antibody. For details see the text. a: $P < 0.001$ as from a Student's *t*-test with respect to 3-month-old AL. b: $P < 0.001$ as from a Student's *t*-test with respect to 24-month-old AL.

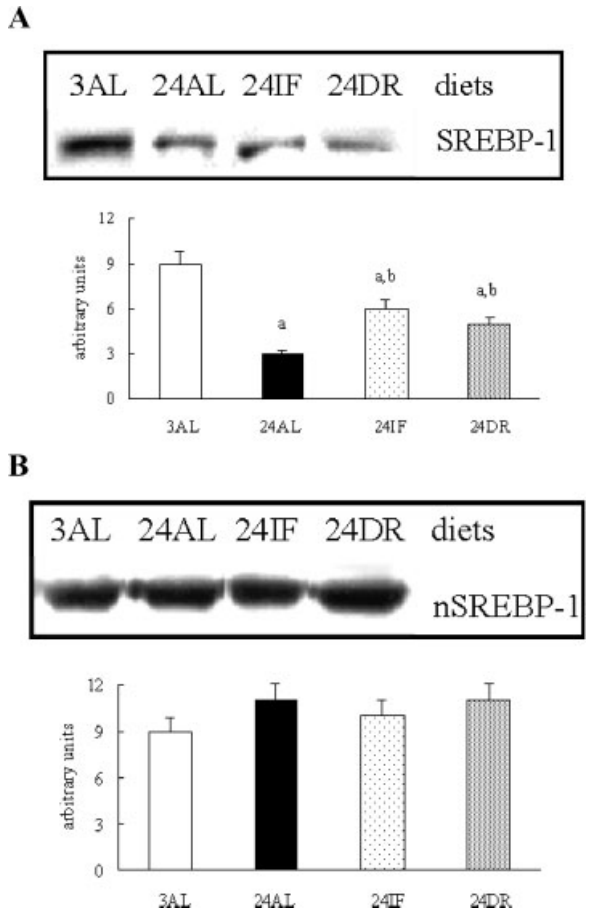

Fig. 3. SREBP-1 protein levels in 3- and 24-month-old AL or IF or DR fed rat liver. **Panel A** illustrates Western blots showing microsomal SREBP-1 with the densitometric analysis obtained from four different experiments. **Panel B** illustrates Western blots showing nuclear SREBP-1 with the densitometric analysis obtained from four different experiments. The experiments were performed in 3- and 24-month-old AL or IF or DR fed rat liver. Microsomes and nuclei were obtained as described in the Materials and Methods section. Proteins from 20 μg microsomes or solubilized nuclei were resolved by SDS–PAGE and subsequently transferred electrophoretically onto nitrocellulose then probed with primary antibodies as described in the text. a: $P < 0.001$ as from a Student's *t*-test with respect to 3-month-old AL. b: $P < 0.001$ as from a Student's *t*-test with respect to 24-month-old AL.

The results obtained on Insig levels, could explain the lowered HMG-CoAR degradation rate occurring both in AL and in CR old rats. On the other hand, the measure of SREBP-2 levels does not help to clarify the unchanged levels of the enzyme observed in AL or CR aged rats. For this reason, the level of another SREBP-2-dependent protein, the LDLr, deeply involved in cholesterol homeostasis was assayed. Figure 5 illustrates the total LDLr level (Panel A) and its

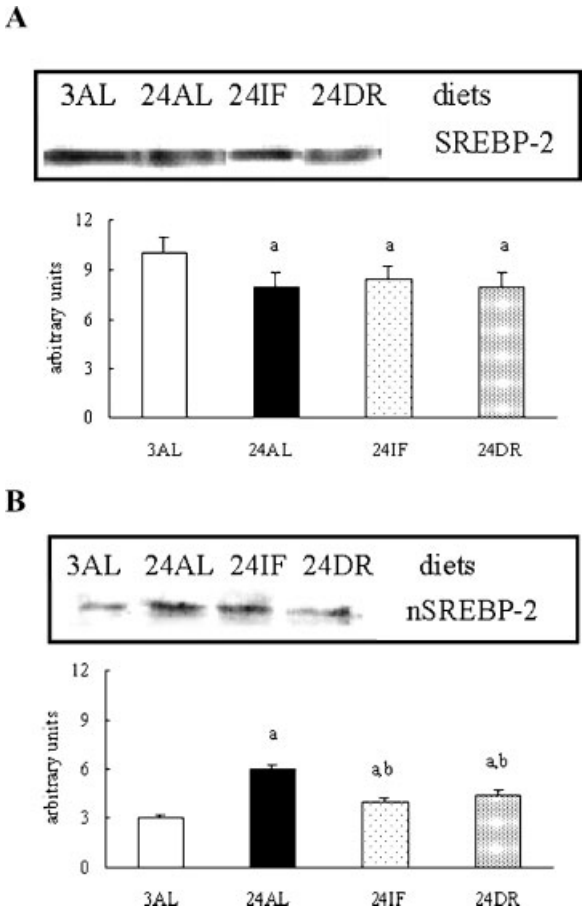

Fig. 4. SREBP-2 protein levels in 3- and 24-month-old AL or IF or DR fed rat liver. **Panel A** illustrates Western blots showing microsomal SREBP-2 with the densitometric analysis obtained from four different experiments. **Panel B** illustrates Western blots showing nuclear SREBP-2 with the densitometric analysis obtained from four different experiments. The experiments were performed in 3- and 24-month-old AL or IF or DR fed rat liver. Microsomes and nuclei were obtained as described in the Materials and Methods section. Proteins from 20 μg microsomes or solubilized nuclei were resolved by SDS–PAGE and subsequently transferred electrophoretically onto nitrocellulose then probed with primary antibodies as described in the text. a: $P < 0.001$ as from a Student's *t*-test with respect to 3-month-old AL. b: $P < 0.001$ as from a Student's *t*-test with respect to 24-month-old AL.

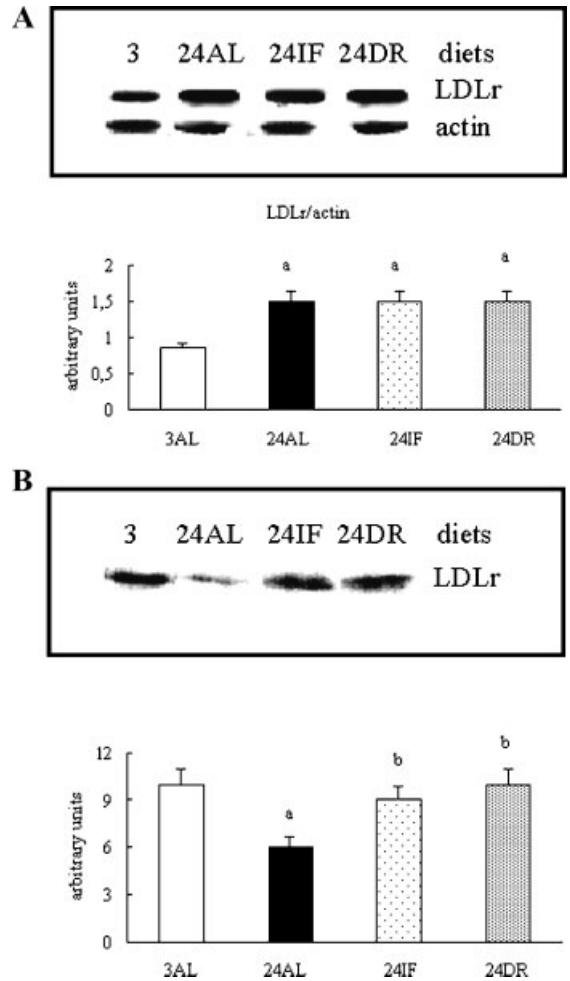

Fig. 5. LDLr protein levels in 3- and 24-month-old AL or IF or DR fed rat liver. **Panel A** shows the LDLr protein levels in total lysate obtained as described in the Materials and Methods section. **Panel B** shows the LDLr protein levels on membrane obtained as described in the Materials and Methods section. Proteins from 20 μg membranes or total lysate were resolved by SDS–PAGE, subsequently transferred electrophoretically onto nitrocellulose and then probed with anti-LDLr antibody. The analysis was performed on four different experiments. a: $P < 0.001$ as from a Student's *t*-test with respect to 3-month-old AL. b: $P < 0.001$ as from a Student's *t*-test with respect to 24-month-old AL.

membrane exposition (Panel B). The data clearly show that the total protein content, in accordance with the nuclear SREBP-2 content has not changed, while the CR totally prevent the large decrease of membrane LDLr observed in AL aged animals. The proper LDLr presence on the hepatic membrane of CR animal could be connected to the already reported improvement of hypercholesterolemia [Marino et al., 2002]. The data shown in Table I confirm that the total, or lipoprotein-linked cholesterol, is reduced into plasma of CR animals, and the HDL/LDL ratio is improved, changing from 1.9 in AL young rats and 2.02 in AL old rats, to 3.6 and 3.9 in IF and DR maintained animals.

Since the decreased membrane expression of LDLr occurring during ageing has been related to the parallel decrement of another SREBPs-dependent protein, the Caveolin-1, we also measured the level of this protein in the hepatic

TABLE I. Plasma Cholesterol Level

	3 AL	24 AL	24 IF	24 DR
Total-Chol (mg/dl plasma)	100 ± 12	415 ± 42^{a}	$210 \pm 20^{a,b}$	$340 \pm 25^{a,b}$
HDL-Chol (mg/dl plasma)	45 ± 9	152 ± 19^{a}	$90 \pm 8^{a,b}$	$110 \pm 10^{a,b}$
LDL-Chol (mg/dl plasma)	23 ± 8	75 ± 12^{a}	25 ± 5^{b}	28 ± 9^{b}

Plasma cholesterol content in young, old, and old diet-restricted rats.
Total Chol, HDL-Chol, and LDL-Chol in plasma of 3- and 24-month-aged AL or IF or DR fed rats. The data represent the mean±SD of at least four different experiments.
[a]$P < 0.001$ as from a Student's *t*-test with respect to 3-month-old AL.
[b]$P < 0.001$ as from a Student's *t*-test with respect to 24-month-old AL.

membranes of CR animals. Figure 6 shows that CR completely prevent the age-related decrease of Caveolin-1.

DISCUSSION

It has been already reported that, in aged rats, the hepatic HMG-CoAR, the rate-limiting enzyme of cholesterol biosynthesis, is fully active and its degradation rate is slow with unexpected unchanged levels [Marino et al., 1998, 2002; Pallottini et al., 2003, 2004]. Moreover, some of the regulating factors of liver cholesterol synthesis and uptake change; in fact during ageing Insig proteins decrease, nuclear SREBPs rise, and LDLr expression on membranes decreases along with Caveolin-1. SREBP-dependent transcription seems to be committed to different target genes; in fact, an increase of LDLr mRNA and a decrease of HMG-CoAR mRNA has been observed [Pallottini et al., 2006]. All these changes could contribute to age-related hypercholesterolemia together with the reported increased cholesterol intestinal absorption [Wang, 2002].

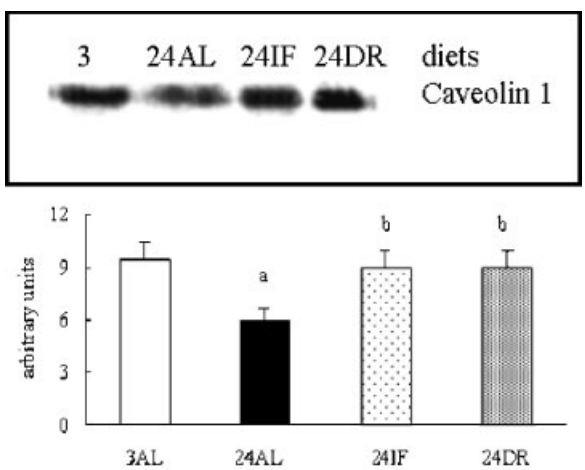

Fig. 6. Caveolin-1 protein level in 3- and 24-month-old AL or IF or DR fed rat liver The figure represents on the top a typical Western blotting, on the bottom the densitometric analysis of the Caveolin-1 levels obtained from four different experiments. The experiments were performed in 3- and 24-month-old AL or IF or DR fed rat liver membranes. Proteins from 20 μg membranes were resolved by SDS–PAGE, subsequently transferred electrophoretically onto nitrocellulose and then probed with anti-Caveolin-1 antibody. For details see the text. a: $P < 0.001$ as from a Student's *t*-test with respect to 3-month-old AL. b: $P < 0.001$ as from a Student's *t*-test with respect to 24-month-old AL.

Multiple evidence accumulating during the past years and a large body of literature have shown the beneficial effects of CR on many typical features of aged animals [Masoro, 2005] but until now this is the only intervention shown to extend lifespan and to delay age-related declines in mammalian functions.

In this study, we have tried to understand the CR mechanism underlying the improving of age-dependent rat hypercholesterolemia and to see if this process involves the protein complex regulating cholesterol synthesis and uptake [Pallottini et al., 2006].

It is well known that CR prevents the reduced sensibility to insulin that occurs during ageing [Fulop et al., 2003; Moller et al., 2003; Masoro, 2005]; in fact, also in the animal models used in this work, IRS activation is less reduced in CR rats. This increase is reflected on the levels of Insig-1 whose moderate enhancement, however, is insufficient to restore either the correct HMG-CoAR degradation rate or the correct balance between immature and mature SREBPs content.

The increased amount of nuclear SREBPs and in particular of SREBP-2, both in aged AL and CR rats, determines an increased total LDLr level. On the contrary its membrane expression follows the same pattern as Caveolin-1, which is another product of a SREBP-dependent gene transcription.

So, the CR attenuates the age-related effects on the factors involved in the synthesis and the degradation rate of HMG-CoAR; moreover this, CR has good effect on the age-related hypercholesterolemia whose reduction seems to depend

both on the correct membrane LDLr localization and on the proper restored HMG-CoAR activity.

These results provide further evidence that age-related hypercholesterolemia is caused by changes in the regulation of intracellular cholesterol metabolism. Furthermore, these modifications could be partially reversed by CR, a condition known to delay the ageing process. Age-related changes in cholesterol metabolism may play a role in triggering and/or aggravating senescence-related disorders characterized by altered cholesterol homeostasis. Thus, the knowledge of the molecular mechanism underlying CR effects could contribute to improve some of the declined physiological processes occurring during ageing.

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