

L. AGUIRRE^{1,2}, E. HIJONA^{3,4}, M.T. MACARULLA^{1,2}, A. GRACIA^{1,2}, I. LARRECHI¹,
L. BUJANDA^{3,4}, L. HIJONA^{3,4}, M.P. PORTILLO^{1,2}

SEVERAL STATINS INCREASE BODY AND LIVER FAT ACCUMULATION IN A MODEL OF METABOLIC SYNDROME

¹Nutrition and Obesity Group, Department of Nutrition and Food Sciences, University of Pais Vasco (UPV/EHU), Vitoria, Spain;

²CIBERObn Physiopathology of Obesity and Nutrition, Institute of Health Carlos III (ISCIII), Madrid, Spain;

^{3,4}Department of Gastroenterology, University of Pais Vasco, Biodonostia Institute, Donostia Hospital,
Centro de Investigacion Biomedica en Enfermedades Hepaticas (CIBERehd), San Sebastian, Spain

Statins are a family of drugs used in hypercholesterolemia. The aim of this study was to analyze the effect of statins on body and liver fat accumulation in obese Zucker rats. Seventy Zucker (*falfa*) rats were divided into seven groups. Rats from six statin groups were treated with pravastatin, simvastatin, atorvastatin, rosuvastatin, fluvastatin and lovastatin respectively, at a dose of 0.6 mg/kg body weight/day. After 6 weeks, liver and white adipose tissue from intra-abdominal and subcutaneous locations were dissected and weighed. Subcutaneous adipose tissue from rosuvastatin, atorvastatin, fluvastatin and lovastatin treated rats was significantly increased. Fatty acid synthase (FAS) activity was increased by the administration of fluvastatin and lovastatin, as was glucose-6-P dehydrogenase (G6PDH) by the administration of atorvastatin and lovastatin. No changes were observed in malic enzyme (ME) activity. Furthermore, heparin-releasable lipoprotein lipase (HR-LPL) was increased in all groups where the subcutaneous depot was increased, and total LPL increased only in rosuvastatin and fluvastatin-treated groups. With regard to liver, there were no changes in weight but the amount of triacylglycerols was increased in rosuvastatin group, as well as its liver damage was higher. In this group FAS and G6PDH activities were increased and no changes were observed in ME, acyl CoA oxidase (ACO) and carnitine palmitoyltransferase-1a (CPT-1a) activities. All statins, with the exception of simvastatin, worsen insulin resistance. These results show that statins have different effects on body fat accumulation. Moreover, rosuvastatin also shows a prosteatotic effect. These results should be taken into account for statin choice in prescription.

Key words : *statins, body fat, liver fat, lipogenesis, fatty acid oxidation, metabolic syndrome, Zucker rat, lipoprotein lipase, adipose tissue*

INTRODUCTION

Cardiovascular diseases are ranked as the number one cause of mortality and are a major cause of morbidity worldwide. Reducing blood cholesterol, which is a risk factor for cardiovascular disease events, is an important goal of medical treatment. In this context, statins are the first-choice agents (1). These drugs and their related combination products have generated \$14.3 billion in expenses in the U.S.A. according to IMS Health (Consumer Reports, 2012).

Statins are a group of drugs which show a potent hypocholesterolaemic effect by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA). This inhibition reduces mevalonate production and decreases the level of liver intracellular cholesterol, thereby stimulating low-density lipoprotein (LDL) receptor activity, and increasing the uptake of non-high-density lipoprotein (non-HDL) particles from the systemic circulation (2). Treatment with statins produces a maximum LDL reduction of about 60% with parallel reductions in triglycerides and a modest rise in HDL through independent mechanisms (3).

When this experimental work was carried out there were six different statins on the market (pravastatin, simvastatin, rosuvastatin, atorvastatin, fluvastatin and lovastatin). These statins present different pharmacokinetics but have the same pharmacodynamic effect by inhibiting HMG-CoA, as explained above. The first statin on the market was lovastatin (1979), produced by *Aspergillus terreus* mold. Pravastatin is produced by the *Norcardia autotrophica* bacterium and the rest are synthetic drugs, fluvastatin being the first statin produced synthetically. There are also differences in their metabolism. Thus, simvastatin, atorvastatin and lovastatin are metabolized by CYP450, rosuvastatin and fluvastatin by CYP2C9 and simvastatin by CYP3A4.

Many side effects associated with the administration of statins have been studied. The most important adverse effects are related to hepatotoxicity (4), such as asymptomatic elevation of serum aminotransferases (alanine aminotransferase, ALT and aspartate aminotransferase, AST (5)), and miotoxicity (6). By contrast, the potential negative effects of statins of body weight, body and liver fat accumulation have been little studied to date.

In this context, the aim of the present study was to analyze the effects of the six available statins on body and liver fat accumulation, as well as to shed light onto the mechanisms underlying these effects. For this purpose we chose Zucker (*fal/fa*) rats, an experimental model which shows metabolic features very commonly found in humans treated with statins, such as hypercholesterolemia and excess body fat accumulation.

MATERIAL AND METHODS

Animals, diets and experimental design

The experiment was conducted with seventy 5-week-old male Zucker (*fal/fa*) rats, purchased from Charles Rives Laboratories (Barcelona, Spain), and took place in accordance with the institution's guide for the care and use of laboratory animals. The rats were individually housed in polycarbonate metabolic cages (Techniplast Gazzada, Guguggiate, Italy) and placed in an air-conditioned room (22±2°C) with a 12 h light-dark cycle. After a 6 day adaptation period, rats were randomly distributed in 7 experimental groups of ten animals each: control, pravastatin (Cinfa, Navarra, Spain), simvastatin (Cinfa, Navarra, Spain), atorvastatin (Cinfa, Navarra, Spain), rosuvastatin (Actavis, Zug, Switzerland), fluvastatin (Cinfa, Navarra, Spain) and lovastatin (Cinfa, Navarra, Spain) respectively, and fed on a standard laboratory diet (Panlab, Barcelona, Spain). Rats in the statin-treated groups were orally administered 0.6 mg body weight kg⁻¹ day⁻¹ of each statin through an orogastric catheter for 6 weeks. Statins were diluted in 1 mL of saline solution. Rats from control group received only the vehicle. All animals had free access to food and water. Food intake and body weight were measured daily.

At the end of the experimental period, the rats were sacrificed between 8 and 12 am, after a fasting overnight period, under isoflurane anaesthesia. Liver and white adipose tissue from different anatomical locations (perirenal, epididymal, and subcutaneous) were dissected, weighed, immediately frozen and stored at -80°C until analysis.

Serum parameters

Serum was obtained from blood samples after centrifugation (1000 g for 10 min at 4°C). Commercial kits were used to measure serum parameters: glucose (BioSystems, Barcelona, Spain), insulin (Millipore, Billerica, MA, USA), free fatty acids (FFA) (Roche Diagnostics GmbH, Mannheim, Germany), triacylglycerols and total cholesterol (BioSystems, Barcelona, Spain).

The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated from insulin and glucose values using Matthews' formula (7).

$$\text{HOMA-IR} = [\text{fasting glucose (mmol/L)} \times \text{fasting insulin (mU/L)}] / 22.5$$

Liver composition

Total lipids were extracted from liver following the method described by Folch *et al.* (8) and the lipid extract was dissolved in isopropanol. Triacylglycerol and cholesterol contents in this extract were measured by using commercial kits (Spinreact, Barcelona, Spain). Protein content was determined by Lowry method (9), and water content was measured gravimetrically by drying samples at 105°C until constant weight (10).

Histological analysis

A piece of liver was placed just after sacrifice in 10% buffered formalin and subsequently embedded in paraffin. Liver

sections were stained with hematoxylin and eosin using standard techniques. Sections were viewed without knowing the treatment group to which each animal belonged. Biopsies were classified into four grades depending on fat accumulation using the Brunt classification (11), assigning grade 0 when no fat was found in the liver, grade 1 when fat vacuoles were seen in less than 33% of hepatocytes, grade 2 when 33–66% of hepatocytes were affected by fat vacuoles, and grade 3 when fat vacuoles were found in more than 66% of hepatocytes. Two experienced pathologists who were masked to the experiment evaluated all samples, and reached an agreement.

Enzyme activities

For lipogenic enzyme analysis, samples of liver (0.5 g) were homogenized in 5 mL of buffer and samples of subcutaneous adipose tissue (1 g) were homogenized in 2.5 mL of the same buffer (pH 7.6) containing 150 mM KCl, 1 mM MgCl₂, 10 mM *N*-acetyl-cysteine and 0.5 mM dithiothreitol. After centrifugation at 100,000 g for 40 min at 4°C, the supernatant fraction was used for quantification of enzyme activities. Fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) activities were measured spectrophotometrically as previously described (12). Enzyme activities were expressed either as nmol NADPH consumed (FAS) and nmol NADPH produced (G6PDH and ME), per minute, per mg of protein. Protein concentration was determined by the Bradford method (13).

For total lipoprotein lipase (LPL) activity determination in adipose tissue, homogenates of subcutaneous adipose depot (300 mg) were prepared in 750 µL of 10 mM HEPES buffer (pH 7.5) containing 1 mM DTT, 1 mM EDTA, 250 mM sucrose and 2 g heparin L⁻¹. For heparin-releasable LPL (HR-LPL) activity determination, 250 mg of subcutaneous adipose tissue were incubated in 750 µL of 10 mM HEPES buffer (described above) at 37°C during 30 min. Enzyme activities were assessed following the method described by Del Prado *et al.* (14), with modifications (15). Total LPL and HR-LPL activities were calculated by subtracting non-LPL lipolytic activity in the presence of NaCl from the total lipolytic activity, determined without NaCl. Both total and HR-LPL activities were expressed as nmol oleate released per minute per gram of tissue.

Acyl CoA oxidase (ACO) and carnitine palmitoyltransferase-1a (CPT-1a) activities were assessed in the liver mitochondrial/peroxisomal fraction. Liver samples (500 mg) were homogenized in 3 vol (wt/vol) of buffer pH 7.4 containing 0.25 mol sucrose L⁻¹, 1 mmol/L EDTA and 10 mmol TrisHCl L⁻¹. Homogenates were centrifuged (700 g for 10 min at 4°C) and supernatant fluid was again centrifuged (12,000 g for 15 min at 4°C). Pellets were resuspended in 70 mmol sucrose L⁻¹, 220 mmol mannitol L⁻¹, 1 mmol EDTA L⁻¹, 2 mmol HEPES buffer L⁻¹, pH 7.4. CPT-1a and ACO activities were assayed as previously reported (16). The pellet protein content was determined according to the Bradford method (13). Both activities were expressed as nmol CoA formed min⁻¹ protein mg⁻¹.

Western blot analysis of subcutaneous and liver transcription factor sterol regulatory-element-binding protein-1c (SREBP-1c)

Subcutaneous adipose tissue and liver nuclear proteins were extracted by using a commercial kit (Nuclear Extraction Kit, Cayman Chemical Company, Ann Arbor, MI). Immunoblot analyses were performed using 40 µg of subcutaneous nuclear extracts and 60 µg of liver nuclear extracts, separated by electrophoresis in a 10% SDS-polyacrylamide gel and transferred to PVDF membranes. The membranes were then blocked with 5% casein PBS-Tween

buffer for 2 hours at room temperature. Subsequently, they were blotted with the appropriate antibodies overnight at 4°C. SREBP-1c levels were detected *via* specific antibodies for SREBP-1c (1:1000) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Afterwards, polyclonal mouse anti- β -actin (1:5000) was incubated for 2 hours at room temperature. β -actin (1:5000) (Sigma, St. Louis, MO, USA) was determined by stripping and reprobing the blot. The bound antibodies were visualized by an ECL system (Thermo Fisher Scientific Inc., Rockford, IL, USA) and quantified by a ChemiDoc MP imaging system (Biorad, Hercules, Ca, USA). The measurements were normalized by β -actin.

Statistical analysis

Results are presented as means \pm standard error of the means. Statistical analysis was performed using SPSS 17.0 (SPSS Inc. Chicago, IL, USA). Normal distribution of data was confirmed by Shapiro-Wilks test. Comparisons between the control group and each statin-treated group were carried out by using Student's *t* test and chi-square test for the histological analysis. Statistical significance was set-up at the $P < 0.05$ level.

RESULTS

White adipose tissue weights and food intake

Rats treated with rosuvastatin, atorvastatin, fluvastatin and lovastatin showed significantly higher subcutaneous adipose

tissue sizes ($P < 0.05$) (Fig. 1A). The percentage of increase in this tissue was 19% for rosuvastatin, atorvastatin and fluvastatin, and 20% for lovastatin. By contrast, intra-abdominal adipose tissues (epididymal and perirenal) were not affected by statin treatments (Fig. 1B). Changes in subcutaneous adipose tissue were not accompanied by changes in final body weight or food intake (Table 1).

Serum parameters

No significant differences were observed among control, pravastatin and atorvastatin groups in serum glucose concentration. By contrast, a significant decrease was observed in simvastatin group and significant increases were found in rosuvastatin, fluvastatin and lovastatin groups when compared to control group. Insulin remained unchanged. When the HOMA-IR index was calculated, significant increases were observed in all the statin-treated groups with the exception of simvastatin, meaning that insulin resistance was increased (Table 2).

As far as serum lipids are concerned, no significant differences were found among experimental groups in TG and FFA concentrations, but a significant reduction in cholesteryl concentration was observed in all statin-treated groups (Table 2).

Liver composition and histological analysis

There were no significant changes in liver weight, protein and water content among the experimental groups. However, the content of triacylglycerols, quantified by the Folch method, was

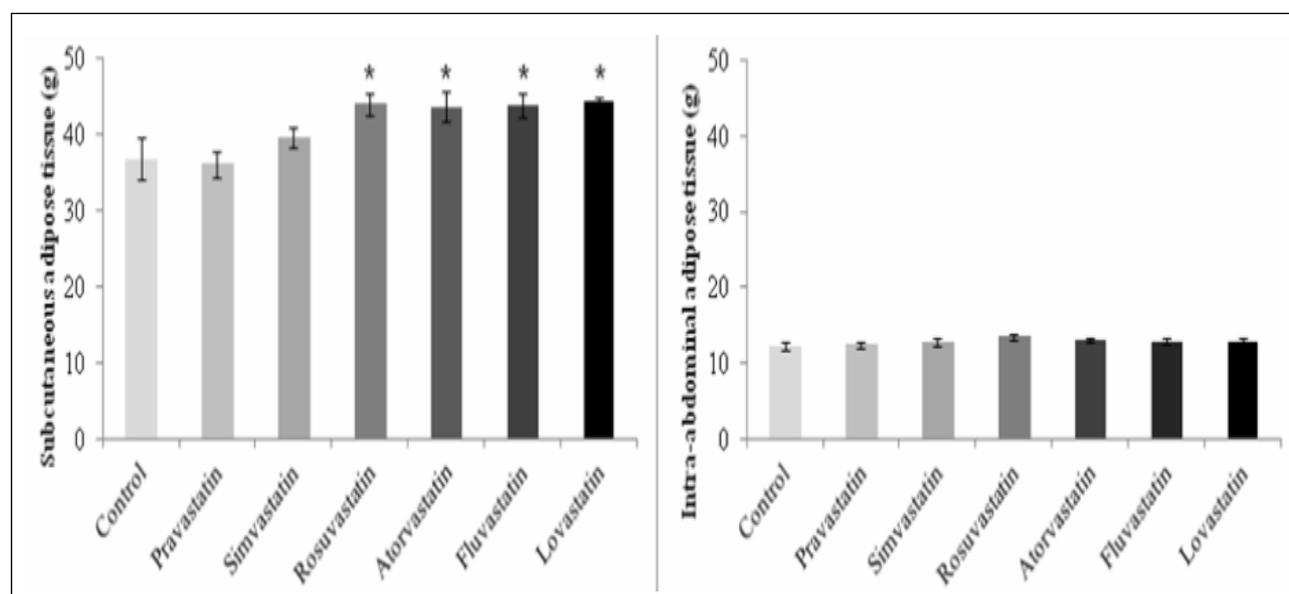


Fig. 1. Subcutaneous adipose tissue (left panel) and intra-abdominal adipose tissues (epididymal and perirenal) from rats of different experimental groups. Values are means \pm S.E.M. ($n=10$). Comparisons are made between control group and each statin-treated group by Student's *t* test. * $P < 0.05$.

Table 1. Final body weight, body weight increase and food intake in rats from the experimental groups.

	control	pravastatin	simvastatin	rosuvastatin	atorvastatin	fluvastatin	lovastatin
Final body weight (g)	407 \pm 13	412 \pm 13	418 \pm 10	422 \pm 6	420 \pm 7	414 \pm 8	411 \pm 7
Body weight increase (g)	214 \pm 10	217 \pm 9	221 \pm 11	226 \pm 6	223 \pm 7	219 \pm 6	214 \pm 6
Food intake (g/d)	26.8 \pm 1.0	26.9 \pm 0.6	27.6 \pm 1.0	27.4 \pm 0.5	26.9 \pm 0.4	27.3 \pm 0.7	27.4 \pm 0.4

Values are means \pm S.E.M. ($n=10$). Comparisons between control group and each statin-treated group are made by Student's *t* test.

Table 2. Serum parameters of rats from the experimental groups.

	control	pravastatin	simvastatin	rosuvastatin	atorvastatin	fluvastatin	lovastatin
Glucose (mg/dL)	215±20	216±14	168±13*	266±13*	218±11	285±6*	258±16*
Insuline (pmol/L)	1140±209	1533±240	1322±275	1559±307	1634±324	1268±234	1321±260
HOMA-IR	15±3	30±6*	14±3	27±5*	31±5*	34±7*	28±4*
TG (mg/dL)	297±59	192±52	208±23	212±18	391±69	188±12	267±41
FFA (µmol/L)	1.12±0.27	0.83±0.15	0.57±0.09	0.82±0.11	0.79±0.11	0.71±0.12	0.70±0.11
Cholesterol (mg/dL)	126±7	116±3*	114±3*	103±4*	111±5*	99±3*	104±1*

Values are means ± S.E.M. (n=10). Comparisons between control group and each statin-treated group are made by Student's *t* test. * P<0.05.

Table 3. Liver weight and liver composition in rats from the experimental groups.

	control	pravastatin	simvastatin	rosuvastatin	atorvastatin	fluvastatin	lovastatin
Liver weight (g)	19.7±0.1	19.1±0.7	18.8±0.8	19.2±0.6	18.2±0.7	18.5±0.7	18.7±0.8
Liver composition							
Triacylglycerols (mg/g)	46±3	49±3	42±3	63±3**	50±2	44±3	54±4
Cholesterol (mg/g)	3.4±0.5	2.9±0.3	1.7±0.1*	1.8±0.2*	2.3±0.2*	2.8±0.2	3.4±0.4
Protein (mg/g)	131±8	141±7	143±6	133±7	137±11	136±9	155±8
Water content (mg/g)	664±91	663±10	669±78	629±16	688±35	635±13	649±13

Values are means ± S.E.M. (n=10). Comparisons between control group and each statin-treated group are made by Student's *t* test. * P<0.05; ** P<0.01.

Table 4. Enzyme activities in subcutaneous adipose tissue from rats in four of the experimental groups.

	control	rosuvastatin	atorvastatin	fluvastatin	lovastatin
FAS (nmol/min/mg prot)	5.79±0.46	6.42±1.17	6.40±1.11	8.68±1.17*	9.23±1.41*
G6PDH (nmol/min/mg prot)	17.5±1.61	16.4±2.8	26.7±2.14*	21.2±2.0	24.23±3.1*
ME (nmol/min/mg prot)	101.75±7.72	115.75±12.85	105.12±6.91	112.84±6.62	122.51±7.53
Total LPL (nmol/min/g tissue)	13.47±1.86	19.87±2.23*	14.67±1.36	19.08±1.68*	16.39±1.3
HR-LPL (nmol/min/g tissue)	5.19±0.48	6.63±0.68*	8.51±0.68**	7.03±0.61**	7.31±0.56**

Values are means ± S.E.M. (n=10). Comparisons between control group and each statin treated group are made by Student's *t* test. * P<0.05; ** P<0.01.

FAS: fatty acid synthase; G6PDH: glucose 6-phosphate dehydrogenase; ME: malic enzyme; LPL: lipoprotein lipase; HR-LPL: heparin-releasable lipoprotein lipase.

significantly increased (36%) by rosuvastatin administration. Cholesterol was significantly reduced by simvastatin, rosuvastatin and atorvastatin (Table 3).

With regard to histological analysis, no significant changes were observed in any group. Nevertheless, in all statin-treated groups, with the exception of simvastatin, a greater number of rats showing grade 3 steatosis was found when compared with control group. This fact was especially clear in the case of rosuvastatin. Of note, the groups in which the number of rats showing grade 3 steatosis was greater, are same that showed increased subcutaneous adipose tissue weight (Figs. 2 and 3).

Enzyme activities

In subcutaneous adipose tissue, enzyme activities were only analyzed in those groups showing an increase in the depot size (rosuvastatin, atorvastatin, fluvastatin and lovastatin). A significant increase in FAS activity was found by the administration of fluvastatin and lovastatin (P<0.05). A similar effect was found in G6PDH activity in atorvastatin and lovastatin groups (P<0.05). By contrast, there were no changes in the activity of ME. In addition, total LPL activity was increased in rosuvastatin and fluvastatin groups (P<0.05), and HR-LPL was increased in rosuvastatin, atorvastatin, fluvastatin and lovastatin groups (Table 3).

As far as liver is concerned enzyme activities were determined in the only group showing an increase in triacylglycerol content (rosuvastatin). FAS and G6PDH activities were increased 60% and 19% respectively in this group, but ME, ACO and CPT-1a activities remained unchanged (Fig. 4).

SREBP-1c protein expression

The measurement of the SREBP-1c protein amount in the nucleus (the mature active form of this transcriptional factor) by Western blot revealed no changes among experimental groups in subcutaneous adipose tissue (Fig. 5A) or liver (Fig. 5B).

DISCUSSION

Statins are powerful drugs very commonly used for hypocholesterolaemia treatment. Several side effects, mainly related to hepatotoxicity and insulin resistance (4, 17-19), have been described. The potential negative effects of these drugs on fat accumulation have scarcely been assessed. Thus, the present study aimed to assess the effects of statins on body fat and liver fat accumulation in Zucker rats (*fa/fa*), an animal model which shows obesity, liver steatosis, dyslipidaemia and insulin resistance (20).

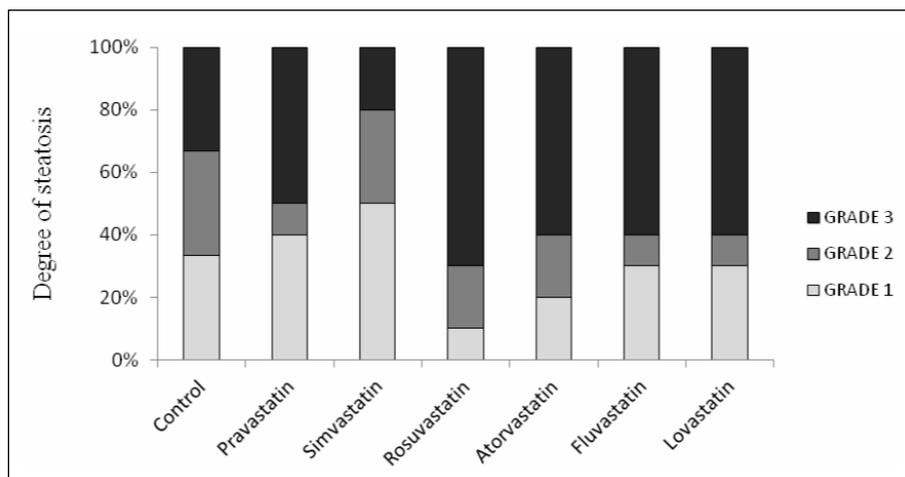


Fig. 2. Histological analysis of liver from rats of different experimental groups. Values are means \pm S.E.M. (n=10). Comparisons are made between by chi-square test.

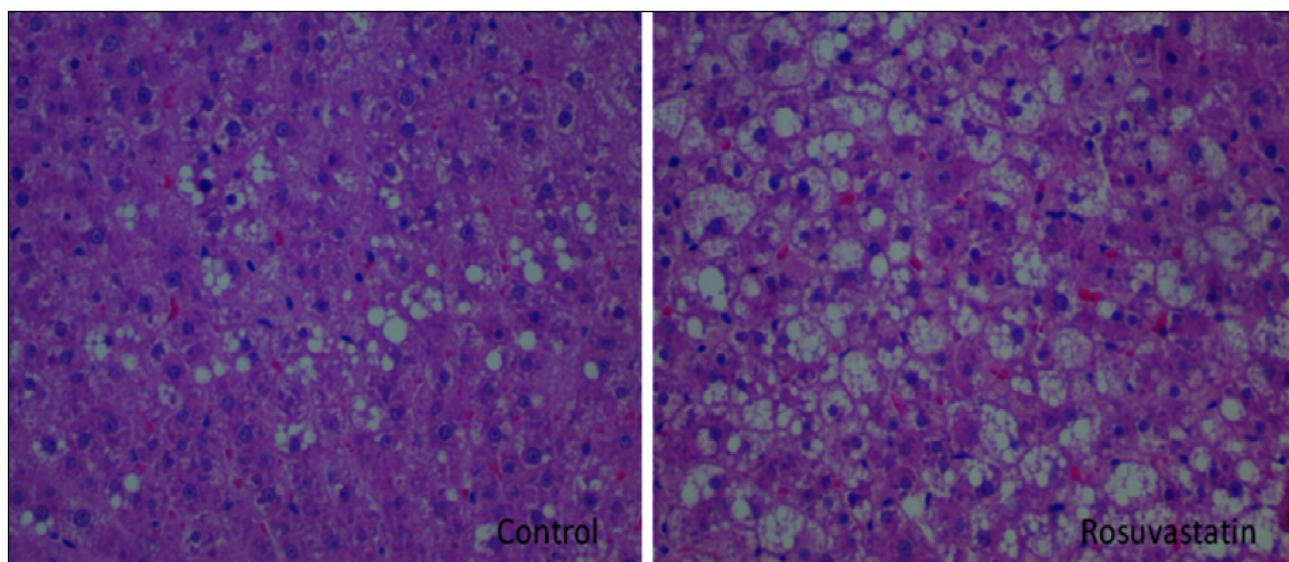


Fig. 3. Liver sections from rats of control and rosuvastatin-treated groups.

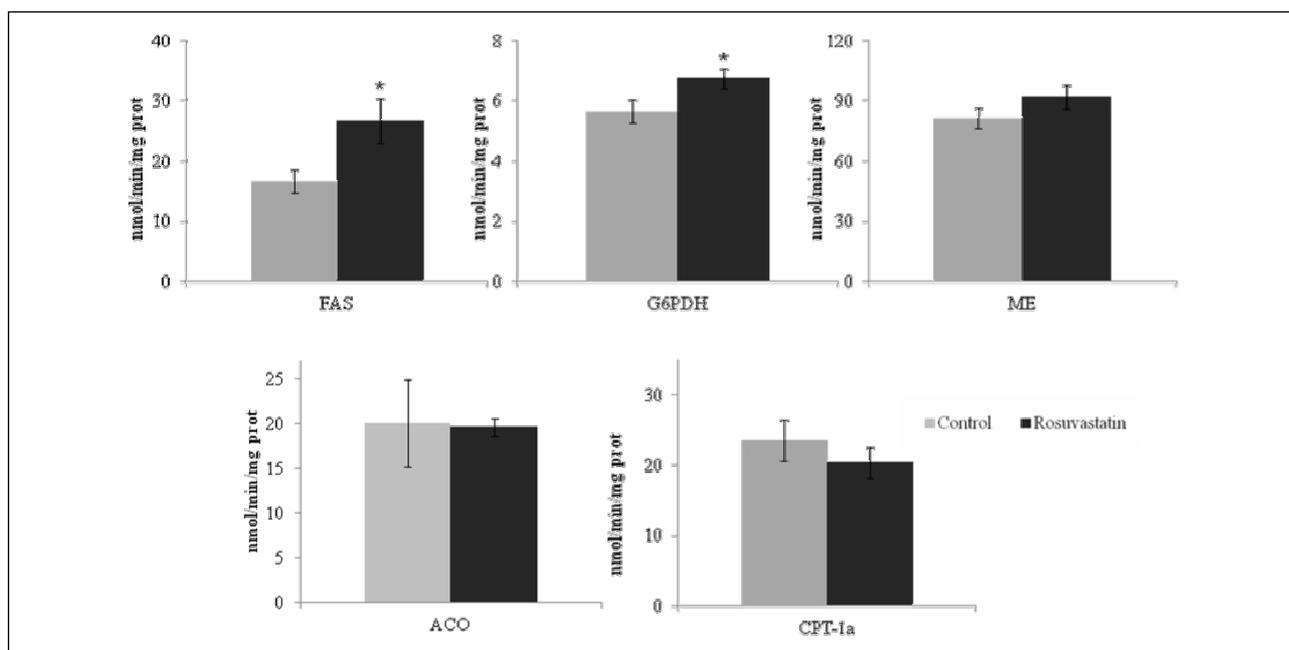


Fig. 4. Enzyme activities in liver from rats of different experimental groups. Values are means \pm S.E.M. (n=10). Comparisons are made between control group and each statin-treated group by Student's *t* test. * $P < 0.05$.

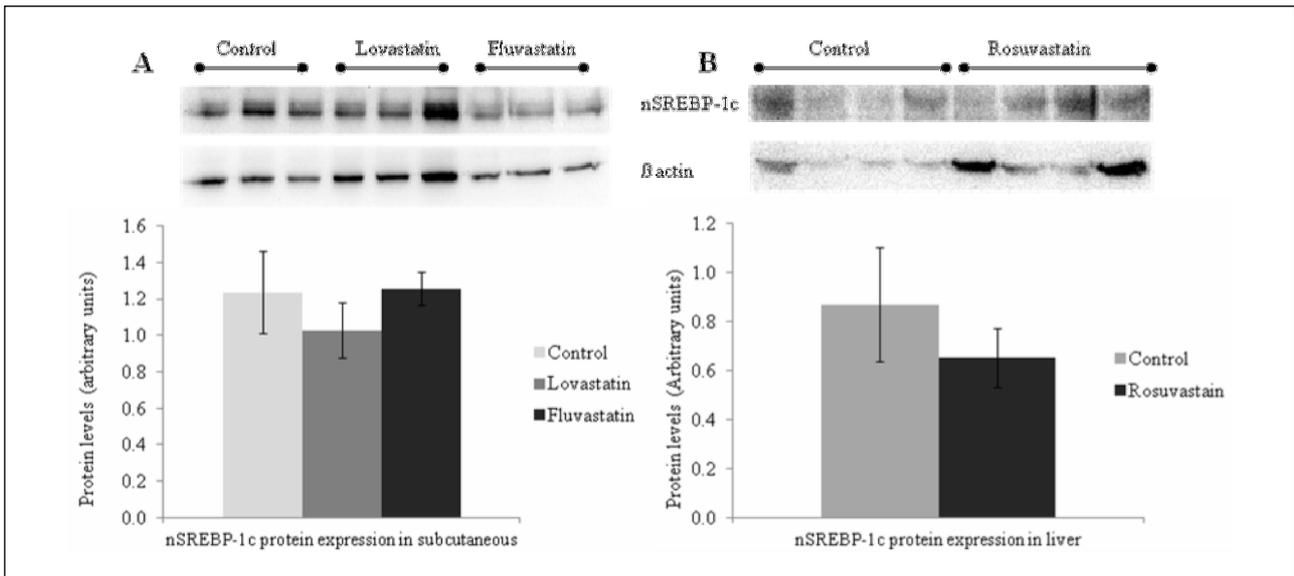


Fig. 5. Active nuclear SREBP-1c protein amount in subcutaneous adipose tissue (A) or liver (B) from rats of different experimental groups. Values are means \pm S.E.M. (n=10). Comparisons are made between control group and each statin-treated group by Student's *t* test.

It is well known that, in humans, statins show different bioavailability and efficacy. In the case of rats these data are not available. Thus, in order to work under the same experimental conditions in all groups, the same dose was used for all statins. Nevertheless, we are aware that this can be a limitation of the present study.

The results here reported show that rosuvastatin, atorvastatin, fluvastatin and lovastatin administration induced an increase in adipose tissue size. However, it is important to point out that this increase was limited to the subcutaneous depot, located directly below the skin, which is less damaging than internal depots. It is well known that visceral obesity, which is centrally located and enclosed by the peritoneum, is the obesity type related to co-morbidities, such as hypertension, diabetes, dyslipidaemia and liver steatosis (21-23). With regard to simvastatin, the results obtained in the present study are not in good accordance with those reported by Salem *et al.* (24), who observed a decreased in body fat in Wistar rats treated with this statin and fed on a high-fat diet. This discrepancy can be due to the fact that in this study the statin was administered at the same time than the obesogenic diet and thus, the effect observed was a partial prevention of obesity induction. By contrast, in the present study obesity was already present in rats at the beginning of the experiment and thus, the study did not focus on obesity prevention. Moreover, in our case a standard rodent diet was supplied to the rats. Altogether these results suggest that the effect of statins on body fat depends on the type of statin as well as on the metabolic status of individuals.

In order to find the mechanisms underlying the increase in this subcutaneous adiposity, the influence of statins on two important pathways which control lipid metabolism in this tissue was assessed: i) the synthesis of fatty acids, known as *de novo* lipogenesis, and ii) the uptake of fatty acids from circulating triacylglycerols. With regard to *de novo* lipogenesis three enzymes, FAS, G6PDH and ME were analyzed. FAS is a multienzymatic complex which elongates a carbon chain starting from an acetyl-CoA primer with molecules of malonyl-CoA provided by ACC in presence of NADPH, mostly supplied by ME and G6PDH. The activity of FAS was enhanced in the groups treated with fluvastatin and lovastatin, and G6PDH activity increased by the administration of atorvastatin and

lovastatin. Thus, it can be suggested that an increase in *de novo* lipogenesis is involved in the fattening effect of these statins.

Triacylglycerol uptake in adipose tissue from circulating lipoproteins is mediated by LPL, which is an enzyme that hydrolyzes triacylglycerols circulating as chylomicrons and very low density lipoproteins (VLDL) into free fatty acids. Total LPL was increased in rosuvastatin and fluvastatin groups and HR-LPL activity, the active form of this enzyme which is placed in adipose tissue endothelium, was increased in all the experimental groups where the subcutaneous depot was increased (rosuvastatin, atorvastatin, fluvastatin and lovastatin). These results are in good agreement with those reported by other authors either *in vitro* or *in vivo* studies. Thus, Bey *et al.* (26) observed an increase in LPL mRNA level in 3T3-L1 pre-adipocytes incubated with atorvastatin, and Sandhya and Rajamohan (27) found an increase in LPL activity in adipose tissue from Sprague-Dawley rats fed on a high-fat high-cholesterol diet supplemented with 0.1 g of lovastatin/100 g diet. Thus, it can be suggested that an increase in fatty acid uptake from circulating triacylglycerols is also involved in the fattening effects of several statins.

Liver in this genetically model of obesity (Zucker *fa/fa*) shows a clear steatosis due to increased *de novo* lipogenesis, potentiated by a huge glycolytic flux which leads to the formation of reduction potential in the form of NADPH, and a strongly reduced fatty acid oxidation (20). In the case of rosuvastatin-treated rats, the hepatic triacylglycerol content was significantly increased. Rodriguez-Calvo *et al.* (28) observed a reduction in liver fat in Sprague-Dawley rats treated with atorvastatin and fed on a high-sucrose diet. In the present study this statin did not induce any change in liver triacylglycerol content. This discrepancy can be explained in the same way than that related to simvastatin and body fat accumulation. That is, in the study reported by Rodriguez-Calvo *et al.* (28) Simvastatin was administered at the same time than a diet which induces liver lipogenesis and thus, this statin prevented triacylglycerol accumulation. In the present study Zucker rats already presented increased liver fat and the diet was a standard one, which did not induced changes in this parameter. As in the case of body fat, these results suggest that the effect of statins on liver fat accumulation depends on the type of statin, as well as on the metabolic status of individuals.

Considering that *de novo* lipogenesis and fatty acid oxidation are two key metabolic pathways in the control of hepatic triacylglycerol content, and hence in fat accumulation in the liver, the effect of rosuvastatin on the activity of several enzymes involved in these pathways was investigated. With regard to *de novo* lipogenesis, an increase in the activity of FAS and G6PDH was observed in rosuvastatin-treated group, suggesting that this metabolic process was activated. CPT-1a a rate-limiting enzyme in mitochondrial fatty acid oxidation, and ACO a rate limiting enzyme of peroxisome fatty acid oxidation, did not change in rosuvastatin group when compared with the control group. These results suggest that increased lipogenesis is, as well as in adipose tissue, at least in part responsible for the enhancement in liver triacylglycerol accumulation induced by this statin.

It is well known that SREBP-1c is the transcriptional factor which regulates FAS activity and that, in turn, is regulated by the intracellular amount of cholesterol (25, 29). Taking into account that in the present study several statins reduced liver cholesterol, an increase in the hepatic active form of SREBP-1c could be expected. However, this effect was not observed, perhaps because the decrease in cholesterol content was not great enough to induce the activation of SREBP-1c, and the same situation took place in the subcutaneous adipose tissue. It is important to point out that although one of the most important mechanisms of FAS regulation occurs at the transcriptional level, other post-transcriptional mechanisms also contribute to enzyme activity regulation (30, 31). It can be hypothesized that this was the case under the present experimental conditions. In addition, it can be proposed that the well known inhibition of HMG-CoA reductase produced by statins could increase the availability of acetyl-CoA, resulting in an activation of *de novo* lipogenesis.

The histological analysis showed that atorvastatin, fluvastatin and lovastatin groups, and more specially rosuvastatin group, presented steatosis degree (grade 3) than the other statin-treated group. Nevertheless, this effect did not reach statistical significance. As observed, no correlation between the histological analysis and the results obtained by using the Folch method was found. This may be due to the fact that fat accumulation is not homogeneous among hepatocytes in liver. Moreover, the histological method is more subjective. This method classifies the lesions in 3 grades: grade 1 (>33% of hepatocytes affected), grade 2 (33–66% of hepatocytes affected) and grade 3 (>66% of hepatocytes affected). Consequently, the boundary of a graduation to another is very high. The biochemical method (Folch method) is more accurate, it has greater specificity, it is more representative and it is the gold standard.

Insulin resistance is related to both obesity and liver steatosis (32–34). On the other hand, recent research has demonstrated that statins might have relevant effect on glucose metabolism in animal models and in humans. However, the reported results are controversial. Some studies have shown that statins had a benefit effect on insulin resistance, others have reported no benefit on insulin action, and others have identified a deterioration in glucose homeostasis (35–38). In this context we considered interesting to evaluate the effects of statins on glycaemic control. HOMA-IR data showed that all statins, with the exception of simvastatin, worsen the insulin resistance already present in Zucker rats.

In summary, the present study provides new useful information concerning potential side-effects of statins. Rosuvastatin, atorvastatin, fluvastatin and lovastatin show a fattening effect, restricted to the subcutaneous area and due, at least in part, to increased lipogenesis and triacylglycerol uptake from circulating in adipose tissue, and it confirms the negative effects on insulin function. Moreover, rosuvastatin also shows a

pro-steatotic effect. Nevertheless, these effects very much depend on the previous metabolic status of individuals. Moreover, taking into account that important limitations exist when extrapolating results from animals to humans, further research is needed to check if these side-effects also take place in human beings in order to bear this information in mind when prescribing one statin or another.

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Author's address: Dr. Leixuri Aguirre, Department of Nutrition and Food Science, Faculty of Pharmacy, University of the Basque Country, 7 Paseo de la Universidad, 01006 Vitoria, Spain.

E-mail: leixuri.aguirre@ehu.es