British Journal of Nutrition (2013), **109**, 1866–1872
doi:10.1017/S000711451200373X

Cholesterol-lowering efficacy of *Lactobacillus plantarum* CECT 7527, 7528 and 7529 in hypercholesterolaemic adults

Mari C. Fuentes[1], Teresa Lajo[2], Juan M. Carrión[3] and Jordi Cuñé[1]*

[1]*AB-BIOTICS S.A., Cerdanyola del Vallès, Edifici Eureka, Campus UAB, 08193 Bellaterra, Spain*
[2]*Department of Endocrinology and Nutrition, Moncloa Hospital, Madrid, Spain*
[3]*Department of Endocrinology and Nutrition, Torrelodones University Hospital, Torrelodones-Madrid, Spain*

(Submitted 30 March 2012 – Final revision received 19 July 2012 – Accepted 21 July 2012 – First published online 28 September 2012)

Abstract

Previous studies have indicated that supplementation with probiotic bacteria may improve lipid metabolism. The present study was aimed at investigating the effects of a mixture of three strains of *Lactobacillus plantarum* (CECT 7527, CECT 7528 and CECT 7529) on cholesterol-lowering efficacy in hypercholesterolaemic patients. A total of sixty volunteers (thirty participants in the placebo group and thirty counterparts in the *L. plantarum* group), aged 18–65 years old, participated in a controlled, randomised, double-blind trial. The study group received one capsule daily containing $1{\cdot}2 \times 10^9$ colony-forming units of *Lactobacillus* strains in a unique dose; the placebo group consumed the same product without bacteria for 12 weeks. A significant reduction of 13·6 % in plasma total cholesterol (TC) levels was observed after 12 weeks of consumption in the *L. plantarum* group when compared with the placebo group. The lipidic outcomes were also analysed based on TC values at baseline: low initial values (LIV, 2000–2500 mg/l) *v.* high initial values (HIV, 2510–3000 mg/l). In the HIV group, the *L. plantarum* treatment showed a reduction after 12 weeks of consumption compared with the placebo group in TC, LDL-cholesterol (LDL-C) and oxidised LDL-C (17·4, 17·6 and 15·6 %, respectively). In the LIV, the *L. plantarum* treatment only showed a reduction after 12 weeks of consumption when compared with the placebo group in TC (9·4 %). The present results showed that the biofunctionality of *L. plantarum* (CECT 7527, CECT 7528 and CECT 7529) is proportional to the cardiovascular risk of the patient, having a better effect in patients with higher levels of cholesterol.

Key words: Probiotic bacteria: *Lactobacillus plantarum*: LDL-cholesterol: CVD

CVD remain the biggest cause of deaths worldwide. More than seventeen million people died from CVD in 2008. More than three million of these deaths occurred before the age of 60 years and could have largely been prevented[1]. Raised blood cholesterol increases the risk of heart disease and stroke[2]. Globally, one-third of IHD is attributable to high cholesterol[3]. Moreover, it has been shown that a 1 % reduction in serum cholesterol is associated with an estimated reduction of 2–3 % in the risk of coronary artery disease[4]. There are different pharmacological agents that are available to treat this condition (e.g. statins or bile acid sequestrants); however, they are often suboptimal and expensive and can have unwanted side effects[5].

There is an increasing interest in non-drug therapies to improve the blood cholesterol profile, particularly when drug treatment is considered unsuitable due to elevated cost, safety reasons or just personal preference. Dietary recommendations and exercise are the first line of therapy for individuals with elevated cholesterol values; however, using these methods, only a modest amelioration can be achieved[6]. Probiotics, in general, are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'[7]. They are regarded as safe for human consumption and numerous (functional) food and nutraceutical products are available in the marketplace[8,9]. In the last years, efforts have been underway to develop probiotics that can help to reduce blood cholesterol and the risk of CVD[10–13].

Strains of lactic acid bacteria were isolated from the faeces of healthy infants as described in Bosch *et al.*[14]. Extensive *in vitro* characterisation of 550 of these strains was carried out to look for candidate strains with the capacity to deconjugate bile acids, to assimilate cholesterol and to produce SCFA, which can then cause a decrease in the systemic levels of blood lipids. *Lactobacillus plantarum* CECT 7527, CECT 7528 and CECT 7529 were selected among the 550 strains for its individual capacity in performing the functionalities

Abbreviations: HDL-C, HDL-cholesterol; HIV, high initial value; LDL-C, LDL-cholesterol; LIV, low initial value; OX-LDL, oxidised LDL-cholesterol; TC, total cholesterol.

* **Corresponding author:** J. Cuñé, fax +34 972 183 213, email cune@ab-biotics.com

mentioned above. The combination of the three strains in mixed cultures produced better results in the different functionalities studied than the individual strains (M Bosch, MC Fuentes, S Audivert, MA Bonachera, S Peiró and J Cuñé; unpublished results). It has been hypothesised that deconjugation of bile acids leads to a reduction in serum cholesterol by increasing cholesterol catabolism during the formation of new bile acids[15]. Thus, the aim of the present study is to perform a controlled evaluation of the effects of AB-LIFE™, a probiotic formula with three different strains of *L. plantarum* (CECT 7527, CECT 7528 and CECT 7529), on the concentration of lipids and other parameters related to cardiovascular risk in hypercholesterolaemic subjects.

Experimental methods

Participants

The present study was carried out according to the Declaration of Helsinki and written informed consent was obtained from all subjects. The protocol was approved by the Ethical Committee of the Hospital Universitario Puerta de Hierro, Madrid, Spain (protocol 106/2009).

A total of sixty subjects were randomly distributed into two groups: placebo or *L. plantarum*. No patient dropped out of the study. Subjects were eligible for the study if male or female (non-pregnant), aged 18–65 years, with total cholesterol (TC) between 2000 and 3000 mg/l (5·16 and 7·64 mmol/l), BMI between 19 and 30 kg/m^2 and LDL-cholesterol (LDL-C) values between 1300 and 1900 mg/l (3·35 and 4·91 mmol/l). Subjects were not included in the case of plasma TAG levels $\geq$3500 mg/l ($\geq$3·85 mmol/l), a previous cardiovascular event within the last 6 months, the presence of secondary dyslipaemias related to thyroid dysfunction or the use of any drug affecting lipid metabolism.

Study design

A single-centre, prospective, randomised, double-blind, placebo-controlled, parallel-group trial was designed. According to the suggestion made by the ethical committee, lifestyle recommendations were given to the participants during the baseline visit of selection (Estrategia Naos, Spanish strategy for nutrition, physical activity and the prevention of obesity); however, they did not receive a specific diet or were institutionalised. The participants agreed to take the product as a dietary supplement and to not change dramatically their regular diets or physical activity in order to study the effect of the supplement in a conventional hypercholesterolaemic lifestyle. As dietary recommendations were given to the patients, to study whether there were some important changes from their conventional diet, dietary intake, including information about total energy, percentage of total fat, percentage of total carbohydrates and percentage of total protein of both groups, was measured at baseline and endpoint (week 12) of the treatment period. On the day of the baseline and endpoint visits, nutritional anamnesis of the participants was collected from the 7 d previous to the visit.

Each participant consumed the probiotic treatment composed of a mixture of three strains in the same proportion of *L. plantarum* (CECT 7527, 7528 and 7529, AB-LIFE™): $1{\cdot}2 \times 10^9$ colony-forming units daily dose, or the control product without bacteria. They were adequately stored before use and therefore the level of lactobacilli was constant throughout the shelf-life of the product. The study consisted of two phases: a treatment period (12 weeks) and a washout period (4 weeks). The study included a baseline visit of selection, a visit at the midpoint and endpoint of the treatment period (weeks 6 and 12, respectively), and a fourth visit after the washout period (week 16).

Blood sampling and biochemical measurements

Blood for assessment of the lipid profile was collected at each visit. Following an overnight fast (12 h), a blood sample was obtained from each participant. Serum samples were analysed enzymatically for TC, LDL-C, oxidised LDL-cholesterol (OX-LDL), HDL-cholesterol (HDL-C) and TAG. Blood for the assessment of the safety profile was collected at the beginning and end of the study. Serum biochemistry was analysed for creatinine, aspartate transaminase, alanine transaminase and γ-glutamyl transpeptidase. Serum analysis was performed on a Dimension RxL biochemistry analyser using appropriate reagent kits (Dade Behring, Siemens).

Statistical analysis

Study data were treated in accordance with the established norms of confidentiality and quality criteria described in the protocol. Statistical analysis of the data was done using SPSS for Windows version 18 software (PASW Statistics; IBM Corporation). Descriptive presentation of the data was performed through means as the measure of the trend of endpoint values measured in the study. To this end, mean values of each studied parameter across time of each experimental day were determined. A comparative analysis of the values obtained throughout the study period was performed. Data are presented as means with their standard errors. The variations in the parameters throughout time for each of the treatments were analysed by the general linear model for repeated measures, both at intra- and inter-group levels, considering the visit as the intragroup factor and treatment as the inter-group factor. Differences in the dietary intake of macronutrients were analysed using a one-way ANOVA. The data of the stratification of the patients (which were not included initially in the protocol) were analysed using 95 % CI. In all hypothesis tests, the null hypothesis of equality between means was rejected when the P value was lower than 0·05, which means that significant differences were considered when α or type I error were $<$0·05.

Results

Baseline characteristics of subjects

The baseline characteristics for the sixty subjects (anthropometric characteristics, values of safety and efficacy variables) were compared in the placebo and *L. plantarum* groups. The two groups produced by randomisation were homogeneous in terms of anthropometric and clinical characteristics (Table 1). Subjects were selected based on fasting serum TC (2000–3000 mg/l) and LDL-C (1300–1900 mg/l). The mean serum concentrations of TC and LDL-C at baseline were not significantly different between the placebo and treatment groups (2526 *v.* 2474 and 1683 *v.* 1666 mg/l, respectively).

Dietary intake

An analysis of total energy, percentage of total lipids, percentage of total carbohydrates and percentage of total proteins of both groups was performed at baseline and endpoint (week 12) of the treatment period (Table 2). There were no significant differences between the placebo and *L. plantarum* groups at baseline or endpoint. Moreover, there were no significant differences between the treatments in relation to weight, BMI, fat-free fat mass and fat mass after 12 weeks of consumption of the probiotic (placebo: 74·9 (SEM 2·1) kg; 25·9 (SEM 0·4) kg/m^2; 58·1 (SEM 1·7) kg; 16·8 (SEM 0·7) kg *v. L. plantarum*: 73·9 (SEM 2·1) kg; 25·5 (SEM 0·4) kg/m^2; 57·1 (SEM 1·7) kg; 16·8 (SEM 0·7) kg).

Serum lipid profile

Global data. The changes in TC, LDL-C, HDL-C, LDL-C:HDL-C, OX-LDL and TAG during the treatment period and after 4 weeks of the washout period are summarised in Table 3. After 6 weeks of consumption, no significant differences were detected in lipid profile variables between the treatments. The values obtained after 12 weeks of consumption in the *L. plantarum* group were significantly lower than those obtained in the placebo group for TC (2138 *v.* 2420 mg/l). In the case of LDL-C and OX-LDL, the *L. plantarum* group after 12 weeks of consumption showed a numerical tendency close to significance for lower values than the placebo group (LDL-C: 1422 *v.* 1585 mg/l; OX-LDL: 47·2 *v.* 55·4 U/l). Likewise, in the group treated with *L. plantarum*, after 12 weeks of consumption, there was a significant reduction compared with the baseline value in TC, LDL-C, LDL-C:HDL-C ratio and OX-LDL (13·6, 14·7, 19·7 and 13·6 %, respectively) and these reductions were higher than the ones observed in the placebo group (4·2, 5·8, 6·8 and 1·8 %, respectively). In relation to HDL-C, although no statistically significant differences between the treatments for none of the follow-up visits were observed, the *L. plantarum* group after 12 weeks of consumption showed a significant increase in HDL-C levels compared with baseline (471 *v.* 442 mg/l), and this effect was not observed in the placebo group.

After 4 weeks of the washout period, the significant differences observed between the treatments after 12 weeks of consumption for TC were maintained. The relative changes observed in the *L. plantarum* group from baseline to week 12 of consumption were not maintained after 4 weeks of the washout period, although there still was a significant reduction compared with the baseline value in TC, LDL-C, LDL-C:HDL-C ratio and OX-LDL (10·9, 12·3, 17·5 and 11·6 %, respectively), and this effect was higher than the one observed in the placebo group (3·9, 5·6, 6·5 and 1·5 %, respectively).

Table 1. Comparison of anthropometric, safety and efficacy variables in the placebo and *Lactobacillus plantarum* treatments at baseline

(Mean values with their standard errors)

	Placebo (*n* 30)		*L. plantarum* (*n* 30)		
	Mean	SEM	Mean	SEM	*P*
Body weight (kg)	75·1	2·02	74·8	2·26	NS
Height (cm)	169·8	1·64	169·6	1·84	NS
BMI (kg/m^2)	26·0	0·44	25·9	0·45	NS
FFM (kg)	58·1	1·59	57·7	1·73	NS
FM (kg)	17·0	0·69	17·1	0·69	NS
Systolic BP (mmHg)	115·8	2·75	114·4	2·87	NS
Diastolic BP (mmHg)	71·9	1·82	71·0	1·53	NS
Pulse (bpm)	76·7	1·74	74·3	1·55	NS
Creatinine (mg/l)	8·4	0·2	8·3	0·2	NS
AST (U/l)	16·4	1·04	17·4	1·04	NS
ALT (U/l)	16·5	1·13	16·7	1·13	NS
GGT (U/l)	18·7	1·29	19·5	1·29	NS
TC (mg/l)	2526	42·9	2474	57·1	NS
LDL-C (mg/l)	1683	35·9	1666	39·5	NS
HDL-C (mg/l)	463	18·7	442	12·6	NS
LDL-C:HDL-C ratio	3·82	0·17	3·85	0·14	NS
OX-LDL (U/l)	56·4	1·82	54·7	2·01	NS
TAG (mg/l)	1896	140	1800	134	NS

FFM, fat-free fat mass; FM, fat mass; BP, blood pressure; bpm, beats per min; AST, aspartate transaminase; ALT, alanine transaminase; GGT, γ-glutamyl transpeptidase; TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; OX-LDL, oxidised LDL-cholesterol.

Table 2. Dietary total energy and macronutrient intake in the placebo and *Lactobacillus plantarum* treatments at baseline and at the end of the treatment period (week 12)

(Mean values with their standard errors)

	Placebo (*n* 30)		*L. plantarum* (*n* 30)		
	Mean	SEM	Mean	SEM	*P*
Total energy (kJ)					
Baseline	7·77	0·19	7·73	0·16	NS
Week 12	7·89	0·19	7·88	0·17	NS
Lipids (%)					
Baseline	37·4	0·09	37·4	0·06	NS
Week 12	39·2	0·10	39·1	0·08	NS
Proteins (%)					
Baseline	19·4	0·72	19·0	0·56	NS
Week 12	20·0	0·75	19·5	0·57	NS
Carbohydrates (%)					
Baseline	43·4	0·12	43·5	0·11	NS
Week 12	40·2	0·16	40·3	0·15	NS

Stratification of the patients

The lipidic outcomes were also analysed based on TC values at baseline: low initial values (LIV) 2000–2500 mg/l *v.* high initial values (HIV) 2510–3000 mg/l (Table 4). After 6 weeks of consumption, no significant differences were detected in lipid profile variables between the treatments in any of the groups. In the HIV group, after 12 weeks of

Table 3. Evolution of lipid parameters in the placebo and *Lactobacillus plantarum* treatments after 6 and 12 weeks of consumption and after the washout period (week 16)†

(Mean values with their standard errors)

	After 6 weeks of consumption					After 12 weeks of consumption					After 4 weeks of washout period				
	Placebo (*n* 30)		*L. plantarum* (*n* 30)			Placebo (*n* 30)		*L. plantarum* (*n* 30)			Placebo (*n* 30)		*L. plantarum* (*n* 30)		
	Mean	SEM	Mean	SEM	*P**	Mean	SEM	Mean	SEM	*P**	Mean	SEM	Mean	SEM	*P**
TC (mg/l)	2393^{B}	42·0	2329^{b}	47·5	NS	2420^{C}	41·4	2138^{c}	34·1	$<0·05$	2429^{C}	42·6	2204^{c}	34·0	$<0·05$
LDL-C (mg/l)	1568^{B}	33·3	1549^{b}	34·1	NS	1585^{C}	33·2	1422^{c}	24·2	NS	1588^{C}	32·3	1461^{c}	25·2	NS
HDL-C (mg/l)	467^{A}	18·2	447^{a}	13·5	NS	467^{A}	18·2	471^{b}	13·4	NS	466^{A}	18·4	471^{b}	13·8	NS
LDL-C:HDL-C ratio	3·51^{B}	0·16	3·56^{b}	0·13	NS	3·56^{B}	0·16	3·09^{c}	0·10	NS	3·57^{B}	0·16	3·18^{c}	0·11	NS
OX-LDL (U/l)	52·7^{B}	1·74	51·4^{b}	1·86	NS	55·4^{C}	1·77	47·2^{c}	1·52	NS	55·6^{C}	1·81	48·3^{c}	1·58	NS
TAG (mg/l)	1824^{A}	137·2	1678^{a}	115·7	NS	1846^{A}	135	1506^{a}	106	NS	1827^{A}	139	1464^{a}	104	NS

TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; OX-LDL, oxidised LDL-cholesterol.
A,B,C Mean values with unlike superscript upper-case letters were significantly different in the placebo group at the intra-group level ($P<0·05$).
a,b,c Mean values with unlike superscript lower-case letters were significantly different in the *L. plantarum* group at the intra-group level ($P<0·05$).
* *P* values indicate significant inter-group differences.
† The baseline values are in Table 1.

consumption, the values observed in the *L. plantarum* group were significantly lower than those observed in the placebo group for TC, LDL-C and OX-LDL (TC: 2286 *v.* 2574 mg/l; LDL-C: 1539 *v.* 1717 mg/l; OX-LDL 53·6 *v.* 60·2). However, in the LIV group, significant differences between the treatments were only detected for TC (*L. plantarum*: 2008 *v.* placebo: 2219 mg/l). In the case of LDL-C, the values observed in the *L. plantarum* group tended to be lower than those observed in the placebo group (placebo 95 % CI 136·30, 146·48 *v.* *L. plantarum* 95 % CI 127·35, 136·53).

In the HIV group, after 12 weeks of consumption, the *L. plantarum* treatment showed a significant reduction from baseline in TC, LDL-C and OX-LDL of 17·4, 17·6 and 15·6 %, respectively, whereas the placebo group only showed a significant increase of 2·0 % in LDL-C. In the LIV group, the *L. plantarum* treatment had a significant reduction from baseline in TC and LDL-C (9·4 and 11·5 %, respectively), and this effect was not observed in the placebo group.

Anthropometric and safety parameters

Anthropometric parameters and biochemical markers of safety were measured at baseline and endpoint and analysed for significant changes. The results showed that the placebo and treatment groups were comparable for anthropometric parameters and biomarkers of safety at the study endpoint. No changes in anthropometric parameters and biochemical markers of safety were considered to be a result of treatment (data not shown).

Discussion

The reduction observed in TC (13·6 %) in the present study in the group supplemented with *L. plantarum* was higher than the ones previously reported in the literature, which has greater clinical relevance. A double-blind, placebo-controlled, randomised, parallel-arm, multi-centre study by Jones *et al.*[16] observed a reduction in TC of only 4·8 % after consumption of a yogurt formulation containing *Lactobacillus reuteri* NCIMB 30242, and Bertolami *et al.*[17] observed a decrease in TC of only 5·3 % after consumption of a fermented milk product containing *Enterococcus faecium* in thirty-two subjects with mild to moderate hypercholesterolaemia. Moreover, in the present study, a numerical tendency close to significance was observed in the *L. plantarum* group compared with the placebo group for LDL-C and OX-LDL. OX-LDL is associated with an increased incidence of the metabolic syndrome[18]. This effect of probiotics on OX-LDL has not been previously described. Furthermore, although there were no significant differences between the treatments in HDL-C levels independently of TC levels, the *L. plantarum* treatment group after 12 weeks of consumption showed an increase in HDL-C levels from baseline. It would be very interesting to get a product with activity in LDL-C and HDL-C, considering that the cardiovascular protective effects of high levels of HDL-C have been widely demonstrated. The present study did not find a significant difference related to these two parameters (LDL-C and HDL-C) in the *L. plantarum* group; however, it

Table 4. Evolution of lipid parameters in the placebo and *Lactobacillus plantarum* treatments after 6 and 12 weeks of consumption and after the wash-out period (week 16) in a stratification of the patients according to the initial concentration of total cholesterol (>2500 or <2500 mg/l)

(Mean values with their standard errors)

	Baseline					After 6 weeks of consumption					After 12 weeks of consumption				
	Placebo (*n* 17)		*L. plantarum* (*n* 14)			Placebo (*n* 17)		*L. plantarum* (*n* 14)			Placebo (*n* 17)		*L. plantarum* (*n* 14)		
	Mean	SEM	Mean	SEM	*P**	Mean	SEM	Mean	SEM	*P**	Mean	SEM	Mean	SEM	*P**
>2500 mg/l															
TC (mg/l)	2703^{A}	30·3	2769^{a}	40·2	NS	2550^{A}	37·1	2555^{b}	42·4	NS	2574^{A}	35·8	2286^{c}	40·3	<0·05
LDL-C (mg/l)	1831^{A}	22·3	1866^{a}	8·3	NS	1699^{B}	25·8	1718^{b}	12·3	NS	1717^{B}	25·8	1539^{c}	14·5	<0·05
HDL-C (mg/l)	456^{A}	20·9	448^{a}	14·9	NS	461^{A}	20·7	451^{a}	17·2	NS	461^{A}	21·2	479^{a}	17·9	NS
LDL-C:HDL-C ratio	4·15^{A}	0·19	4·23^{a}	0·14	NS	3·81^{A}	0·19	3·88^{a}	0·15	NS	3·85^{A}	0·19	3·28^{a}	0·14	NS
OX-LDL (U/l)	61·5^{A}	1·56	63·5^{a}	1·14	NS	57·5^{A}	1·51	59·5^{a}	1·04	NS	60·2^{A}	1·56	53·6^{b}	1·14	<0·05
TAG (mg/l)	2054^{A}	173·4	2231^{a}	198	NS	1961^{A}	172·7	2009^{a}	169	NS	2007^{A}	170	1781^{a}	158	NS
	Placebo (*n* 13)		*L. plantarum* (*n* 16)			Placebo (*n* 13)		*L. plantarum* (*n* 16)			Placebo (*n* 13)		*L. plantarum* (*n* 16)		
	Mean	SEM	Mean	SEM	*P**	Mean	SEM	Mean	SEM	*P**	Mean	SEM	Mean	SEM	*P**
<2500 mg/l															
TC (mg/l)	2295^{A}	28·2	2217^{a}	33·5	NS	2189^{A}	36·0	2131^{a}	34·7	NS	2219^{A}	36·7	2008^{b}	24·3	<0·05
LDL-C (mg/l)	1490^{A}	29·2	1491^{a}	35·2	NS	1397^{A}	26·8	1401^{a}	31·2	NS	1414^{A}	25·5	1319^{b}	21·9	<0·10
HDL-C (mg/l)	472^{A}	34·4	438^{a}	20·1	NS	475^{A}	33·2	444^{a}	20·9	NS	475^{A}	32·6	464^{a}	20·1	NS
LDL-C:HDL-C ratio	3·38^{A}	0·27	3·52^{a}	0·20	NS	3·13^{A}	0·23	3·27^{a}	0·18	NS	3·18^{A}	0·24	2·92^{a}	0·14	NS
OX-LDL (U/l)	49·8^{A}	2·78	47·0^{a}	2·28	NS	46·5^{A}	2·69	44·3^{a}	2·15	NS	49·1^{A}	2·72	41·7^{a}	1·73	NS
TAG (mg/l)	1690^{A}	225·0	1423^{a}	123	NS	1644^{A}	220	1387^{a}	119	NS	1635^{A}	213	1265^{a}	116	NS

TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; OX-LDL, oxidised LDL-cholesterol.

A,B Mean values with unlike superscript upper-case letters were significantly different from baseline in the placebo group at the intra-group level ($P < 0.05$).

a,b,c Mean values with unlike superscript lower-case letters were significantly different from baseline in the *L. plantarum* group at the intra-group level ($P < 0.05$).

* *P* value indicates significant inter-group differences.

is likely that in future studies with a higher number of participants, this effect could be observed.

When examining the cholesterol-lowering trend over the course of the study, it is apparent that the time to maximal therapeutic effect may be longer than other cholesterol-lowering therapies[19]. In the present study, a significant reduction in TC (5·9 %) was observed after 6 weeks of consumption in the *L. plantarum* group when compared with the baseline value. However, there was not a significant effect when compared with the placebo group because a similar reduction in TC after 6 weeks of consumption was also observed in the placebo group (5·3 %). The maximal therapeutic effect in the *L. plantarum* group was reached after 12 weeks of consumption of the probiotic (TC 13·6 %). These results indicate that the effect of *L. plantarum* is progressive and accumulative along time, with a significant therapeutic effect reached after 12 weeks of consumption. This suggests that the time to maximal therapeutic effect may be longer than in other cholesterol-lowering probiotics, as Jones *et al.*[16], Bertolami *et al.*[17] and Agerbaek *et al.*[20] observed that probiotic reduced cholesterol after 6, 8 and 6 weeks of consumption, respectively.

A more profound analysis of the results showed that in patients with HIV of TC, the reduction in TC and LDL-C after 12 weeks of consumption compared with the baseline values was higher than in patients with LIV of TC (17·4 and 17·6 % *v.* 9·4 and 11·5 %, respectively). This finding may suggest that patients with higher levels of TC may benefit from higher reductions in TC and LDL-C after treatment with *L. plantarum* than after any others. Therefore, the biofunctionality of *L. plantarum* could be proportional to the cardiovascular risk of the patient.

Finally, the analysis of safety parameters did not show any deleterious effects associated with *L. plantarum* consumption. Therefore, *L. plantarum* AB-LIFE™ could fulfil all the requirements of safety and efficacy in the treatment of hypercholesterolaemia.

L. plantarum (CECT 7527, CECT 7528 and CECT 7529) strains may reduce cholesterol levels by different mechanisms (M Bosch, MC Fuentes, S Audivert, MA Bonachera, S Peiró and J Cuñé; unpublished results): favouring the reduction of plasma cholesterol through the reduction of the enterohepatic circulation of bile salts (due to the bile salt hydrolase activity); reducing the bioavailability of cholesterol from the diet; producing large quantities of propionic acid which can then cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from the plasma to the liver[21]; producing large quantities of butyric acid, which is an important source of energy for the colonocytes[22]. Bile salt hydrolase activity allows the strains to be able to metabolise the bile salts excreted by the gallbladder during digestion, thereby preventing their reabsorption[23]. As a consequence, the liver requires a higher mobilisation of systemic cholesterol for the *de novo* synthesis of bile salts for the next digestive cycle, favouring a major reduction in plasma cholesterol. It is known that some drugs that are used in the treatment of hypercholesterolaemia may cause many adverse side effects, sometimes dangerous[24]. There are other possibilities of treatment, especially when the increase in LDL-C is not very high. In these situations, clinicians often use dietary phytosterols. Phytosterols lower blood concentrations of cholesterol by inhibiting intestinal absorption of cholesterol by mean of competing for the cholesterol space in mixed micelles, which are the form of lipid delivery for absorption into the mucosal cells[25]. Taken into account that approximately 25 % of the plasma cholesterol production rate is due to absorbed dietary cholesterol and 75 % is accounted for by endogenously synthesised cholesterol[26], the effect of phytosterol on circulating LDL-cholesterol could be limited. Moreover, the consumption of high doses of plant sterols significantly reduces the blood levels of carotenoids and, to a lesser extent, of other essential fat-soluble nutrients[27]. This is why European Union regulations limit exposure to a maximum of 3 g/d in order to avoid intakes above the recommended limits[27].

Considering these topics, new therapies that combine efficacy and safety could be useful for many patients. *L. plantarum* (CECT 7527, CECT 7528 and CECT 7529) is safe at high doses, affects dietary cholesterol but mostly affects enterohepatic cholesterol and reduces systemic inflammation markers, positively affecting cardiovascular health.

In summary, the results of the present study show that supplementation of the diet with *L. plantarum* may contribute significantly to the reduction of serum cholesterol in hypercholesterolaemic patients, having a better effect in patients with higher levels of cholesterol. *L. plantarum* CECT 7527, CECT 7528 and CECT 7529 seem to be a safe and superior alternative to traditional probiotic therapy in the treatment of hypercholesterolaemia.

Acknowledgements

We thank Ana Jurczynska for her technical assistance during this study. We also thank Marco Puma Duque for assisting in the manuscript preparation. This study received external funding from the Ministry of Education and Science of Spain (PTQ05-02-02782), the CDTI-Neotec Project IDI-2006-0244 'Development of probiotic products with specific effects' and the CDTI-PID Project IDI-20101629 'Clinical Assays of AB LIFE, AB 13.1 and AB FORTIS'. M. C. F. prepared the manuscript; T. L. and J. M. C. conducted the research; J. C. conducted the research, designed the study and performed the statistical analysis. All authors read and approved the final manuscript. J. C. and M. C. F. are employed by AB-BIOTICS, SA and report a conflict of interest. All other contributors have no conflicts of interest to report.

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