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To cite this article: Juan Mielgo-Ayuso, Pilar S Collado, Aritz Urdampilleta, José Miguel Martínez-Sanz & Jesús Seco (2013) Changes induced by diet and nutritional intake in the lipid profile of female professional volleyball players after 11 weeks of training, Journal of the International Society of Sports Nutrition, 10:1, 55, DOI: [10.1186/1550-2783-10-55](https://doi.org/10.1186/1550-2783-10-55)

To link to this article: <https://doi.org/10.1186/1550-2783-10-55>



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Published online: 01 Apr 2022.



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RESEARCH ARTICLE

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Changes induced by diet and nutritional intake in the lipid profile of female professional volleyball players after 11 weeks of training

Juan Mielgo-Ayuso^{1*}, Pilar S Collado^{2,3}, Aritz Urdampilleta⁴, José Miguel Martínez-Sanz⁵ and Jesús Seco^{3,6}

Abstract

Background: The relationship between cardiovascular disease and lipid profile is well known. Apart from a heart-healthy diet, exercise is the primary factor that can modify this lipid-associated cardiovascular risk. The aim of the study was to evaluate potential changes in the levels of triglycerides, total cholesterol (TC), low-density lipoprotein-cholesterol (LDLc), and high-density lipoprotein-cholesterol (HDLc), as well as atherogenic indices (TC/HDLc and LDLc/HDLc), and also to analyse the diet over 11 weeks of training in female professional volleyball players.

Methods: The lipid profile of 22 female professional volleyball players was analysed on Day T0 (pre-preseason) and Day T11 (after 11 weeks of training). The consumption of fats by the players was estimated using a food frequency questionnaire, confirmed by seven days of full dietary records.

Results: By the end of the study, the LDLc levels and both atherogenic indices of the players had decreased ($p < 0.05$) compared to the values obtained at baseline. In addition, the diet of the players contained $35.5 \pm 3.2\%$ of fats (saturated fatty acid: $11.1 \pm 1.2\%$, monounsaturated fatty acid: $14.3 \pm 1.9\%$, and polyunsaturated fatty acid: $7.0 \pm 1.1\%$) and 465 ± 57 mg of dietary cholesterol. Their score for the (monounsaturated + polyunsaturated fatty acid)/saturated fatty acid ratio was 1.9 ± 0.4 , less than the recommended ≥ 2 .

Conclusion: These data indicate that the activity of the female professional volleyball players during the first 11 weeks of training in the season was heart healthy, because their lipid profile improved, despite an inadequate intake of fats.

Keywords: Lipids, Atherogenic indices, Sports nutrition, Team sports, Elite athletes, Dietary intake, Female athletes

Background

Physical activity and a heart-healthy diet, such as the Mediterranean diet [1], have been highlighted as major factors in preventing cardiovascular disease (CVD) [2]. Therapeutic lifestyle changes, including nutrition and exercise, are recommended as the front-line strategy for addressing cardiovascular risk factors. Moreover, the positive relationship between CVD and concentrations of low-density lipoprotein cholesterol (LDLc) and the negative relationship between concentrations of high-density lipoprotein cholesterol (HDLc) and cardiovascular risk have been clearly established in numerous clinical

trials [3]. Extensive physical activity is one of the factors that have been shown to be associated with high concentrations of HDLc, which may in part explain the lower risk of coronary heart disease in physically active people [4]. Furthermore, the influence of diet on plasma lipid levels is well known, in particular, the fact that the impact on cardiovascular risk is dependent on the saturated or unsaturated nature, as well as on the number of carbon atoms in the chain, of the fatty acids consumed [5]. In a recent meta-analysis, Kelley et al. [6] concluded that a proper diet along with a programme of aerobic exercise (brisk walking, swimming, cycling, aerobics, or racquet sports) improved the lipid profile (LP), thanks to decreased levels of LDLc, triglycerides (TG), and total cholesterol (TC).

In women's volleyball, as in other sports, the first part of the season is a period of heavy training loads that aim

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to develop technical and tactical skills, as well as achieve adequate physical fitness for the competition period [7]. It is difficult to establish the effects of training on the LP of professional volleyball players. This is because, apart from the personal characteristics of each player, particular features of their training, especially those focused on competition, can substantially modify the LP [8], but we have found no studies that analyse the interaction of these factors. Ruiz et al. [9] commented that volleyball is a sport with a strong component of physical stress, so that playing it leads to lower levels of undesirable plasma lipids and lipoproteins than in the case of other less stressful sports. Witek et al. [10] suggested that changes in the LP over the course of a season could be regarded as transient, with no impact on CVD risk, because the lipid levels remained within normal physiological ranges. Both these studies were, however, conducted in men [9,10].

Thus, the primary aim of this study was to evaluate potential changes in the LP (TG, TC, LDLc, HDLc and atherogenic indices, TC/HDLc and LDLc/HDLc) that might be induced by 11 weeks of training in female volleyball players (FVPs). The secondary aim was to collect baseline data on nutrient intake, in order to advise FVPs from the Spanish Super League concerning the fat content and quality of their diet during this period.

Methods

The study was designed in compliance with the recommendations for clinical research of the World Medical Association Declaration of Helsinki [11]. The protocol was reviewed and approved by the clinical research ethics committees University of León and the University of Basque Country. The experimental procedures, associated risks, and benefits were explained to eligible players before they gave written informed consent to participate.

Subjects

The study group consisted of 22 FVPs, undertaking 25 hours per week of performance training (Table 1). All

the participants were required to attend the laboratory at two specific points: (a) Day T0 (baseline, prior to their general preparation phase of training); and (b) Day T11 (11 weeks later, after 6 weeks of general preparation and 5 weeks of the specific preparation, as well as 6 matches in the regular women's volleyball season).

All the participating players also completed a diet record to record their food intake during the study and had two sets of anthropometric measurements taken (detailed below).

Anthropometric measurements

All anthropometric measurements were conducted on Days T0 and T11 by the same Level 2 certified anthropometrist following the protocol of the International Society for the Advancement of Kinanthropometry (ISAK) [12]. Body weight (BW) was measured in kilograms using a SECA® scale, to the nearest 0.1 kg., and height using a stadiometer to the nearest 0.5 cm. Body mass index (BMI) was then calculated using the formula $BW/height^2$ (kg/m^2). A total of six (triceps, abdominal, supra-iliac, sub-scapular, front thigh and calf) skin-fold measurements were taken in millimetres with a Harpenden® skin-fold calliper, to the nearest 0.2 mm and their sum ($\Sigma 6SF$) calculated. Body Fat mass (FM) was calculated using the Faulkner equation [13].

Blood collection and analysis

Venous blood samples were drawn after 12 hours of fasting from the ante-cubital fossa of the forearm, between 8.00 and 9.00 a.m. on days T0 and T11. None of the players trained the day before the samples were taken. The TG, TC, and HDLc levels were measured by an enzymatic spectrophotometric technique with an auto-analyser (COBAS FARA; Roche Diagnostics, Basel, Switzerland). These values were then used to calculate the LDLc with the Friedewald equation [14]: $LDLc = (TC - HDLc) - TG/5$; and the atherogenic indices (TC/HDLc and LDLc/HDLc).

Table 1 Example of a week of training

	Morning		Afternoon	
MONDAY	10:00	STRENGTH WORKOUTS All players (1)	18:30	TECH-TAC
TUESDAY		FREE TIME	18:30	TECH-TAC
WEDNESDAY	9:30	STRENGTH WORKOUTS Hitters and Libero (2)	15:30	STRENGTH WORKOUTS Setters and middle blockers (2)
	10:30	Specific TECH-TAC Setters and middle blockers	18:30	Specific TECH-TAC Hitters and Libero
THURSDAY		FREE TIME	18:30	TECH-TAC
FRIDAY	9:30	13:00	18:30	TECH-TAC
	TECH-TAC	Video		
SATURDAY		OFFICIAL TRAINING		MATCH
SUNDAY		FREE TIME		FREE TIME

TECH-TAC: Technical/Tactical training; (1): Basic strength training (maximal strength through hypertrophy); (2): Specific strength training (explosive strength and plyometrics).

Dietary control

The participating players were taught how to accurately assess their food intake by dieticians. First, after the T11 anthropometric measurements, the participants were requested to complete a validated food frequency questionnaire (FFQ) for the female Spanish population [15], previously used in other studies conducted in Spain [16,17]. This FFQ, which asked the subjects to recall their average consumption over the previous 11 weeks, included 139 different foods and drinks, arranged by food type and meal pattern. Frequency categories were based on the number of times that items were consumed per day, week or month. Daily consumption in grams was determined by dividing the reported intake by the frequency in days.

Second, as a check on the answers to the FFQ, the participants completed a 7-day dietary record the week prior to starting training (T0) and during week 11 (T11), these questionnaires being distributed on the day the anthropometric measurements were taken. The results obtained by the FFQ were found to be highly reproducible regarding the frequency and amount of foods consumed compared to the data from the 7-day dietary records. When it was not possible to weigh food, serving sizes consumed were estimated from either product names, the place of food consumption, standard weights of food items or the portion size indicated in a picture booklet of 500 photographs of foods. Food values were converted into intakes of total energy, fats, different fatty acids, and cholesterol by a validated software package developed by the Spanish Centre for Higher Studies in Nutrition and Dietetics (CESNID), which is based on Spanish tables of food composition [18].

Third, on Day T11, the participants completed a validated 14-point Mediterranean Diet Adherence Screener (MEDAS) [19]. This included 10 items to measure the frequency of consumption of beneficial foods pertaining to the typical Mediterranean diet (virgin olive oil, vegetables, fresh fruits, legumes and pulses, fish, nuts, white meat, and wine in moderate quantities). It also had four items to measure the consumption of foods that should be limited in or eliminated from the diet (red and processed meats; cream, butter, and margarine; carbonated and/or sugary beverages; and commercial bakery products such as cakes or pastries). One point was assigned to each of the 14 items, so that the total MEDAS score ranged from 0 to 14 points, as a continuous measure, and scores above 9 were considered to indicate good adherence to the Mediterranean diet.

Statistical analysis

All data are reported as means \pm standard deviations. Statistical analysis was performed using SPSS, version 19.0 (SPSS, Chicago). A comparison was made of anthropometric characteristics (BW, BMI, Σ 6SF, and FM) and their LP parameters (TG, TC, HDLc, and LDLc, as well as the

atherogenic indices) on Days T0 and T11, using the Student's *t*-test or Mann–Whitney *U*-test, after normality of the data had been confirmed with the Shapiro–Wilk test. The percentage of change in the outcome variables after 11 weeks was calculated as Δ (%): $[(T11 - T0)/T0] \times 100$. The differences were considered statistically significant when $p < 0.05$.

Results

The mean characteristics of the players are summarised in Table 2. Regarding the anthropometric parameters, significant decreases ($p = 0.027$) in Σ 6SF were observed over the 11 weeks of the study.

The levels of serum lipids and associated indices are listed in Table 3. There were significant decreases in the levels of LDLc ($p = 0.034$), TC/HDLc ($p = 0.027$) and LDLc/HDLc ($p = 0.030$) after the 11 weeks of training.

Table 4 compares energy and fat intakes and the recommended allowances for each of these nutrients. Total fat intake, SFA, W6 and cholesterol intakes were above, and MUFAs were below the recommended allowances for adults in the general population, whilst PUFAs and W3 intakes were adequate.

With regard to the diet quality of the players (Table 5), the MEDAS score, and W6/W3 and (MUFA + PUFA)/SFA ratios indicated that they consumed a healthy diet, but the MUFA/SFA ratio was below the recommended figure.

Finally, Table 6 shows the daily food intake by the players over the 11-week study and the recommended amounts for the general population and for athletes. Relative to the recommended allowances for athletes, the FVPs consumed smaller quantities of cereals, potatoes, legumes and pulses, and larger amounts of pastries, margarine, fatty meat and cold meats.

Discussion

The data collected in this study are of interest because, although the FVPs had a diet rich in fats, cholesterol and SFAs, it was found that their LP did improve. Specifically, LDLc and the atherogenic indices declined, whilst HDLc increased, after 11 weeks of training.

Table 2 The anthropometric characteristics of the female volleyball players at T0 and T11 and the percentage changes

	T0 (n = 22)	T11 (n = 22)	% Change	p T0-T11
Weight (kg)	69.6 \pm 9.4	70.1 \pm 9.2	0.8 \pm 3.1	0.274
BMI	21.8 \pm 2.0	21.9 \pm 1.8	0.8 \pm 3.1	0.311
Σ 6SF (mm)	93.2 \pm 26.7	87.5 \pm 24.4	-5.2 \pm 6.4	0.027
Fat mass (kg)	14.3 \pm 4.3	13.9 \pm 3.9	-2.0 \pm 10.1	0.240

Data are expressed as mean \pm standard deviation. BMI: body mass index; Σ 6SF: Sum of 6 skinfolds.

% Change calculated as: $((T11-T0) \times 100/T0)$.

p T0-T11: baseline vs. after 11 weeks of training.

Table 3 The lipid profile in the female volleyball players at T0 and T11 and the percentage changes

		% Change	p T0-T11
TG (mg/dL)			
T0	71 ± 35	0.3 ± 29.3	0.329
T11	65 ± 16		
TC (mg/dL)			
T0	182 ± 36	-2.7 ± 15.2	0.284
T11	175 ± 18		
HDLc (mg/dL)			
T0	65 ± 16	7.3 ± 22.6	0.089
T11	71 ± 20		
LDLc (mg/dL)			
T0	102 ± 38	-7.0 ± 18.1	0.034
T11	91 ± 23		
TC/HDLc			
T0	3.0 ± 1.0	-9.5 ± 11.4	0.004
T11	2.7 ± 0.9		
LDLc/HDLc			
T0	1.7 ± 0.9	-13.2 ± 15.4	0.011
T11	1.5 ± 0.7		

Data are expressed as mean ± SD. TG: triglycerides; TC: total cholesterol; HDLc: HDL cholesterol; LDLc: LDL cholesterol. % change calculated as: $(T11 - T0)/T0 \times 100$. p T0-T11: baseline vs. after 11 weeks of training.

There is strong evidence that aerobic exercise is associated with favourable shifts in blood triglycerides and HDLc; further, data from intervention studies [20] and numerous meta-analyses [21,22] also support the view that there is an LDLc lowering response to exercise training, though this is a less well-characterized and seems to be variable. Furthermore, independent of diet, exercise was found to have beneficial effects on the concentration and size of low-density lipoprotein cholesterol particles, concentration of high-density lipoprotein cholesterol, size of high-density lipoprotein cholesterol particles, and triglycerides [23].

Table 5 Quality indices for the diet of the female volleyball players (n = 22)

	Per day	Recommended healthy diet
W6/W3	6.6 ± 6.4	5-10:1 ^a
MUFA/SFA	1.4 ± 0.2	≥ 0.5 ^a
(MUFA + PUFA)/SFA	1.9 ± 0.4	≥ 2 ^a
Mediterranean diet adherence	9.3 ± 2.3	≥ 9 ^b

Data are expressed as mean ± standard deviation. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; W3: omega-3 fatty acids; W6: omega-6 fatty acids. ^aRecommended healthy diet [41]; ^bRecommended good Mediterranean diet adherence [19].

A recent meta-analysis [24] showed that continuous exercise (training) produces a 5 to 8% increase in HDLc levels. This is attributable to an increase in the activity of lecithin-cholesterol acyltransferase (LCAT), which increases the synthesis of HDLc, and a reduction in the activity of hepatic lipase, which is involved in the catabolism of these lipids. The effects of physical activity on LCAT and hepatic lipase depend on the type, intensity, frequency, and duration of the physical activity [25]. Paraoxonases are also associated with HDLc because they induce the hydrolysis of lipid peroxide and they provide protection against atherosclerosis [25]. Additionally, a reduction of up to 20% in paraoxonase levels has been reported in sedentary people [26]. HDLc serum levels are inversely associated with the risk of CVD [8]. In the present study, a slight increase of $7.3 \pm 22.6\%$ ($p > 0.05$) was observed in the levels of HDLc in the FVPs after 11 weeks of training. Though the change was not significant, it is interesting to note that an increase of this order of magnitude would decrease their risk of CVD by 16 to 24% [24].

In contrast to HDLc, high levels of LDLc favour the onset and development of CVD [8]. This is why many studies have been conducted to determine which factors lower LDLc levels [6,24,27]. Tambalis et al. [8], in a recent systematic review, pointed out striking evidence that resistance and combined exercise both lower LDLc levels.

Table 4 Energy and macronutrient intake by female volleyball players (n = 22) during the study and the dietary reference recommendations

Nutrient	Per day	Per kg BW	% total energy	Dietary reference recommendations
Energy (kcal)	2840 ± 268	41 ± 6	100	45-50 g/kg BM/day ^a
Fat (g)	113 ± 20	1.6 ± 0.4	35.6 ± 4.8	15-30% ^b
SFA (g)	35.4 ± 9.8	0.5 ± 0.2	11.1 ± 2.3	< 10% ^b
MUFA (g)	46.9 ± 4.7	0.7 ± 0.1	14.9 ± 2.0	15-20% ^b
PUFA (g)	21.0 ± 7.5	0.3 ± 0.1	6.6 ± 2.0	5-8% ^b
W3 (g)	1.6 ± 0.6	0.04 ± 0.01	0.5 ± 2.0	1-2% ^b
W6 (g)	10.4 ± 3.7	0.4 ± 0.2	4.7 ± 10.0	5-8% ^b
Cholesterol (mg)	443 ± 72	6.6 ± 1.5		< 300 mg/day ^b

Data are expressed as mean ± standard deviation. BW: body weight; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; W3: omega-3 fatty acids; W6: omega-6 fatty acids. ^aRecommended energy and carbohydrate intakes [31]; ^bRecommended lipid intake in the adult population to reduce cardiovascular diseases [2].

Table 6 Servings consumed daily by the female volleyball players (n = 22) during the study and the reference recommendations

Food groups	Daily ingested servings	Recommended servings ATHLETES ^a
Cereals and potatoes	3.3 ± 0.4	6-11/day
Dairy products	3.1 ± 0.9	3-4/day
Fruits	3.1 ± 0.9	2-4/day
Vegetables	3.8 ± 0.6	3-5/day
Olive oil	1.2 ± 0.4	2-4/day
Other oils	0.3 ± 0.1	Not mentioned
Legumes and pulses	0.5 ± 0.2	2-3/week or frequently (1/day)
Dried fruits	0.4 ± 0.2	2-3/week or frequently (1/day)
Fish	0.9 ± 0.2	2-3/day and alternating these food groups
Lean meats and poultry	1.8 ± 0.4	
Eggs	0.5 ± 0.1	
Fatty meat and cold meats	0.5 ± 0.1	A few times per month
Pastries and margarines	2.1 ± 0.5	
Wine and beer	0.3 ± 0.2	Not mentioned

Data are expressed as mean ± standard deviation of the number of ingested servings for each food group per person per day. ^aProposal to adapt the food pyramid to an athlete's diet [31].

Likewise, the *Lipid Research Clinics Program* [28] revealed that long-term physical activity, undertaken in a frequent and continuous manner, could decrease LDLc and TC levels. In the FVPs, we observed a slight decrease (by $2.7 \pm 15.2\%$; $p > 0.05$) in TC and a significant decrease (by $7.0 \pm 18.1\%$; $p = 0.034$) in LDLc, changes which add up to an improvement in the LP. The fall in LDLc in the players is attributable to their physical activity having the effect on skeletal muscles of increasing the amount and activity of lipoprotein lipase (LPL). This is an enzyme responsible for hydrolysing TG-rich lipoprotein, thereby reducing VLDL (very low-density lipoprotein) cholesterol and LDLc [29].

Furthermore, it appears that the number of weekly workouts is correlated with increased levels of HDLc and decreased LDLc/HDLc and TC/HDLc atherogenic indices [30]. Specifically, the positive effects of exercise on lipid metabolism were found to last 48 hours [30]. Consistent with this, in our study, the FVPs did two workouts a day, six days a week and significant decreases were observed in their LDLc/HDLc ($p = 0.011$) and TC/HDLc ($p = 0.004$) indices, of 13.2 ± 15.4 and 9.5 ± 11.4 respectively. These decreases in their atherogenic indices can be considered a useful outcome, since high values are strongly associated with the risk of CVD [10].

The daily energy intake of the FVPs during the 11 weeks of study was 41 ± 6 kcal/kg of BW per day. González-Gross et al. [31] advocated an intake of 45 to 50 kcal/kg/day for athletes who train for more than 75 to 90 min/day, as was the case of the FVPs in our study. However, the 39 to 44 kcal/kg/day recommended by Volek et al. [32] for women who engage predominately in resistance exercise

training seems more adequate for the first 11 weeks of training in the season in the case of women's volleyball, because the subjects' BW remained stable while their FM fell (kg). This was indicated by a significant reduction ($p = 0.027$) in the $\Sigma 6SF$, skin-fold thicknesses being used as indicators of body FM [33].

It is worth mentioning that total energy intake may also be directly related to the levels of TG, TC, HDLc, and LDLc, especially the amount and type of fat ingested [4]. Fat accounted for $35.5 \pm 3.2\%$ of total energy intake by the FVPs, in line with what has been reported by several other authors [34-38], but higher than the data reported by Beals et al. [39] and also higher than the 20 to 35% of the total energy consumed that is recommended for team athletes and for the general adult population [33].

Additionally, the amount of cholesterol and SFA intake was found to be positively correlated with the TC and LDLc [40]. The amount of cholesterol ingested by the FVPs was high (465 ± 57 mg) compared to the 300 mg recommended for the general population [2], similar to the 460 mg reported by Anderson et al. in 15 FVPs at the start of the season after a dietary and nutritional intervention [35] and lower than the 104 mg found by Papadopoulou et al. in teenage FVPs [34]. In addition to these data, we note that the intake of SFAs by the FVPs was also high ($11.1 \pm 1.2\%$) compared to the $< 10\%$ that has been suggested to be appropriate the general adult population to reduce cardiovascular diseases [2].

This high cholesterol and SFA intake may be due to the players drinking full-fat milk (3.1 ± 0.9 servings/day), even though their daily number of servings was within the recommendations for athletes [31]. In addition, the

FVPs consumed relatively large amounts of pastries and butter, foods containing a considerable quantity of SFAs [18], whose consumption is not recommended more often than a few times per month [31] and particularly not more than once daily, as was the case for the players in this study (2.1 ± 0.5 servings/day). For athletes' nutrition, semi-skimmed or skimmed milk is considered preferable, so as to reduce the intake of cholesterol and calories from SFAs. It is known that the cholesterol metabolism has some negative feedback, in the sense that if large amounts of cholesterol are ingested, the body produces less (in a normal physiological situation). However, an increase in the consumption of SFAs would cause activation of the cholesterol metabolism, with a possible increase in TC [3].

Additionally, the intake of MUFAs ($14.3 \pm 1.9\%$) was below the ideal recommended allowance (15 to 20%) [41]. MUFAs have healthy effects on the heart by increasing HDLc levels [5]. It was also established that the ratios between different fatty acids, as measured by the PUFA/SFA (1.4 ± 0.2) and W6/W3 (6.6 ± 6.4) ratios, were within the recommendations (≥ 0.5 and 5–10:1, respectively), while the PUFA + (MUFA/SFA) intake was below the recommended level (1.9 ± 0.4 vs. ≥ 2) for a healthy diet [41].

An inappropriate dietary intake jeopardizes sports performance and the benefits of training. It is crucial to plan a diet education programme to optimise the pattern of food and drink consumed (in this case, increasing the consumption of carbohydrates while decreasing that of fats and proteins) and hence improve athletes' sporting performance and health.

Future studies should aim to explore LP, as a function of sex, the sport played and the phase of the season (with respect to pre-season, specific preparatory periods, and competitions) and whether there are changes in the profile with diet programmes or supplementation, and in addition should involve hyperlipidaemic subjects.

The limiting factor in this study is the small sample size. For results in future research to be significant, the samples should be larger, or the period of the study should be extended. On the other hand, this study is the first in which the LP of professional sportswomen has been compared with their dietary intake and even these provisional data have allowed us to identify some significant trends that motivate future research.

Conclusions

According to the data recorded, physical activity during the first 11 weeks of training in the professional women's volleyball season is heart-healthy because it improves the LP (with a decrease in the LDLc and TC/HDLc and LDLc/HDLc indices). This was true despite the intakes of fats by the players being inadequate, in terms of both quality and quantity. In addition, the exercise carried out

by the players during the 11-week study seemed to improve their HDL levels.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors read and extensively reviewed and contributed to the final manuscript as follows MAJ: Conception and design, analysis and interpretation of the data, drafting and critically reviewing the manuscript. SCP: Interpretation of the data, drafting and critically reviewing the manuscript. UA: Drafting and critically reviewing the manuscript. MSJ: Drafting and critically reviewing the manuscript. SJ: Conception, interpretation of the data, drafting and critically reviewing the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

The authors wish to thank the players involved for their participation in the study and Dr. Juan Miguel Orta Costea for his help in the collection of blood samples.

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Received: 9 August 2013 Accepted: 5 December 2013

Published: 11 December 2013

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doi:10.1186/1550-2783-10-55

Cite this article as: Mielgo-Ayuso *et al.*: Changes induced by diet and nutritional intake in the lipid profile of female professional volleyball players after 11 weeks of training. *Journal of the International Society of Sports Nutrition* 2013 **10**:55.

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