



## High-Density Lipoprotein Cholesterol Subfractions in Collegiate Female Volleyball Players

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### Abstract

Much of the published data on the lipid profiles of athletes are based on studies of endurance athletes. Data on female volleyball players are rare. The purpose of this study was thus to examine serum high-density-lipoprotein cholesterol (HDL-C) subfractions in collegiate female volleyball players. Twenty-six female collegiate volleyball players were compared with 26 age- and body mass index-matched control subjects. Dietary information was obtained with a food frequency questionnaire. The subjects were all non-smokers and were not taking any drug known to affect lipid and lipoprotein metabolism. The volleyball players showed significantly higher mean HDL<sub>2</sub>-C than the control group. There were no significant differences in HDL-C and HDL<sub>3</sub>-C between the 2 groups. The results indicate that favorable lipid and lipoprotein profiles could be obtained by vigorous volleyball training and significantly higher HDL<sub>2</sub>-C could be obtained without observing significant differences in HDL-C and HDL<sub>3</sub>-C.

### Keywords

Female athlete; Lipoprotein cholesterol; Physical activity; Volleyball

### Introduction

It has been shown that high-density-lipoprotein cholesterol (HDL-C) concentrations are inversely associated with the risk of coronary heart disease [1]. It has also been shown that increased concentrations of HDL<sub>2</sub>-C and HDL<sub>3</sub>-C [2] or decreased HDL<sub>3</sub>-C [3] were associated with decreased risk of myocardial infarction. High physical activity is one of the factors shown to be associated with high HDL-C concentrations [4], which can explain in part the decreased risk of coronary heart disease in physically active people [5]. Many of the studies on the lipid profile of athletes examined endurance activities [6-8], whereas the lipid profile of volleyball players [9,10], whose training schedule consists of aerobic and anaerobic exercises, is less known. Ruiz et al. [9], using 29 swimmers, 17 volleyball players, 23 soccer players, and 26 controls, reported that the volleyball and soccer players had less favorable lipid profiles than the control subjects, whereas swimmers had a more favorable lipid profile. On the other hand, Tsopanakis et al. [10] compared lipoprotein and lipid

profiles of elite athletes in Olympic sports and reported that volleyball players showed significantly lower total lipids, total cholesterol (TC), and low-density-lipoprotein cholesterol (LDL-C) and higher HDL-C. However, these 2 studies [9,10] on volleyball did not measure HDL-C subfractions. Altena et al. [11] reported that normolipidemic individuals can exhibit an improved lipoprotein profile equally with continuous exercise and intermittent exercise by a reduction of TC through the sum of changes in LDL-C subfractions, increased mean LDL particle size, and increased HDL<sub>2</sub>-C. Furthermore, the 2 above-mentioned studies of volleyball players [9,10] used male subjects. It has been suggested that women respond to exercise with significantly smaller changes in lipid and lipoprotein concentrations than do men [12]. Against this background, the purpose of this study was to examine serum HDL-C subfractions in Japanese collegiate female volleyball players.

### Methods

#### Subjects

The study protocol was approved by the ethics committee of the university. Informed consent was obtained from each participant.

Twenty-six female collegiate volleyball players from the same team were compared with 26 age- and body mass index (BMI)-matched control subjects. The volleyball players had maintained their training schedule, which consisted of aerobic and anaerobic exercises all year round (at least 6 d/wk, 3-4 training h/d), for more than 4 y. The mean ( $\pm$  SD) duration of experience of playing volleyball among the players was 11.1 $\pm$  2.0 y. The volleyball team had competed in the All Japan Collegiate Championship and was among the top 16 teams. All data were obtained in December and January, which were considered representative of the athletes' physiologic status at the end of season training. The control group had been sedentary, except when taking physical education class once a week, for at least 1 y. The subjects were all non-smokers and were not taking any drug known to affect lipid and lipoprotein metabolism.

Body weight and height were measured with the subjects in underwear to the nearest 0.1 kg and 0.1 cm, respectively. BMI was determined as weight/height<sup>2</sup> (kg/m<sup>2</sup>).

All subjects were interviewed by experienced dietitians using a food frequency questionnaire (FFQ), which is based on 29 food groups and 10 types of cooking, for estimating the energy and nutrient intakes of each subject during the past 1 to 2 months [13]. The FFQ was validated by a comparison with weighed dietary records for 7 continuous days [14]. From the FFQ, the selected mean daily dietary and nutrient intakes were calculated according to the Tables of Japanese Foodstuff Composition [15]. Information on nutrient supplement and/or on diet was obtained via a self-administered questionnaire. The accuracy of the questionnaire was checked through individual interviews.

Physical exercise and beverages other than water were not allowed 36 hours prior to the blood sampling. Subjects arrived at the laboratory by 0800 h. The temperature of the laboratory was set at 25°C. Fasting (12 h) blood samples were drawn from the antecubital vein after each subject had been seated quietly for at least 30 min.

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The samples were immediately stored in a cooler box, which was kept at 4°C until centrifuged in a refrigerated centrifuge at 4°C. Samples were analyzed by a local commercial laboratory (Labotech Inc., Nagasaki, Japan). The results were reported within 2 wk. TC (L-type Wako CHO•M kit, Wako Pure Chemical Industries Ltd., Tokyo, Japan) [16], triglycerides (TG) (Pureauto S TG-N kit, Sekisui Medical Co., Ltd., Tokyo, Japan) [17], and LDL-C (Cholestest LDL kit, Sekisui Medical Co., Ltd., Tokyo, Japan) [18] were analyzed by enzymatic methods. HDL-C (Cholestest N HDL kit, Sekisui Chemical Industries, Ltd., Tokyo, Japan) [19] was analyzed by direct assay using a selective inhibition method. TC, TG, HDL-C, and LDL-C were analyzed using auto analyzers (LABOSPECT 008K, Hitachi High-Tech, Tokyo, Japan, and BioMajesty JCA-BM8060, Japan Electron Optics Laboratory, Ltd., Tokyo, Japan). HDL<sub>2</sub>-C and HDL<sub>3</sub>-C (L-type Wako CHO•H kit, Wako Pure Chemical Industries Ltd., Tokyo, Japan) were analyzed by an ultracentrifugation method using an autoanalyzer (JCA-BM2250, Japan Electron Optics Laboratory, Ltd., Tokyo, Japan). Lipoprotein (a) (Lp (a)) (Lp (a) RG kit, FUJIREBIO Inc., Tokyo, Japan) was analyzed by turbidimetric immunoassay [20] using autoanalyzers (BioMajesty JCA-BM8060, Japan Electron Optics Laboratory, Ltd., Tokyo, Japan).

### Statistical analysis

The SPSS statistical software 22.0J (Chicago, IL) was used to analyze the data. Descriptive statistics included means and SD. One-sample Kolmogorov-Smirnov test was performed to examine whether or not each parameter was normally distributed. Logarithmic transformation of TG and Lp (a) were used to normalize the grossly skewed (p<0.05) distribution of the parameter. The mean differences between the 2 groups were analyzed by non-paired t-test. Two-sided p <0.05 was considered to be statistically significant.

### Results

The mean characteristics of the subjects are shown in Table 1. The volleyball players showed significantly higher mean body height and weight than the control group. BMI did not differ significantly between the 2 groups.

The selected mean daily dietary and nutrient intakes are shown in Table 2. There were no significant differences between the 2 groups in the mean daily dietary and nutrient intakes shown in the table.

The mean values of serum lipids and lipoproteins are shown in Table 3. The volleyball players showed significantly higher HDL<sub>2</sub>-C than the control group, while HDL<sub>3</sub>-C did not differ significantly between the two groups. The difference in HDL-C between the 2 groups showed a tendency to be significant (p < 0.072).

### Discussion

It has been shown that increased concentrations of HDL<sub>2</sub>-C and HDL<sub>3</sub>-C [2] or decreased HDL<sub>3</sub>-C [3] were associated with decreased risk of myocardial infarction. In the present study, the volleyball players showed significantly higher HDL<sub>2</sub>-C than the control group, while HDL<sub>3</sub>-C did not differ significantly between the two groups. The difference in HDL-C between the 2 groups did not differ significantly although it showed a tendency to be significant.

The question arises of whether these favorable lipid profiles of the volleyball players actually due to regular vigorous exercise or are simply the result of other confounding factors common to active people. Cigarette smoking has been shown to be negatively associated

with HDL-C [21], while alcohol consumption appears to be positively associated with it [22]. BMI has been shown to be positively related to LDL-C and TG, and negatively correlated with HDL-C [23]. Saturated fatty acids, cholesterol, and excess caloric intake raise serum LDL-C [24], and the consumption of fruit and vegetables is inversely related to LDL-C [25]. Individuals consuming a high-carbohydrate diet tend to show lower HDL-C than those who consume a low-carbohydrate one [26]. In the present study, the subjects were all non-smokers, and there were no significant differences in alcohol intake, BMI, and selected nutrient and dietary intakes between the 2 groups. Thus, the influences of these confounding factors appear to be limited.

One limitation of the present study needs to be mentioned. The menstrual cycle can also influence lipid metabolism. An increase of HDL-C at ovulation in healthy women has been reported [27]. However, no information on menstrual cycle was available in the present study.

Regarding the lipid profile of volleyball players, Ruiz et al. [9] investigated the plasma lipid profile of 28 swimmers, 17 volleyball players, 23 soccer players, and 26 sedentary controls. All of the sports players participated in official national competitions. All groups were matched according to age, BMI, and nutritional status. However, the exercise regimens of swimming (continuous, aerobic component = 95%, anaerobic component = 5%), volleyball (high intensity and intermittent, aerobic component = 60%, anaerobic component = 40%), and soccer (high intensity and intermittent, aerobic component=70%, anaerobic component = 30%) differed significantly. The results indicated that the swimmers showed significantly lower TC than the volleyball players, and significantly lower LDL-C and apo B100 and higher HDL-C than the volleyball and soccer players. The swimmers also showed significantly higher apo A-I than the other 3 groups. The soccer players showed significantly higher lipoprotein (a) than the other 3 groups. The results of this study showed that

Table 1: Characteristics of the subjects.

	Volley ball group (n=26)	Control group (n=26)
Age (yrs)	20.3 ± 1.1	19.7 ± 1.1
Height (cm)	16.7 ± 6.0	154.8 ± 5.0*
Weight (kg)	62.6 ± 7.1	52.4 ± 5.9*
BMI (kg/m <sup>2</sup> )	22.3 ± 2.0	21.8 ± 2.1

Values are the mean ± SD.

\*p < 0.05

Abbreviations; BMI, body mass index

Table 2: Mean daily nutrient and dietary intakes.

	Volleyball group		Control group	
	(n=26)		(n=26)	
Energy (kcal)	1620	± 339	1456	± 407
Fat (g)	51.5	± 15.3	49.4	± 17.0
Carbohydrate (g)	227.1	± 57.2	199.7	± 60.7
Saturated fat (g)	17.4	± 5.5	15.4	± 5.6
Polyunsaturated fat (g)	8.2	± 2.4	9.6	± 4.3
Y&G vegetables (g)	51	± 37	40	± 32
Other vegetables (g)	144	± 79	123	± 77
Fruits (g)	52	± 45	58	± 91
Alcohol (g)	26	± 45	32	± 69

Values are the mean ± SD.

\*p<0.05

Abbreviations; Y&G vegetables, yellow & green vegetables.

**Table 3:** Mean ( $\pm$  SE) values of serum lipids and lipoproteins.

	Volleyball group			Control group		
	(n=26)			(n=26)		
HDL-C (mg/dl)	69.2	$\pm$	13.8	62.5	$\pm$	12.4
HDL <sub>2</sub> -C (mg/dl)	44.4	$\pm$	9.9	38.0	$\pm$	9.2*
HDL <sub>3</sub> -C (mg/dl)	15.7	$\pm$	2.0	15.7	$\pm$	2.0
LDL-C (mg/dl)	101.4	$\pm$	25.2	105.6	$\pm$	29.4
TC (mg/dl)	185.2	$\pm$	30.3	181.0	$\pm$	36.5
TG (mg/dl)	72.7	$\pm$	34.5	77.2	$\pm$	51.5
logTG	1.82	$\pm$	0.18	1.83	$\pm$	0.20
LP (a) (mg/dl)	14.1	$\pm$	13.1	11.9	$\pm$	9.3
logLP (a)	0.96	$\pm$	0.42	0.94	$\pm$	0.36
TC / HDL-C	2.76	$\pm$	0.60	2.94	$\pm$	0.53

Values are the mean  $\pm$  SD.

\* p<0.05

Abbreviations: HDL-C, high-density lipoprotein cholesterol;

LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol;

TG, triglycerides; Apo, apolipoprotein; L-CAT, lecithin:cholesterol acyltransferase;

LP (a), lipoprotein (a)

log, logarithmic transformation

individuals who practice sports involving a high level of physical exertion (volleyball and soccer players) had a less favorable lipid profile. In contrast, swimmers had a more favorable lipid profile. The authors concluded that stressful physical exertion can lead to abnormalities in plasma lipid profile. On the other hand, Tsopanakis et al. [10] compared lipoprotein and lipid profiles of elite athletes in Olympic sports and reported that volleyball players showed significantly lower total lipids, TC, and LDL-C and higher HDL-C. However, the above-mentioned studies in volleyball [9,10] did not measure HDL-C subfractions. In the present study, although HDL-C did not differ significantly between the 2 groups, the volleyball players showed significantly higher HDL<sub>2</sub>-C than the control group. Altena et al. [11] reported that normolipidemic individuals can exhibit an improved lipoprotein profile equally with continuous exercise and intermittent exercise by a reduction of TC through the sum of changes in LDL-C subfractions, increased mean LDL particle size, and increased HDL<sub>2</sub>-C. The divergent results obtained in these studies could be due to the differences in training status, cardiorespiratory fitness levels, nutrient intake, and variations in the frequency, duration, and intensity of training.

In conclusion, the volleyball players showed significantly higher mean HDL<sub>2</sub>-C than the controls, while HDL<sub>3</sub>-C did not differ significantly between the two groups. The results indicate that favorable lipid and lipoprotein profiles could be obtained by vigorous volleyball training and significantly higher HDL<sub>2</sub>-C could be obtained without significant differences in HDL-C and HDL<sub>3</sub>-C.

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