• CLINICAL RESEARCH •

# **Effects of exercise on lipid metabolism and musculoskeletal fitness in female athletes**

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# **Abstract**

**AIM:** This study investigated the effects of intense training on lipid metabolism, bone metabolism and bone mineral density (BMD) in female athletes.

**METHODS:** Sixty-six female subjects participated in this study, age ranging from 18 to 55 years. The sample group included thirty-six athletic subjects and the control group comprised thirty non-athletic individuals. Five athletes competed with national level (5/36) and nine non-athletic subjects (9/30) were postmenopausal women. The assessment items included body composition, radius BMD, calcaneus BMD, lung function, muscular endurance, renal and liver function, bone marker assay and hormone status. All data were analysed, using SPSS 10.0 software, and were presented as mean rank statistical difference, using the Kurskal-Wallis (K-W) test. After that the non-parameter statistics were used. Either *K* value or *P* value below 0.05 was considered significant.

**RESULTS:** Urine deoxypyridinoline/creatinine (Dpd/Cre) levels increased significantly (5.93±2.31 *vs* 6.85±1.43, *K*<0.01), sit-reach (29.30±9.48 cm *vs* 41.31±9.43 cm, *K*<0.001, *P*<0.001), 1 minute sit-ups with bended knees (1 min situps) (17.60±9.34 count *vs* 30.00±10.38 count, *K*<0.001, *P*<0.001), and vertical jump (25.27±6.63 cm *vs* 34.69±7.99 cm, *K*<0.001, *P*<0.001) improved significantly in the athletes group. The athletes group also had a significantly increased level of estriol (E3) (0.14±0.13 pg/mL *vs* 0.07±0.04 pg/mL, *K*<0.01, *P*<0.01), radius BMD (1.37±0.49 gm/cm<sup>2</sup> *vs* 1.19±0.40 gm/cm<sup>2</sup> , *K*<0.05) and calcaneus BMD (0.57±0.17 gm/cm<sup>2</sup> *vs* -0.20±0.17 gm/cm<sup>2</sup> , *K*<0.01, *P*<0.05) compared with those of the controls. The high density lipoprotein (HDL) (65.00±14.02 mg/dL *vs* 52.26±4.84 mg/dL, *K*<0.05, *P*<0.05) was significantly lower in postmenopausal inactive athletes (5/36) than premenopausal active athletes (31/36). On the other hand, low-density lipoprotein (LDL) (98.35±23.84 mg/dL *vs* 131.00±21.63 mg/dL, *K*<0.05, *P*<0.01), cholesterol (CHO) (164.03±27.01 mg/dL *vs* 193.00±23.48 mg/dL, *K*<0.05, *P*<0.05), triglyceride (TG) (63.00±26.39 mg/dL *vs* 147.00± 87.21 mg/dL, *K*<0.01), body fat % (BF%) (28.16±4.90% *vs* 34.84±4.44%, *K*<0.05, *P*<0.001) and body mass index (BMI) (21.98±2.98 kg/m<sup>2</sup> *vs* 26.42±5.01 kg/m<sup>2</sup> , *K*<0.05, *P*<0.001) were significantly higher in postmenopausal inactive athletes (5/36) than premenopausal active athletes (31/36). TG (90.22±39.82 mg/dL *vs* 147.00±87.21 mg/dL), CHO (186.44±24.90 mg/dL *vs* 193.00±23.48 mg/dL) were higher, but the HDL was significantly lower (62.18±10.68 mg/dL *vs* 52.26±4.84 mg/dL, *P*<0.05) in postmenopausal athletes (5/36) group than in postmenopausal control group (9/30).

**CONCLUSION:** Postmenopausal athletes (5/36) who no longer took competing exercises had reduced levels of physical activity, faced increased risk of cardiovascular disease compared to active athletes (31/36) and the postmenopausal controls (9/30). We may thus concluded that long term exercise effectively improves musculoskeletal fitness and prevents BMD loss in female athletes.

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# **INTRODUCTION**

Weightlessness orimmobilization, as experienced by astronauts in space, is a well known cause of significant and rapid bone mineral loss<sup>[1-24]</sup>. Furthermore sedentary individuals generally have a lower bone mass than physically active individuals, moderate exercise is known to increase skeletal mass [3] . The above effect is most obvious in sports that place a significant stress on the skeleton. Investigations of athletes have identified physical activity as a major determinant of bone mass in the general population.

Physical fitness significantly influences quality of life. In Taiwan, medical care quality and public health environment have improved markedly over recent decades. The incidence of fatal infectious diseases thus has reduced significantly and the life span of Taiwanese has enlongated. Simultaneously, the incidence of chronic diseases has increased, yet people remain ignorant of the importance of exercise<sup>[1-5]</sup>.

Exercising for 20-60 min per day, three days per week, at moderate intensity level of 3-6 Metabolic Equivalent units (METs) for most individuals derives at least some health-related benefits, including improved cardiorespiratory fitness, muscle strength and endurance, flexibility and body composition, as well as associated psychological benefits. Consequently, lifelong physical exercise is recommended to optimize health-related benefits<sup>[2-8]</sup>. And the influence of physical activity and exercise training on BMD in females previously has been assessed in cross-sectional, retrospective longitudinal and controlled trial studies<sup>[3-13]</sup>.

Even though no relationship about growth hormone and BMD was found. But the effect of  $E_3$  significantly improved BMD by inhibiting bone resorption, female athletes with low estradiol  $(E_2)$  level take a risk for increased lipid peroxidation following exercise<sup>[11-15]</sup>. Thus hormone status and lipid metabolism may play an important role in the protection against cardiovascular disease, this physiological response has implications for risks of heart disease. Longitudinal information on associations between life style factors and age-related bone loss remains quite controversial. Some studies have found no relationship between bone loss and body composition or body weight, while others

have shown them to predict bone mass changes<sup>[3-16]</sup>.

Therefore, the purpose of this study was to explore the physiological function of female athletes, including BMD, renal function, liver function, hormone status, bone marker assay, lipid metabolism and muscle biology related to the effectiveness of exercise intervention for the health status of female athletes compared with controls.

# **MATERIALS AND METHODS**

#### *Subjects*

Sixty-six female subjects participated in thisinvestigation, with ages ranging between 18 and 55 yrs. The sample group was the athlete group  $(n=36)$ , while the control group comprised non-athletic individuals (*n*=30). Inclusion criteria were that the female athletes had participated in high-intensity resistance or impact activities (e.g., basketball, dancing). Exclusion criteria for both the subjects and the controls were that the subjects had no major medical illnesses, including coronary artery disease which could influence lipid metabolism, and were free of other risk factors that are associated with influencing lipid metabolism, such as smoking or ethanol intake or treatment within the last two years with systemic glucomineralocorticoids, anticonvulsants, bisphosphonates, oestrogen, or raloxifene. Five athletes competed with national level (5/36) and nine non-athletic subjects(9/30) were included in the analysis of postmenopausal women. The parameters to be measured included body composition, radius BMD and calcaneus BMD, lung function, muscular endurance, renal function, liver function and hormone status.

# *Anthropometric measurement of body composition*

Anthropometric measurements were taken based on conventional criteria. The measurement procedures of body weight (Wt) and body height (Ht) were estimated to the nearest 0.1 kg and 0.5 cm, respectively. Finally BMI was calculated using the formula: BMI  $(kg/m^2)$ =Wt  $(kg)/Ht$  (m<sup>2</sup>).

#### *Health related fitness*

They were tested using a modified Guthrie R test<sup>[6]</sup>. Health related fitness tests included vertical jump, 3 min steps, sitreach, hand grip and 1 min sit-ups items.

# *Lung function*

Respiratory muscle strength and pulmonary function were assessed by spirometry. The flow volume and respiratory muscle forces were measured using a Fukuda, microspiro HI-501 model spirometer.

#### *Renal and liver function*

Sixty-six blood samples per subject were drawn from an antecubital vein with the subjects in the seated position. Routine complete blood counts(CBC) were taken using a Sysmex-E9000 (TOA Electronic,Inc., Tokyo,Japan) and renal and liverfunction tests were performed using a Hitachi 7170 instrument (Hitachi Electronic, Inc., Tokyo, Japan) by clinical chemistry laboratory staff at Li-Shin Hospital, Taoyuan County, Taiwan.

#### *Hormone status*

 $E_2, E_3$ , triiodothyronine  $(T_3)$ , thyroxine  $(T_4)$ , thyroid stimulating hormone (TSH), parathyroid hormone (PTH), cortisol and human growth hormone (HGH) were assayed in basal conditions, using commercial radioimmunoassay (RIA) and enzymeimmunoassay (EIA) Kits.

#### *Bone marker assay*

Serum bone specific alkaline phosphatase (BAP) activity was

measured using an EIA kit obtained from Metra Biosystems (Monutain View, CA, USA). Urine Dpd level was measured using enzyme immunoassay (Ciba-Corning ACS-180) kits purchased from Bayer international (Bayer Diagnostics, Tarrytown, NY, USA).

#### *BMD determination*

Calcaneus site BMD was measured via speed of sound (SOS) equipped for a bone mineral densitometry (Aloka Medical Ltd, modelAOS-100, Tokyo, Japan) and all BMD values were also expressed as a T-score, accurately reflecting the BMD. Distal site BMD was measured using the osmometer DTX-100 (SPA, Single Photon Absorptiometry, Osmometer, Rodovre, Denmark). The scanners were calibrated daily against the standard calibration block supplied by the manufacturer to control baseline drift.

#### *Statistical analysis*

All data were analysed, using SPSS 10.0 software, and were presented as mean rank statistical difference, using the Kurskal-Wallis (K-W) test. After that the non-parameter statistics were be used. The confidence interval was set at 95% and the significance level used was *K*<0.05 (two sides). All statistical analyses were carried out with SPSS statistical package. The Kruskal-Wallis test does not use any information on the relative magnitude of each observation when compared with every other observation in the combined sample. This comparison is replaced in each observation by its rank in the poolsample. The smallest observation is replaced by its rank 1, the next smallest by rank 2, and so on, the largest by its rank *n*. Since the test is an extension of the Mann-Whitney-Wilcoxon (M-W-W) test. Either K value or *P* value below 0.05 is considered significant.

# **RESULTS**

#### *No difference in body composition*

The thirty-six female athletes enrolled in this cross- sectional study did not differ significantly in terms of BF, BF%, BMI and resistance compared with the control group (Table 1).

**Table 1** Body composition of two groups

	66 females									
Variables	Control group $n=30$	Athlete group $n = 36$	K-Value							
mean rank										
Body fat	31.68	35.01	0.483							
BF%	33.83	33.22	0.898							
BMI	32.45	34.38	0.685							
Resistance	34.95	32.29	0.575							

*Exercise improvements muscular endurance in female athletes* These two different groups did not differ significantly in muscular endurance. The hand grip (28.06±6.14 kg *vs* 26.85±5.73 kg), 3 min steps(55.32±6.90 count/min *vs* 57.82±7.21 count/min) and vital capacity (86.86±15.98 L *vs* 88.23±12.05 L) in athlete group were better than those in control group, but did differ significantly in terms of sit- reach (29.3±9.48 cm *vs* 41.31±9.43 cm, *K*<0.001, *P*<0.001), 1 min sit-ups(17.60±9.34 count *vs* 30.00±10.38 count, *K*<0.001, *P*<0.001) and vertical jump (25.27±6.63 cm *vs* 34.69±7.99 cm, *K*<0.001, *P*<0.001) as listed in Table 2.

# *Lipid metabolism*

Table 3 shows that the results were not significantly different between both groups. But lipid metabolism including HDL (59.36±12.23 mg/dL *vs* 63.23±13.83 mg/dL) and Hb (12.95±1.22 g/dL *vs* 13.43±1.09 g/dL) in the athlete group was higher than

in the control group. However, LDL (105.93±30.76 mg/dL *vs* 102.89±25.92 mg/dL), TG (81.53±49.53 mg/dL *vs* 74.60±48.31 mg/dL) and CHO (170.20±32.20 mg/dL *vs* 168.06±28.13 mg/dL) were lower in the athlete group than those of the control group. Thus exercise could improve the lipid metabolism, and it is good for health.

**Table 3** No significant differences in blood CHO and lipid variables between both groups



**Table 4** Serum enzyme activities related to renal and liver metabolism



<sup>a</sup>*K*<0.05 *vs* statistically significant when compared with control group. <sup>b</sup>*P*<0.05 *vs* statistically significant when compared with control group. <sup>c</sup>*P*<0.01 *vs* statistically significant when compared with control group.

#### *Renal and liver function*

Table 4 shows that no difference between the data (data not shown here) of the two groups in terms of blood enzymes such

#### **Table 2** Muscular strength and endurance assessment among controls and athlete groups



<sup>a</sup>*K*<0.001 *vs* statistically significant when compared with control group. <sup>b</sup>*P*<0.001 *vs* statistically significant when compared with control group.

#### **Table 5** BMD, urine electrolytes, blood electrolytes in two groups



<sup>a</sup>*K*<0.05 *vs* statistically significant when compared with control group. <sup>b</sup>*K*<0.01 *vs* statistically significant when compared with control group. <sup>c</sup>*K*<0.001 *vs* statistically significant when compared with control group. <sup>d</sup>*P*<0.05 *vs* statistically significant when compared with control group.

**Table 6** Hormonal findings in athletes with significance by non-parameter statistics test compared with controls



<sup>a</sup>*K*<0.01 *vs* statistically significant when compared with control group. <sup>b</sup>*P*<0.01 *vs* statistically significant when compared with control group.

**Table 7** Biochemical bone turnover markers and BMD in athletes with significance by non-parameter statistics test as compared with controls



<sup>a</sup>*K*<0.05 *vs* statistically significant when compared with control group. <sup>b</sup>*K*<0.01 *vs* statistically significant when compared with control group. <sup>c</sup>*K*<0.001 *vs* statistically significant when compared with control group. <sup>d</sup>*P*<0.05 *vs* statistically significant when compared with control group. <sup>e</sup>*P*<0.01 *vs* statistically significant when compared with control group. <sup>f</sup>*P*<0.001 *vs* statistically significant when compared with control group.

**Table 8** Postmenopausal female athlete lipid metabolism compared to premenopausal active athletes



<sup>a</sup>*K*<0.05 *vs* statistically significant when compared with premenopausal group. <sup>b</sup>*K*<0.01 *vs* statistically significant when compared with premenopausal group. <sup>c</sup>*P*<0.05 *vs* statistically significant when compared with premenopausal group. <sup>d</sup>*P*<0.01 *vs* statistically significant when compared with premenopausal group. <sup>e</sup>*P*<0.001 *vs* statistically significant when compared with premenopausal group.

as glutamic oxalocetic transminase (GOT), glutamic pyruvic transminase (GPT), blood urea nitrogen (BUN), uric acid (UA), total protein (TP), globulin (GLO) and bilirubin (BIL). But the control group displayed significantly lower alkaline phosphatase (ALP) (61.03±13.99 U/L *vs* 70.81±15.23 U/L, *K<*0.05, *P*<0.01), ALB (4.52±0.18 g/dL *vs* 4.62±0.27 g/dL, *K*<0.05), Cre (0.75±0.09 mg/dL *vs* 0.81±0.10 mg/dL, *P*<0.05, *K*<0.05) and direct bilirubin (DBIL) (0.25±1.11 mg/dL *vs*  $0.29\pm0.8$  mg/dL,  $K<0.05$ ) than the athlete group.

#### *Electrolytes and BMD*

According to non-parameter statistical tests, both the radius BMD (1.37±0.49 gm/cm<sup>2</sup> *vs* 1.19±0.40 gm/cm<sup>2</sup> ,*K*<0.05) and calcaneus BMD (0.57±0.17 gm/cm<sup>2</sup> *vs* -0.20±0.17 gm/cm<sup>2</sup> , *K*<0.01, *P*<0.05), increased significantly in the athlete group compared with those of the control group. Moreover, the athlete group's body electrolytes such as urine-Cre (132.22±72.30 mg/dL *vs* 166.83±62.52 mg/dL, *K*<0.05, *P*<0.05), blood calcium (Ca) (8.76±0.32 mg/dL *vs* 8.43±0.37 mg/dL, *K*<0.01) and chloride (Cl) (99.94±2.41 meq/L *vs* 102.83±1.97 meq/L, *K*<0.001) significantly decreased compared to the control group.

#### *Hormone status*

 $HGH$  and  $T_4$  were lower in the athlete group than in the control group (8.95±1.51 μg/dL *vs* 9.38±1.51 μg/dL), but cortisol (11.39±4.03 μg/dL *vs* 10.75±3.42 μg/dL), E<sup>2</sup> (88.82±66.42 pg/mL *vs* 80.56±63.10 pg/mL), T<sup>3</sup> (112.07±13.52 ng/dL *vs* 114.78±17.16 ng/dL) and PTH (39.07±16.97 pg/mL *vs* 34.70±11.66 pg/mL) levels were higher. Notably, E<sup>3</sup> level (0.14±0.13 pg/mL *vs* 0.07±0.04 pg/mL, *K*<0.01, *P*<0.01) significantly increased in the athlete group compared to those of the control group.

## *Bone marker assay and BMD*

All biochemical and bone turnover markers, for example, (67.97±39.67 nmol/mmol *vs* 102.63±46.97 nmol/mmol, *K*<0.01, *P*<0.01), Dpd/Cre ratio (5.93±2.31 *vs* 6.85±1.43, *K*<0.01), and BAP (14.04±3.31 μg/L *vs* 20.93±6.17 μg/L, *K*<0.001, *P*<0.001) significantly increased in the female athlete group compared to those of the control group. The athletes displayed positive correlation of regional radius BMD  $(K<0.05)$  and calcaneus BMD  $(K<0.01, P<0.05)$  with these results (Table 7).

#### *Lipid metabolism in postmenopausal athletes*

Table 8 displays levels of LDL (98.35±23.84 mg/dL *vs* 131.00±21.63 mg/dL, *K*<0.05, *P*<0.01), CHO (164.03±27.01 mg/dL *vs* 193.00±23.48 mg/dL, *K*<0.05, *P*<0.05), TG (63.00 ±26.39 mg/dL *vs* 147.00±87.21 mg/dL, *K*<0.01), BF% (28.16±4.90% *vs* 34.84±4.44%, *K*<0.05, *P*<0.001) and BMI (21.98±2.98 kg/m<sup>2</sup> *vs* 26.42±5.01 kg/m<sup>2</sup> , *K*<0.05, *P*<0.001) increased in the postmenopausal (5/36) inactive athletes group compared to the premenopausal (31/36) active athletes. Then the level of HDL (65.00±14.02 mg/dL *vs* 52.26±4.84 mg/dL, *K*<0.05, *P*<0.05) markedly decreased in the postmenopausal (5/36) inactive athletes.

**Table 9** Lipid metabolism of postmenopausal female athletes

Postmenopausal group	n	BMI	HDL LDL CHO			TG.	Hh
Control group (mean rank)	9	6.56		$9.00$ $7.11$ $7.06$		6.44	7.61
Athletes group (mean rank)	5	9.20	4.80 <sup>a</sup>	8.20	8.30	9.40	7.30

<sup>a</sup>*P*<0.05 *vs* statistically significant when compared with postmenopausal control group.

# *Lipid metabolism in postmenopausal females*

Results from this study show higher levels of TG (90.22±39.82 mg/dL *vs* 147.00±87.21 mg/dL), CHO (186.44±24.90 mg/dL *vs* 193.00±23.48 mg/dL), but lower levels of HDL (62.18±10.68 mg/dL *vs* 52.26±4.84 mg/dL, *P*<0.05), Hb (13.82±0.88 g/dL *vs*  $13.52 \pm 0.21$  g/dL) in postmenopausal athletes (5/36) group compared with the postmenopausal control group (9/30). This implies that the effect is a cardiovascular disease risk for postmenopausal retired female athletes (Table 9).

# **DISCUSSION**

The data in this study were expressed as mean  $\bar{x} \pm s$ . Statistical significance in the mean values was evaluated by the Student's *t* test. But in ourstudy, only sixty-six female subjects participated in this investigation. Therefore, we use K-W test to analyze the results of all tests. The Kruskal-Wallis test does not use any information on the relative magnitude of each observation when compared with every other observation in the combined sample. This comparison is replaced in observation by its rank in the pool sample. The smallest observation is replaced by its rank 1, the next smallest by rank 2, and so on, the largest by its rank *n*. Since the test is an extension of the M-W-W test. Either *K* value or *P* value below 0.05 was considered significant.

Exercise is important for maintaining skeletal health. However, the ability of exercise to influence bone might not be entirely related to hormone status. This study has shown that hormones and exercise interact to influence bone adaptations, and thus raise  $E_3$  level related to increased BMD following exercise in female athletes. For example, serum  $E_2$ , cortisol, PTH and  $T_3$  levels in the athlete group were higher than those of the controls, and the major finding of this study was that increased radius BMD (*K*<0.05) and calcaneus BMD (*K*<0.01, *P*<0.05) were significantly and positively related to serum  $E_3$  (*K*<0.01, *P*<0.01) concentrations<sup>[10-12]</sup>. Therefore, a clear understanding the interaction suggested by the present data between  $E_3$  concentration and the adaptation of bone to exercise is important, and provides an interaction through which the estrogen receptors involved in the early response of bone cells might increase their responsiveness to loading<sup>[11,12]</sup>.

These results indicate that physical exercise positively affects the maintenance of radius BMD (*K*<0.05), calcaneus BMD  $(K<0.01, P<0.05)$  in female athletes, thus increased  $E_3$  level can prevent BMD loss and possible risk of osteoporosis<sup>[12]</sup>. The athletes have higher levels of all the biomarkers than the controls, including Dpd (*K*<0.01, *P*<0.01), urine-Cre (*K*<0.05, *P*<0.05), Dpd/Cre ratio (*K*<0.005), BAP (*K*<0.001, *P*<0.001) and lower levels of blood-Ca (*K*<0.01), blood-Cl (*K*<0.001) these results were associated with markedly increased radius BMD and calcaneus BMD<sup>[13-22]</sup>.

Further studies are required to examine a larger population, and also to consider the effects of BMD marker assay (for example insulin-like growth factors).

Physical inactivity has been designated by the American Heart Association as a major modifiable risk factor for cardiovascular disease. Numerous studies have examined individual morbidity and mortality from cardiovascular disease. The results presented here indicate that exercise can improve physiological characteristics, such the lowering levels of serum CHO and TG in female athletes, all of which may improve cardiovascular fitness and reduce morbidity and mortality from cardiovascular disease [12-16,23-26] .

But the findings regarding the renal function, liver function and lipid metabolism of retired female athletes were surprising. Enzyme activity indicates that this group (5/36) may not have the same health benefits from physical exercise as the control subjects. Specifically, this group displayed decreased HDL (*K*<0.05, *P*<0.05), Hb and increased LDL (*K*<0.05, *P*<0.01),

CHO (*K*<0.05, *P*<0.05), TG (*K*<0.01) compared to the premenopausal active athletes (31/36). Postmenopausal retired female athletes (5/36) engaged in less physical activity than previously, displayed increase rates of liver and renal dysfunction, which require further investigation<sup>[17,23-28]</sup>.

An understanding of the dyslipidemia and ensuing atherosclerosis has implications for the pathophysiology of coronary heart disease (CHD). Risk of cardiac morbidity and mortality is directly related to concentration of plasma total CHO or LDL. Lipid lowering therapy has been shown to reduce the risk of cardiovascular events in both high risk individuals and patients with manifest  $CHD<sup>[17-22,24-28]</sup>$ . The present study has found that postmenopausal retired female athletes (5/36) who were no longer engaged in strenuous physical activity, they had a significantly higher BF% (*K*<0.05, *P*<0.001) and BMI (*K*<0.05, *P*<0.001) compared to the active female athletes (31/36) group, specifically, in lipid dysfunction marker with the postmenopausal retired female athletes. Results from this study show higher levels of TG, CHO, but lower levels of HDL, Hb in athletes (5/36) group compared with the control group (9/30). Then, five postmenopausal athletes (5/36), who had retired from competition, and were engaged in less physical activity than previously, had significantly higher BF%, BMI and lipid dysfunction markers had a significantly decreased level of HDL (*P*<0.05) compared to the controls (9/30). This suggest that the effect is a cardiovascular disease risk for postmenopausal retired female athletes.

Future studies should recruit more numbers of female athletes, who have retired from competition butstill maintained high levels of physical activity, and then compare this group with the low physical activity group that serves as the control group. Lipid metabolism related apolipoprotein E (*ApoE)* genotypes with an allele specific oligonucleotide (ASO) based microarray system may interact with exercise training to affect their plasma lipid profiles. To clarify the atherogenic risk of different lipoprotein phenotypes, the relations among total CHO, LDL, HDL and CHD risk in older female athletes should be investigated.

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