UNIVERSITY OF COPENHAGEN

Effect of menopause and exercise training on plasma apolipoprotein M and sphingosine-1-phosphate

Yafasova, Adelina; Mandrup, Camilla Maria; Egelund, Jon; Nyberg, Michael Permin; Stallknecht, Bente; Hellsten, Ylva; Nielsen, Lars Bo; Christoffersen, Christina

1   **Effect of menopause and exercise training on plasma apolipoprotein M and sphingosine-1-phosphate**

2

3   Adelina Yafasova[1], Camilla M. Mandrup[2], Jon Egelund[3], Michael Nyberg[3], Bente Stallknecht[2], Ylva

4   Hellsten[3], Lars B. Nielsen[1,2,4], Christina Christoffersen[1,2,5,*]

5

6   [1]Department of Clinical Biochemistry, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark

7   [2]Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen,

8   Denmark

9   [3]Department of Nutrition, Exercise and Sports, University of Copenhagen, Nørre Allé 51, 2200 Copenhagen,

10   Denmark

11   [4]Department of Clinical Medicine, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen, Denmark

12   [5]Department of Clinical Biochemistry, Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen,

13   Denmark

14

15   * To whom correspondence should be addressed

16   Christina Christoffersen, MD, PhD, DMSc, Department of Clinical Biochemistry, Rigshospitalet,

17   Blegdamsvej 9, 2100 Copenhagen, Denmark. Tel: +45 35453946. Fax: +45 35452880

18   E-mail: christina.christoffersen@regionh.dk

19

20   Running title: Menopause, training and the apoM/S1P axis

21

22

1

23 **Abstract**

24 **Objective:** The axis of apolipoprotein M (apoM) and sphingosine-1-phosphate (S1P) is of importance to

25 plasma lipid levels, endothelial function, and development of atherosclerosis. Menopause is accompanied by

26 dyslipidemia and an increased risk of atherosclerosis, which can be lowered by exercise training. The aim of

27 this study was to explore if effects of menopause and training are paralleled by changes in the apoM/S1P

28 axis.

29 **Methods:** Healthy, late premenopausal (n=38, age 49.2±2) and recent postmenopausal (n=37, age 53.3±3)

30 women from the Copenhagen Women Study participated in a three-month, aerobic high-intensity exercise

31 intervention.

32 **Results:** Before training, plasma apoM was higher in postmenopausal (1.08±0.2 µmol/l (mean±SD))

33 compared to premenopausal (0.82±0.2 µmol/l) women (p<0.0001). Plasma S1P was similar in the two

34 groups (0.44±0.1 and 0.46±0.1 µmol/l, respectively). Hence, the pre-training S1P/apoM ratio was 26% lower

35 in postmenopausal than premenopausal women (p<0.0001). After the training program, plasma apoM

36 increased from 0.82±0.2 to 0.90±0.3 µmol/l in premenopausal women and from 1.08±0.2 to 1.16±0.3 µmol/l

37 in postmenopausal women (p<0.05). Plasma S1P increased from 0.44±0.1 to 0.47±0.1 µmol/l in

38 premenopausal women and from 0.46±0.1 to 0.48±0.1 µmol/l in postmenopausal women (p<0.05).

39 **Conclusions:** The results suggest that menopause is accompanied by higher plasma apoM but not S1P

40 concentrations, and that exercise training increases plasma apoM and S1P in healthy middle-aged women

41 irrespective of menopausal status.

42

43 **Keywords**

44 Apolipoproteins, lipoprotein metabolism, lipids, sphingolipids, atherosclerosis, menopausal transition,

45 cardiovascular training

46

47

2

48   **New & Noteworthy**

49   The ApoM/S1P complex is involved in maintaining a healthy endothelial barrier function. Our study is the

50   first to show how menopause affects apoM/S1P axis. The results suggest that the menopause is accompanied

51   by higher plasma apoM but not S1P concentrations. Secondly, the study is also the first to show that exercise

52   training increases both apoM/S1P in women irrespective of menopausal status.

Introduction

Menopause is associated with an elevated risk of developing endothelial dysfunction (33) and metabolic

syndrome, including dyslipidemia and atherosclerosis (7). Long-term hormone therapy is not generally

recommended for postmenopausal women due to increased risk of cancer and athero-thrombotic disease

(32). Currently, there is a lack of mechanistic understanding of changes that cause dyslipidemia,

atherosclerosis, and endothelial dysfunction in postmenopausal women.

Apolipoprotein M (apoM) is a lipocalin mainly bound to plasma high density lipoprotein (HDL)

particles (11). ApoM has an important role in protecting the endothelial barrier function (8, 12) and affects

several potential anti-atherogenic pathways, such as reverse cholesterol transport (10, 19), formation of preβ-

HDL (10, 34, 47), and removal of reactive oxygen species (10, 18). Moreover, variations in the apoM gene

are associated with risk of cardiovascular disease (CVD) and altered plasma lipids (5). Further investigations

are however needed to clarify the exact role of apoM in atherosclerosis and dyslipidemia.

The bioactive lipid sphingosine-1-phosphate (S1P) is carried by apoM (12). ApoM-containing HDL

carries ~65 % of plasma S1P, while albumin carries ~35 % (27, 35). Hence, apoM is a chaperone, and its

biological effects are likely provided by S1P (36, 40). S1P acts through five G-protein-coupled receptors

(S1P$_{r1-5}$) (24), affecting diverse processes such as protection of the endothelial barrier (8, 12, 45), regulation

of angiogenesis (16), promotion of lymphocyte trafficking (39), and activation of endothelial nitric oxide

synthase (eNOS) (23). Accordingly, S1P has been implicated in several diseases, including inflammatory

diseases and atherosclerosis (30).

Exercise training decreases CVD risk by effects on endothelial function (21) and plasma lipids,

including in postmenopausal women (4, 28, 42). This finding is of particular interest as the menopausal

transition is accompanied by increased prevalence of dyslipidemia (7, 14, 15, 25), and endothelial

dysfunction (33). The apoM/S1P axis is associated with endothelial function (8, 12) and plasma lipids (5),

which are all influenced negatively by the menopausal transition and can be improved with exercise training.

The effect of menopause and exercise training on the apoM/S1P axis itself is unknown, and further

investigation is needed in order to understand if apoM and S1P are involved in menopausal and exercise-

induced changes. This has prompted the present study on how plasma apoM and S1P concentrations are

4

80   affected by exercise training in early postmenopausal compared with late premenopausal women, as well as

81   how apoM and S1P concentrations differ in pre-and postmenopausal women.

82

83

Materials and methods

The data presented in this study are part of the Copenhagen Women Study (31, 37), which is an interdisciplinary study on the effects of exercise training in the late premenopausal and early postmenopausal phase. The study was approved by the Ethics Committee in the capital region of Denmark (protocol number: H-1-2012-150) and hosted institute. All participants were given informed consent at time of inclusion, and the study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Participants - In the present study, we used samples from 38 premenopausal women and 37 postmenopausal women from the Copenhagen Women Study (31, 37). All included participants were healthy non-smokers with no excessive alcohol intake and a body mass index of 18.5-30 kg/m^2. The premenopausal women had regular menstrual cycles and were not using hormonal contraceptives. The postmenopausal women had not experienced a menstrual cycle for at least 1 year and were not receiving hormone therapy. All women were physically active less than 2 hours per week prior to the training intervention.

Study design - As described previously (31, 37), all women underwent testing before and after three months of high-intensity exercise training on a cycling ergometer for one hour three times per week. The training sessions were supervised by instructors, and exercise intensity was monitored and increased gradually during the three-month period. As described by Nyberg et al (37), the heart rate was at 71-95% of the maximal heart rate almost 90% of the time. On test days, all women were fasting and had not exercised for 24 hours.

Plasma lipids - Blood samples taken with a BD Vacutainer system (Becton-Dickinson, Plymouth, UK) were analyzed at the Department of Clinical Biochemistry at Rigshospitalet, Denmark. Plasma aliquots were used for analysis of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and triglycerides with enzymatic absorption photometry (Cobas 8000, c702 module, F. Hoffmann-La Roche Ltd., Rotkreuz, Switzerland). Other blood samples for apoM and S1P measurements were centrifuged for 5 minutes at 4000g and stored at -80°C. Blood samples for S1P measurements were placed on ice immediately after collection.

6

108 *Plasma apoM* - ApoM was measured with ELISA as described by *Bosteen et al.* with intra and inter assay

109 coefficients of variation of 3.2% and 7.9%, respectively (6). Plasma for measurement of apoM was available

110 for 38 premenopausal women (38 pre-training samples and 36 post-training samples) and 37 postmenopausal

111 women (37 pre- and post-training samples). For the two-way repeated measures ANOVA, we could only

112 include subjects with paired values obtained before and after exercise training (pre-menopause n=36;

113 postmenopause n=37).

114 *Plasma S1P* - S1P was measured with an HPLC-based method as described by *Christoffersen et al.* (12).

115 Plasma for measurement of S1P was available for 37 premenopausal women (36 pre-training samples and 31

116 post-training samples) and 35 postmenopausal women (32 pre-training samples and 33 post-training

117 samples). For two-way repeated measures ANOVA, we could only include subjects with paired values

118 obtained before and after exercise training (premenopause n=30; postmenopause n=30).

119 *Statistical Analysis* - Data were analyzed using GraphPad Prism 4 software. The significance level (alpha)

120 was set at $p < 0.05$. The effects of menopausal status and exercise training were assessed using two-way

121 repeated measures ANOVA; only women with measurements before and after the training intervention were

122 included in the two-way repeated measures ANOVA. Correlations were assessed using Pearson correlation

123 analysis. Normally distributed data are presented as mean±SD. Data that are not normally distributed are log-

124 transformed and presented as median (25-75 percentile). After the log-transformation, all data were normally

125 distributed.

126

7

Results

Characteristics of the participants - Before the training intervention, the premenopausal women were 49.2±2

years old. The postmenopausal women were 53.3±3 years old and 3.1±1 years past their final menstruation.

Effects of menopausal status - Before training, the plasma apoM concentration was 32 % higher in the

postmenopausal than in the premenopausal women (1.08±0.2 μmol/l and 0.82±0.2 μmol/l, respectively)

(p<0.0001) (Figure 1A). The plasma S1P concentration was similar in the two groups (p>0.05) (Figure 1B).

Hence, the S1P/apoM ratio was 26 % lower in postmenopausal than premenopausal women before training

(0.45±0.1 and 0.61±0.2, respectively) (p<0.001) (Figure 2A). In accordance with previous findings from the

Copenhagen Women Study (40), plasma TC (p<0.0001), HDL-C (p<0.001), and LDL-C (p<0.01)

concentrations were higher in this subset of postmenopausal compared with premenopausal women, while

the plasma triglyceride concentration was similar in the two groups (p>0.05) (Table 1). The plasma

apoM/HDL-C ratio was also similar in the two groups (p>0.05) (Figure 2B).

Effects of exercise training - The three-month high-intensity training intervention increased the plasma apoM

concentration from 0.82±0.2 to 0.90±0.3 μmol/l in premenopausal women and from 1.08±0.2 to 1.16±0.3

μmol/l in postmenopausal women (p<0.05) (Figure 1A). The plasma S1P concentration increased from

0.44±0.1 to 0.47±0.1 μmol/l in premenopausal women and from 0.46±0.1 to 0.48±0.1 μmol/l in

postmenopausal women (p<0.05) (Figure 1B). The plasma S1P/apoM ratio was not affected by training

(p>0.05) (Figure 2A). As previously reported (40), plasma TC (p=0.01) and LDL-C (p<0.01) concentrations

decreased after the training period, whereas plasma HDL-C and triglyceride concentrations did not change

(p>0.05) (Table 1). The plasma apoM/HDL-C ratio was increased from 0.50±0.1 to 0.54±0.2 in

premenopausal women and from 0.56±0.2 to 0.60±0.2 in postmenopausal women, which was borderline

statistically significant (p=0.05) (Figure 2B).

Correlations between apoM and lipids in plasma - The plasma apoM concentration correlated positively

with plasma TC, HDL-C, and LDL-C concentrations both before and after the training intervention, while no

correlation was found between the plasma apoM and triglyceride concentration (Figure 3). Also, there was

8

152   no correlation between plasma apoM and S1P concentrations (p>0.05) (Figure 4). Additionally, plasma S1P

153   concentrations did not correlate with plasma TC, HDL-C, LDL-C, or triglyceride concentrations (p>0.05)

154   (data not shown).

9

Discussion

There were two main findings in the present study. Firstly, recent postmenopausal women had higher plasma apoM than late premenopausal women. Secondly, exercise training increased plasma apoM and S1P in both late premenopausal and recent postmenopausal women.

Recent postmenopausal women had 32 % higher plasma apoM concentrations compared with late premenopausal women before the training intervention. There are at least two possible explanations for this. The first possible explanation for the higher postmenopausal apoM concentration is a difference in lipid levels. The postmenopausal women had higher TC (by 15 %), HDL-C (by 21 %), and LDL-C (by 14 %) compared with the premenopausal women before the training intervention. In accordance with previous findings, apoM correlated positively with plasma TC, HDL-C, and LDL-C (1). ApoM also correlates negatively with the fractional catabolic rate of LDL (9), suggesting that LDL-C should be increased when apoM is increased, which is consistent with our findings. As discussed previously (31), there is a general agreement that TC and LDL-C are elevated in postmenopausal women compared with premenopausal women, rendering a more atherogenic profile (7, 14, 25). In contrast, findings on the relationship between menopause and HDL-C and triglycerides are inconsistent (7, 14, 15, 25, 38). The Study of Women's Health Across the Nation (SWAN) found that HDL-C was higher in recent postmenopausal women ($\leq$24 months after the final menstrual period [FMP]), but then declined to the premenopausal level in late postmenopausal women (>24 months after the FMP) (14). In the present study, no significant correlation between lipid levels or apoM levels and age was found (data not shown). A study on a sub-cohort from SWAN also found that an increase in HDL-C over the menopausal transition was associated with a greater development of atherosclerosis (17). This is an interesting observation as HDL particles are generally considered anti-atherogenic (20). The finding that plasma apoM was higher in the postmenopausal women might be explained by a concomitant increase in HDL-C, illustrated by a stable apoM/HDL-C ratio between pre- and postmenopausal women. Thus, one may speculate that apoM-containing HDL particles may lose their endothelium-protective and anti-atherogenic potential over the menopausal transition. Nyberg et al., found that the early postmenopausal phase was associated with a marked reduction in vascular function in the Copenhagen Women Study (37), and observed that several biomarkers of vascular function were adversely

10

182 altered in a similar cohort (38). Further studies are however still needed to conclude whether apoM-

183 containing HDL plays a role in this reduction. Second, it is possible that the higher apoM concentration can

184 be explained by the changes in hormone levels that occur over the menopausal transition and possibly even a

185 direct effect of sex hormones on apoM. *Axler et al.* found that apoM concentrations correlate positively with

186 age for women only, with women aged 18-49 years having lower apoM concentrations than women aged 50

187 years or older (1). This observation does not prove a link between apoM and menopause, but the study

188 supports the notion that apoM concentrations could be dependent on hormone levels, as the positive

189 correlation with age is seen for women only. Few studies have addressed the effect of sex hormones on

190 apoM, but it has been shown that estrogen upregulates apoM expression in vitro and in vivo in rats (44). This

191 suggests that plasma apoM concentrations should be higher in pre- than postmenopausal women, which was

192 not the case in the present study. Further, we did not find any correlation between levels of estrogen and

193 apoM (data not shown). Thus, present findings suggest that the difference in apoM levels between groups

194 may not be related to estrogen alone, but rather to a combination of age, hormonal status, and other unknown

195 variables.

196   Plasma S1P was similar in pre- and postmenopausal women, causing the S1P/apoM ratio to be

197 significantly lower in postmenopausal women. A recent study found that apoM without S1P did not have

198 anti-inflammatory properties (41); this further supports the notion that the higher apoM concentrations in

199 postmenopausal women do not necessarily provide an atheroprotective effect as the S1P concentration did

200 not differ between the two groups. This finding is in contrast to an earlier study which found that plasma S1P

201 in premenopausal women. The study found S1P to be negatively correlated with age in both men and women

202 (22). A disadvantage of the study is a large age difference between the subjects (~30 years), and lack of

203 follow up on the same subject before, during, and after postmenopausal transition. While the Copenhagen

204 Women Study neither is a prospective study, it has the strength of a minimal age difference (~4 years)

205 between the two groups. In the future, it would be relevant to examine the effect of menopause on the

206 apoM/S1P axis by following the same cohort throughout the menopausal transition since the pre-menopausal

207 women in the present study could be at varies pre-transitional ages.

11

208   The training intervention increased plasma apoM and plasma S1P. Importantly, the S1P/apoM ratio

209   did not change in contrast to the menopause-related changes, implying that the training-induced increase is

210   different from and possibly renders a more atheroprotective profile than the menopause-induced changes.

211   There are at least two possible explanations for the post-training increase in apoM. First, it is possible that

212   the exercise-induced changes can be explained by changes in plasma lipids. The general consensus is that

213   exercise training increases HDL-C in healthy adults, providing an atheroprotective effect (13, 29). However,

214   previous findings in postmenopausal women have shown that exercise training decreases TC and LDL-C

215   without changing HDL-C (4), which is in accordance with the present findings. Thus, it does not seem likely

216   that the post-training increases in apoM and S1P can be explained solely by changes in plasma lipids.

217   Another possible explanation could be that training also lowered plasma insulin during the oral glucose

218   tolerance test (31) which could lead to an increase in plasma apoM as insulin inhibits the expression of apoM

219   through a Foxa2-mediated mechanism (46). The increase in S1P with training agrees with a previous study

220   showing that plasma S1P was 37 % higher in endurance trained (average experience of 4.3±1.7 years of long

221   distance running) than in untrained healthy, young males (2). However, another study found no difference in

222   plasma S1P between endurance-trained athletes and obese, sedentary controls (3). The reason for this

223   discrepancy between studies is unclear, but the variation may be due to differences in study setups, including

224   gender, age, and duration of the training period.

225   *Potential Clinical Value* – Currently, there is a lack of mechanistic understanding of changes that occur

226   during the menopausal transition. Also, few intervention studies have been conducted addressing the effects

227   on plasma apoM levels in humans. One study has shown that 8 weeks of statin treatment decreases apoM by

228   7% (26). In the present study the range of changes are comparable observed between pre-and

229   postmenopausal women. However, the higher apoM in postmenopausal women without a concomitant

230   increase in S1P may contribute to understanding how previously atheroprotective apoM-containing HDL

231   particles can lose their anti-atherogenic and endothelium-protective potential in the menopausal transition.

232   The finding that postmenopausal women have a lower S1P/apoM ratio gives rise to the question of whether

233   S1P analogues – which are currently released on the market for treatment of multiple sclerosis (43) – can be

12

234   beneficial in treating risk factors for endothelial dysfunction and atherosclerosis related to the

235   postmenopausal phase. Finally, exercise increased both plasma apoM and S1P in pre-and postmenopausal

236   women. It is likely that an extended period of training could increase the plasma apoM/S1P levels further. To

237   maintain a high level of apoM – and S1P containing HDL particles could be of clinical value due to its anti-

238   atherogenic and endothelial protective value.

13

14

250	**References**

251	1.	**Axler O, Ahnstrom J, and Dahlback B**. An ELISA for apolipoprotein M reveals a strong correlation

252	to total cholesterol in human plasma. *J Lipid Res* 48: 1772-1780, 2007.

253	2.	**Baranowski M, Charmas M, Dlugolecka B, and Gorski J**. Exercise increases plasma levels of

254	sphingoid base-1 phosphates in humans. *Acta Physiol (Oxf)* 203: 373-380, 2011.

255	3.	**Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH, Sanders P, Siddall P, Kuo**

256	**MS, and Perreault L**. Serum sphingolipids: relationships to insulin sensitivity and changes with exercise in

257	humans. *Am J Physiol Endocrinol Metab* 309: E398-E408, 2015.

258	4.	**Binder EF, Birge SJ, and Kohrt WM**. Effects of endurance exercise and hormone replacement

259	therapy on serum lipids in older women. *J Am Geriatr Soc* 44: 231-236, 1996.

260	5.	**Borup A, Christensen PM, Nielsen LB, and Christoffersen C**. Apolipoprotein M in lipid

261	metabolism and cardiometabolic diseases. *Curr Opin Lipidol* 26: 48-55, 2015.

262	6.	**Bosteen MH, Dahlback B, Nielsen LB, and Christoffersen C**. Protein unfolding allows use of

263	commercial antibodies in an apolipoprotein M sandwich ELISA. *J Lipid Res* 56: 754-759, 2015.

264	7.	**Carr MC**. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* 88:

265	2404-2411, 2003.

266	8.	**Christensen PM, Liu CH, Swendeman SL, Obinata H, Qvortrup K, Nielsen LB, Hla T, Di LA,**

267	**and Christoffersen C**. Impaired endothelial barrier function in apolipoprotein M-deficient mice is

268	dependent on sphingosine-1-phosphate receptor 1. *FASEB J* 30: 2351-2359, 2016.

269	9.	**Christoffersen C, Benn M, Christensen PM, Gordts PL, Roebroek AJ, Frikke-Schmidt R,**

270	**Tybjaerg-Hansen A, Dahlback B, and Nielsen LB**. The plasma concentration of HDL-associated apoM is

271	influenced by LDL receptor-mediated clearance of apoB-containing particles. *J Lipid Res* 53: 2198-2204,

272	2012.

273	10.	**Christoffersen C, Jauhiainen M, Moser M, Porse B, Ehnholm C, Boesl M, Dahlback B, and**

274	**Nielsen LB**. Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low

275	density lipoprotein receptor knock-out mice. *J Biol Chem* 283: 1839-1847, 2008.

15

276 11. **Christoffersen C, Nielsen LB, Axler O, Andersson A, Johnsen AH, and Dahlback B**. Isolation and

277 characterization of human apolipoprotein M-containing lipoproteins. *J Lipid Res* 47: 1833-1843, 2006.

278 12. **Christoffersen C, Obinata H, Kumaraswamy SB, Galvani S, Ahnstrom J, Sevvana M, Egerer-**

279 **Sieber C, Muller YA, Hla T, Nielsen LB, and Dahlback B**. Endothelium-protective sphingosine-1-

280 phosphate provided by HDL-associated apolipoprotein M. *Proc Natl Acad Sci U S A* 108: 9613-9618, 2011.

281 13. **Cornelissen VA, and Fagard RH**. Effects of endurance training on blood pressure, blood pressure-

282 regulating mechanisms, and cardiovascular risk factors. *Hypertension* 46: 667-675, 2005.

283 14. **Derby CA, Crawford SL, Pasternak RC, Sowers M, Sternfeld B, and Matthews KA**. Lipid

284 changes during the menopause transition in relation to age and weight: the Study of Women's Health Across

285 the Nation. *Am J Epidemiol* 169: 1352-1361, 2009.

286 15. **Do KA, Green A, Guthrie JR, Dudley EC, Burger HG, and Dennerstein L**. Longitudinal study of

287 risk factors for coronary heart disease across the menopausal transition. *Am J Epidemiol* 151: 584-593, 2000.

288 16. **Du W, Takuwa N, Yoshioka K, Okamoto Y, Gonda K, Sugihara K, Fukamizu A, Asano M, and**

289 **Takuwa Y**. S1P(2), the G protein-coupled receptor for sphingosine-1-phosphate, negatively regulates tumor

290 angiogenesis and tumor growth in vivo in mice. *Cancer Res* 70: 772-781, 2010.

291 17. **El Khoudary SR, Wang L, Brooks MM, Thurston RC, Derby CA, and Matthews KA**. Increase

292 HDL-C level over the menopausal transition is associated with greater atherosclerotic progression. *J Clin*

293 *Lipidol* 10: 962-969, 2016.

294 18. **Elsoe S, Ahnstrom J, Christoffersen C, Hoofnagle AN, Plomgaard P, Heinecke JW, Binder CJ,**

295 **Bjorkbacka H, Dahlback B, and Nielsen LB**. Apolipoprotein M binds oxidized phospholipids and

296 increases the antioxidant effect of HDL. *Atherosclerosis* 221: 91-97, 2012.

297 19. **Elsoe S, Christoffersen C, Luchoomun J, Turner S, and Nielsen LB**. Apolipoprotein M promotes

298 mobilization of cellular cholesterol in vivo. *Biochim Biophys Acta* 1831: 1287-1292, 2013.

299 20. **Gordon T, Castelli WP, Hjortland MC, Kannel WB, and Dawber TR**. High density lipoprotein as

300 a protective factor against coronary heart disease. The Framingham Study. *Am J Med* 62: 707-714, 1977.

301 21. **Green DJ, Maiorana A, O'Driscoll G, and Taylor R**. Effect of exercise training on endothelium-

302 derived nitric oxide function in humans. *J Physiol* 561: 1-25, 2004.

16

303   22.   **Guo S, Yu Y, Zhang N, Cui Y, Zhai L, Li H, Zhang Y, Li F, Kan Y, and Qin S**. Higher level of

304   plasma bioactive molecule sphingosine 1-phosphate in women is associated with estrogen. *Biochim Biophys*

305   *Acta* 1841: 836-846, 2014.

306   23.   **Igarashi J, and Michel T**. S1P and eNOS regulation. *Biochim Biophys Acta* 1781: 489-495, 2008.

307   24.   **Ishii I, Fukushima N, Ye X, and Chun J**. Lysophospholipid receptors: signaling and biology. *Annu*

308   *Rev Biochem* 73: 321-354, 2004.

309   25.   **Jensen J, Nilas L, and Christiansen C**. Influence of menopause on serum lipids and lipoproteins.

310   *Maturitas* 12: 321-331, 1990.

311   26.   **Kappelle PJ, Ahnstrom J, Dikkeschei BD, de Vries R, Sluiter WJ, Wolffenbuttel BH, van Tol A,**

312   **Nielsen LB, Dahlback B, and Dullaart RP**. Plasma apolipoprotein M responses to statin and fibrate

313   administration in type 2 diabetes mellitus. *Atherosclerosis* 213: 247-250, 2010.

314   27.   **Karuna R, Park R, Othman A, Holleboom AG, Motazacker MM, Sutter I, Kuivenhoven JA,**

315   **Rohrer L, Matile H, Hornemann T, Stoffel M, Rentsch KM, and von EA**. Plasma levels of sphingosine-

316   1-phosphate and apolipoprotein M in patients with monogenic disorders of HDL metabolism.

317   *Atherosclerosis* 219: 855-863, 2011.

318   28.   **Kim JW, and Kim DY**. Effects of aerobic exercise training on serum sex hormone binding globulin,

319   body fat index, and metabolic syndrome factors in obese postmenopausal women. *Metab Syndr Relat Disord*

320   10: 452-457, 2012.

321   29.   **Lin X, Zhang X, Guo J, Roberts CK, McKenzie S, Wu WC, Liu S, and Song Y**. Effects of

322   Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic

323   Review and Meta-Analysis of Randomized Controlled Trials. *J Am Heart Assoc* 4: pii:e00214, 2015.

324   30.   **Maceyka M, Harikumar KB, Milstien S, and Spiegel S**. Sphingosine-1-phosphate signaling and its

325   role in disease. *Trends Cell Biol* 22: 50-60, 2012.

326   31.   **Mandrup CM, Egelund J, Nyberg M, Lundberg Slingsby MH, Andersen C, Logstrup S,**

327   **Bangsbo J, Suetta C, Stallknecht B, and Hellsten Y**. Effects of high-intensity training on cardiovascular

328   risk factors in pre- and postmenopausal women. *Am J Obstet Gynecol* 216: e1-e11, 2016.

17

329   32.   **Marjoribanks J, Farquhar C, Roberts H, and Lethaby A**. Long term hormone therapy for

330   perimenopausal and postmenopausal women. *Cochrane Database Syst Rev* CD004143, 2012.

331   33.   **Moreau KL, Hildreth KL, Meditz AL, Deane KD, and Kohrt WM**. Endothelial function is

332   impaired across the stages of the menopause transition in healthy women. *J Clin Endocrinol Metab* 97: 4692-

333   4700, 2012.

334   34.   **Mulya A, Seo J, Brown AL, Gebre AK, Boudyguina E, Shelness GS, and Parks JS**.

335   Apolipoprotein M expression increases the size of nascent pre beta HDL formed by ATP binding cassette

336   transporter A1. *J Lipid Res* 51: 514-524, 2010.

337   35.   **Murata N, Sato K, Kon J, Tomura H, Yanagita M, Kuwabara A, Ui M, and Okajima F**.

338   Interaction of sphingosine 1-phosphate with plasma components, including lipoproteins, regulates the lipid

339   receptor-mediated actions. *Biochem J* 352 Pt 3: 809-815, 2000.

340   36.   **Nofer JR, Bot M, Brodde M, Taylor PJ, Salm P, Brinkmann V, van BT, Assmann G, and**

341   **Biessen EA**. FTY720, a synthetic sphingosine 1 phosphate analogue, inhibits development of atherosclerosis

342   in low-density lipoprotein receptor-deficient mice. *Circulation* 115: 501-508, 2007.

343   37.   **Nyberg M, Egelund J, Mandrup CM, Nielsen MB, Mogensen AS, Stallknecht B, Bangsbo J, and**

344   **Hellsten Y**. Early Postmenopausal Phase Is Associated With Reduced Prostacyclin-Induced Vasodilation

345   That Is Reversed by Exercise Training: The Copenhagen Women Study. *Hypertension* 68: 1011-1020, 2016.

346   38.   **Nyberg M, Seidelin K, Andersen TR, Overby NN, Hellsten Y, and Bangsbo J**. Biomarkers of

347   vascular function in premenopausal and recent postmenopausal women of similar age: effect of exercise

348   training. *Am J Physiol Regul Integr Comp Physiol* 306: R510-R517, 2014.

349   39.   **Pappu R, Schwab SR, Cornelissen I, Pereira JP, Regard JB, Xu Y, Camerer E, Zheng YW,**

350   **Huang Y, Cyster JG, and Coughlin SR**. Promotion of lymphocyte egress into blood and lymph by distinct

351   sources of sphingosine-1-phosphate. *Science* 316: 295-298, 2007.

352   40.   **Poti F, Costa S, Bergonzini V, Galletti M, Pignatti E, Weber C, Simoni M, and Nofer JR**. Effect

353   of sphingosine 1-phosphate (S1P) receptor agonists FTY720 and CYM5442 on atherosclerosis development

354   in LDL receptor deficient (LDL-R(-)/(-)) mice. *Vascul Pharmacol* 57: 56-64, 2012.

18

355   41.   **Ruiz M, Frej C, Holmer A, Guo LJ, Tran S, and Dahlback B**. High-Density Lipoprotein-

356   Associated Apolipoprotein M Limits Endothelial Inflammation by Delivering Sphingosine-1-Phosphate to

357   the Sphingosine-1-Phosphate Receptor 1. *Arterioscler Thromb Vasc Biol* 37: 118-129, 2016.

358   42.   **Swift DL, Weltman JY, Patrie JT, Saliba SA, Gaesser GA, Barrett EJ, and Weltman A**.

359   Predictors of improvement in endothelial function after exercise training in a diverse sample of

360   postmenopausal women. *J Womens Health (Larchmt)* 23: 260-266, 2014.

361   43.   **Ward MD, Jones DE, and Goldman MD**. Overview and safety of fingolimod hydrochloride use in

362   patients with multiple sclerosis. *Expert Opin Drug Saf* 13: 989-998, 2014.

363   44.   **Wei J, Shi Y, Zhang X, Feng Y, Luo G, Zhang J, Mu Q, Tang Y, Yu Y, Pan L, Nilsson-Ehle P,**

364   **and Xu N**. Estrogen upregulates hepatic apolipoprotein M expression via the estrogen receptor. *Biochim*

365   *Biophys Acta* 1811: 1146-1151, 2011.

366   45.   **Wilkerson BA, Grass GD, Wing SB, Argraves WS, and Argraves KM**. Sphingosine 1-phosphate

367   (S1P) carrier-dependent regulation of endothelial barrier: high density lipoprotein (HDL)-S1P prolongs

368   endothelial barrier enhancement as compared with albumin-S1P via effects on levels, trafficking, and

369   signaling of S1P1. *J Biol Chem* 287: 44645-44653, 2012.

370   46.   **Wolfrum C, Howell JJ, Ndungo E, and Stoffel M**. Foxa2 activity increases plasma high density

371   lipoprotein levels by regulating apolipoprotein M. *J Biol Chem* 283: 16940-16949, 2008.

372   47.   **Wolfrum C, Poy MN, and Stoffel M**. Apolipoprotein M is required for prebeta-HDL formation and

373   cholesterol efflux to HDL and protects against atherosclerosis. *Nat Med* 11: 418-422, 2005.

374

375

376

377

19

Figure legends

Figure 1. Effect of menopausal status and exercise training on plasma apoM and S1P concentrations.

(**A**) Plasma apoM concentrations in pre- (n=36) and postmenopausal (n=37) women. Changes between

groups were analyzed using Two-way repeated measures ANOVA: menopause*exercise, P>0.05; effect of

menopause, P<0.0001; effect of training, P<0.05. (**B**) Plasma S1P concentration in pre- (n=30) and

postmenopausal (n=30) women. Changes between groups were analyzed using Two-way repeated measures

ANOVA: menopause*exercise, P>0.05; effect of menopause, P>0.05; effect of training, P<0.04. Only

women with measurements before and after training have been included. *P<0.05: significantly different

from premenopausal. #P<0.05: significantly different from before training. ApoM, apolipoprotein M; S1P,

sphingosine-1-phosphate.

Figure 2. Effect of menopausal status and exercise training on plasma S1P/apoM and apoM/HDL-C

ratios. (**A**) Plasma S1P/apoM ratio in pre- (n=30) and postmenopausal (n=30) women. Changes between

groups were analyzed using Two-way repeated measures ANOVA: menopause*exercise, P>0.05; effect of

menopause, P<0.001, effect of training P>0.05. (**B**) Plasma apoM/HDL-C ratio in pre- (n=34) and

postmenopausal women (n=36). Changes between groups were analyzed using Two-way repeated measures

ANOVA: menopause*exercise, P>0.05; effect of menopause, P>0.05, effect of training P=0.05. Only

women with measures before and after training have been included. *P<0.05: significantly different from

premenopausal. ApoM, apolipoprotein; S1P, sphingosine-1-phosphate; HDL-C, high-density lipoprotein

cholesterol.

Figure 3. Linear correlation between plasma apoM and lipid concentrations. Correlations between

plasma apoM and TC (**A**, **B**), HDL-C (**C**, **D**), LDL-C (**E**, **F**), and triglyceride (**G**, **H**) concentrations in all

women before training (n=73) and after training (n=72). Data were evaluated by Pearson's correlation.

ApoM, apolipoprotein; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-

density lipoprotein cholesterol.

20

402 **Figure 4. Linear correlations between plasma apoM and S1P concentrations.** (**A**) Pre- and

403 postmenopausal women before training (n=68). (**B**) Pre- and postmenopausal women after training (n=64).

404 Data were evaluated by Pearson´s correlation. ApoM, apolipoprotein M; S1P, sphingosine-1-phosphate.

21

406   **Table 1: Lipid levels (modified from Mandrup et al. (40))**

	Premenopausal		Postmenopausal	
	Before training **(n=36)**	**After training** **(n=36)**	**Before training** **(n=37)**	**After training** **(n=36)**
TC (mmol/l)	4.89 ± 0.7	4.75 ± 0.7#	5.64 ± 0.7*	5.48 ± 0.6*#
HDL-C (mmol/l)	1.65 ± 0.4	1.70 ± 0.4	2.00 ± 0.4*	2.03 ± 0.4*
LDL-C (mmol/l)	2.88 ± 0.7	2.72 ± 0.6#	3.26 ± 0.6*	3.17 ± 0.6*#
TRIG (mmol/l)	0.83 (0.6-1.1)	0.86 (0.7-1.1)	0.85 (0.7-1.1)	0.78 (0.7-1.1)

407   Parametric data are given as mean±SD, and non-parametric data are given as median (25-75 percentiles).

408   Data are for all available measurements. Changes between groups were analyzed using Two-way repeated

409   measures ANOVA. Only women with measurements before and after training were included in the two-way

410   repeated measures ANOVA. No significant interactions for menopause*exercise were found ($P > 0.05$) for

411   any parameter. *$P < 0.05$: significantly different from premenopausal. #$P < 0.05$: significantly different from

412   before training. TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density

413   lipoprotein cholesterol; TRIG, triglycerides.

414

415

416

417

418

22

A

B

Figure 1, Yafasova et al

A

B
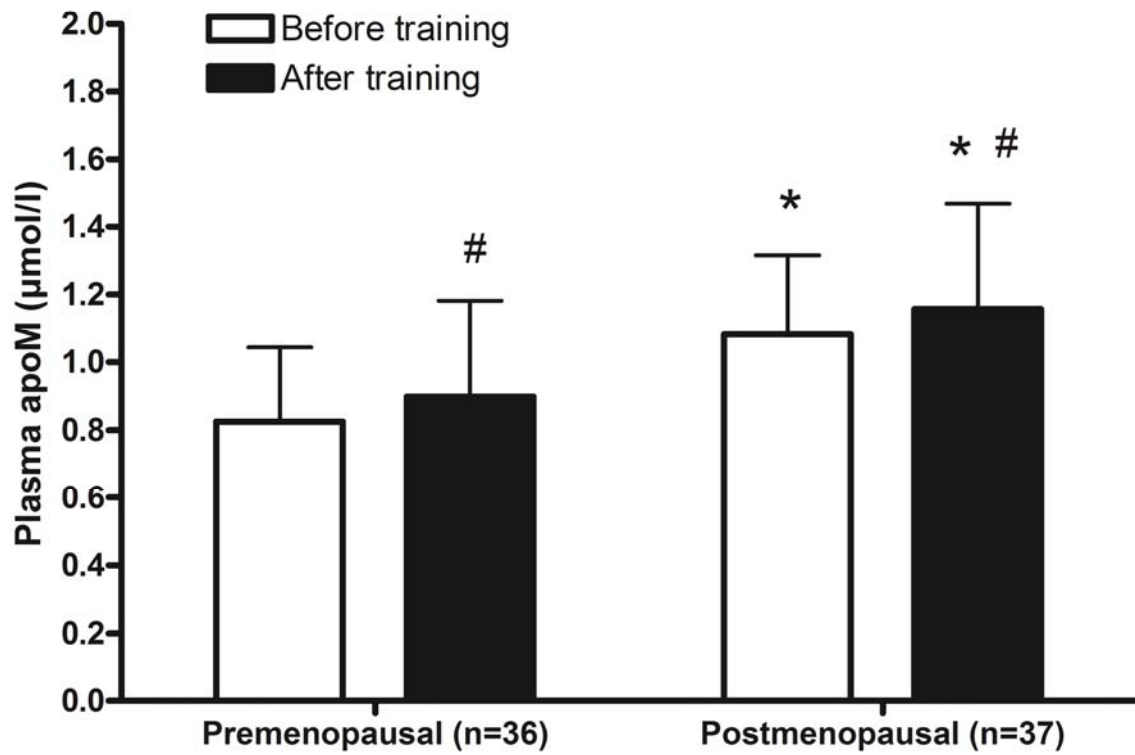
Figure 2, Yafasova et al
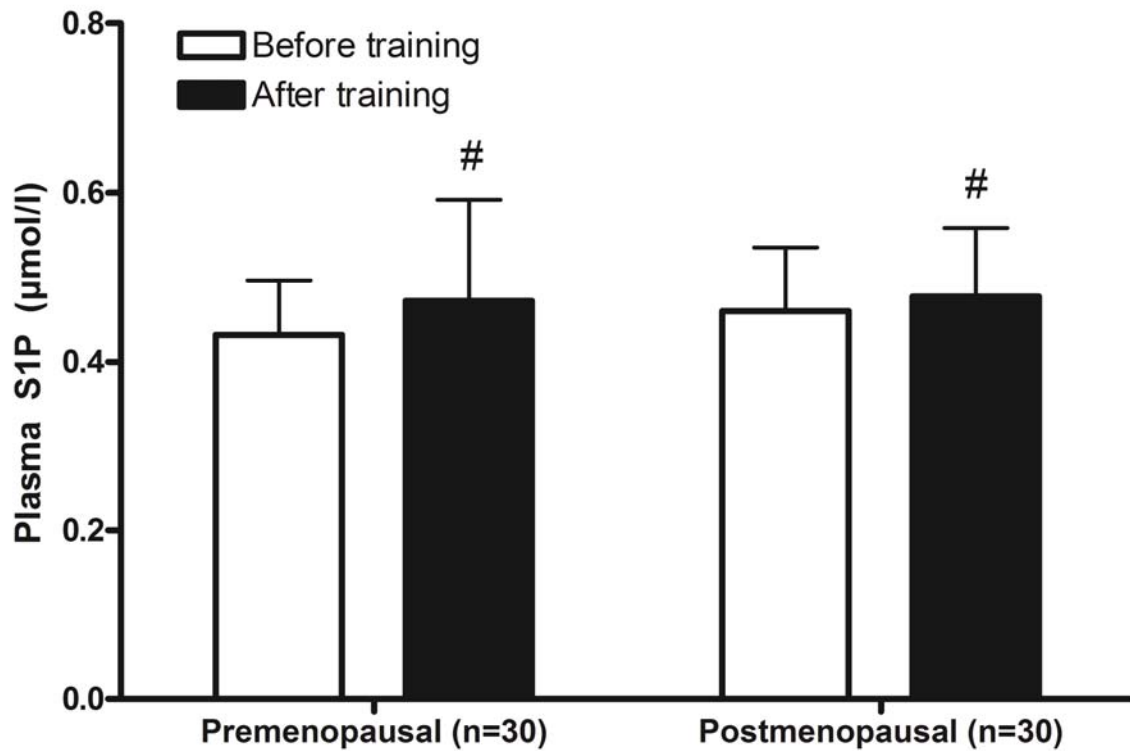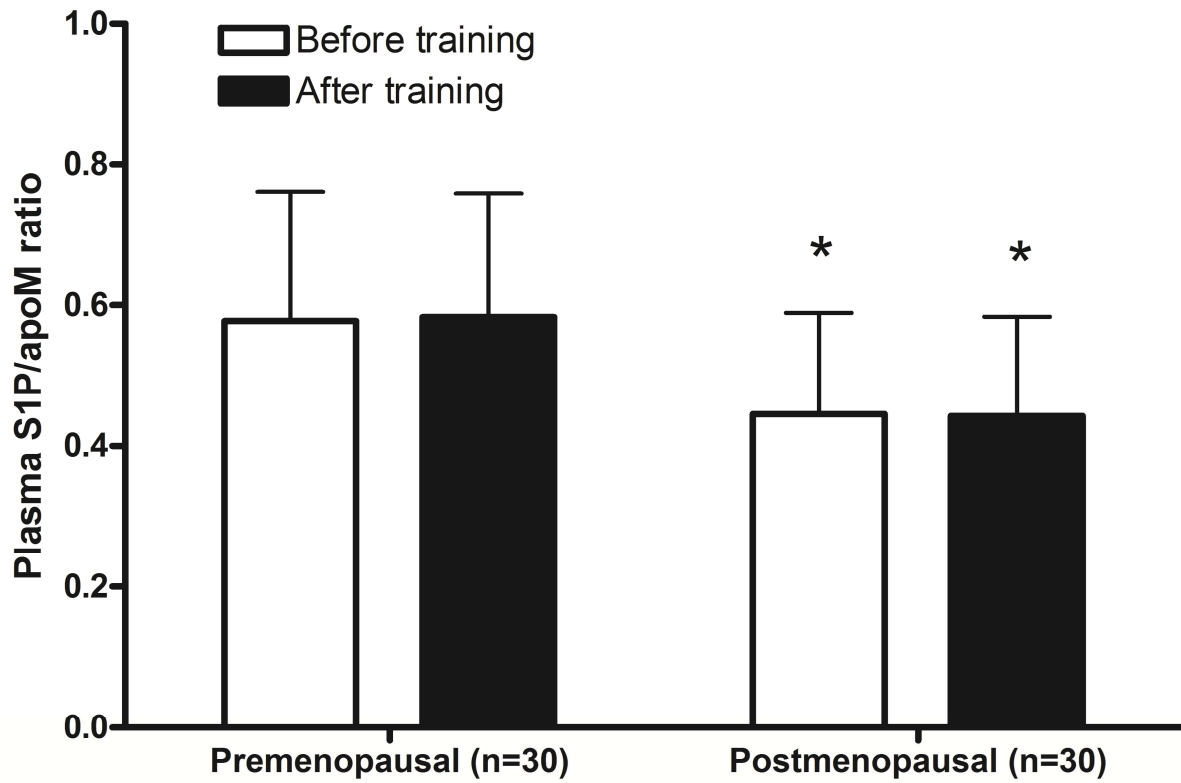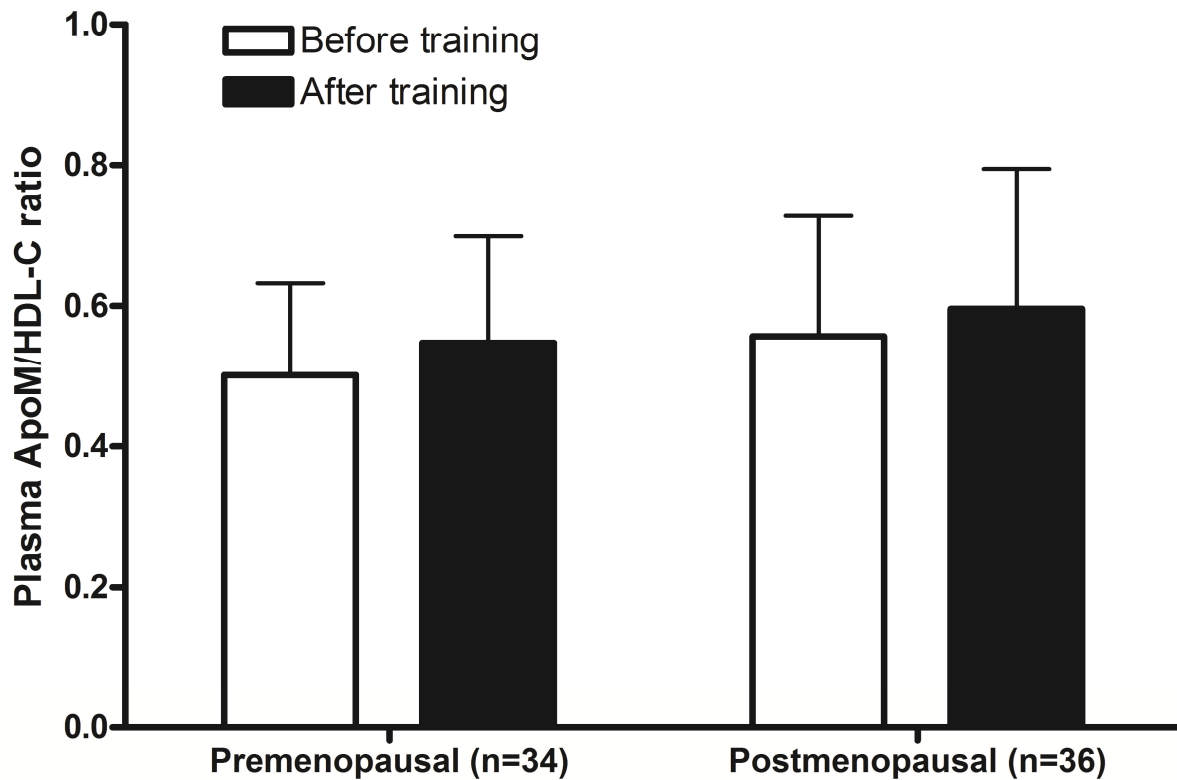
Before training (n=73)   ## After training (n=72)
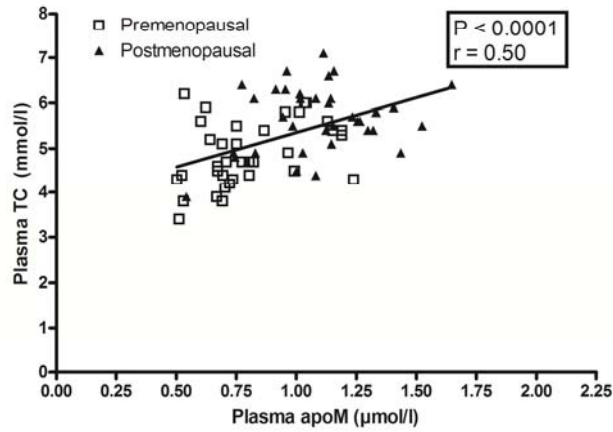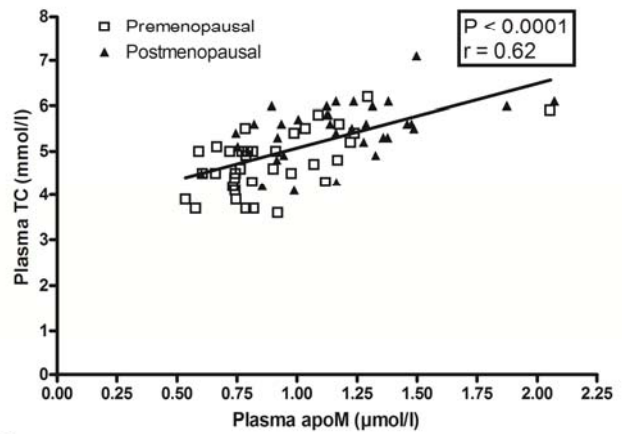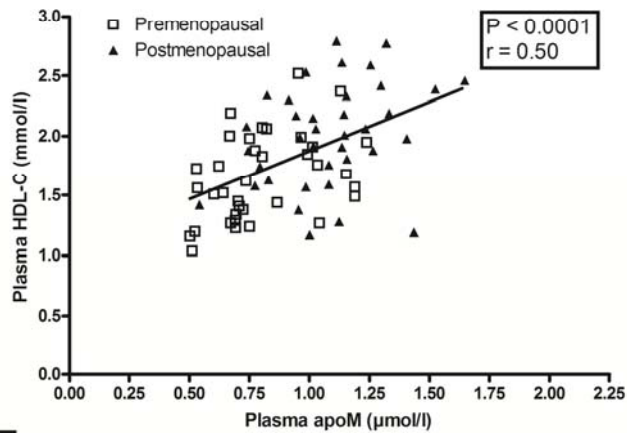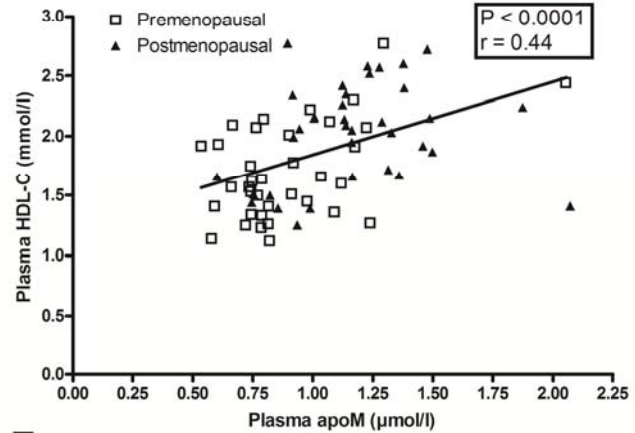
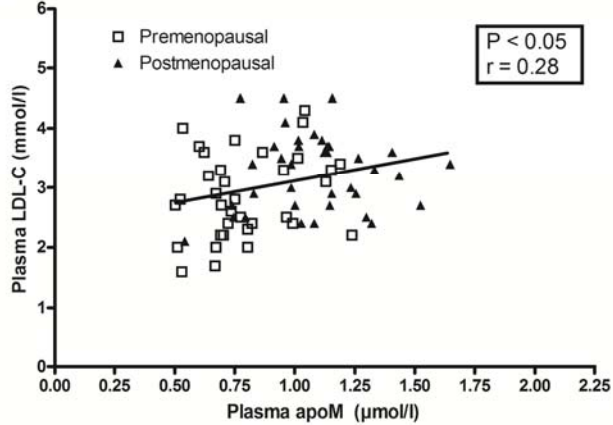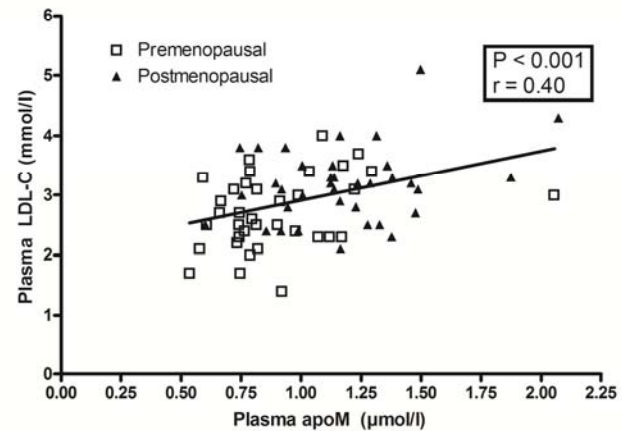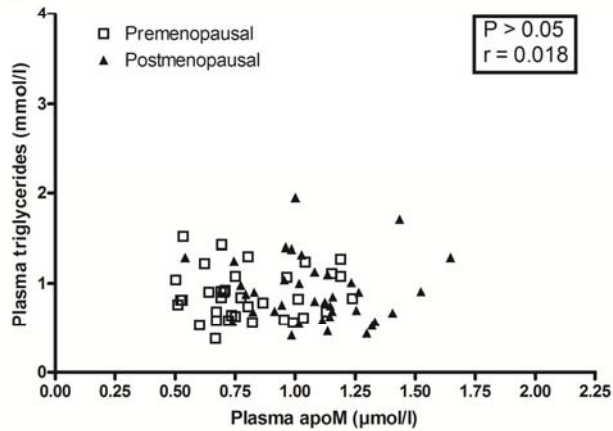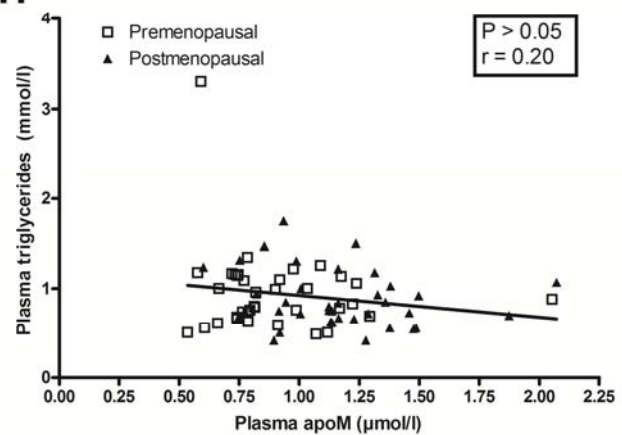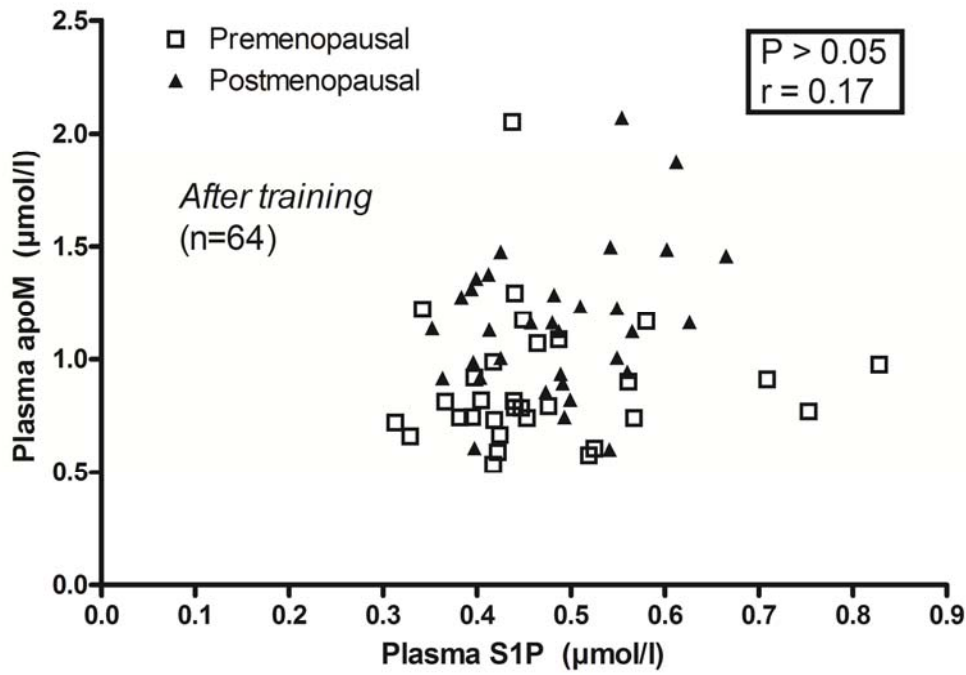**Figure 3. Yafasova et al**

A

B

Figure 4, Yafasova et al.