

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

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

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22

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.

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43 **Keywords**

44 Apolipoproteins, lipoprotein metabolism, lipids, sphingolipids, atherosclerosis, menopausal transition,
45 cardiovascular training

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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.

53 **Introduction**

54 Menopause is associated with an elevated risk of developing endothelial dysfunction (33) and metabolic
55 syndrome, including dyslipidemia and atherosclerosis (7). Long-term hormone therapy is not generally
56 recommended for postmenopausal women due to increased risk of cancer and athero-thrombotic disease
57 (32). Currently, there is a lack of mechanistic understanding of changes that cause dyslipidemia,
58 atherosclerosis, and endothelial dysfunction in postmenopausal women.

59 Apolipoprotein M (apoM) is a lipocalin mainly bound to plasma high density lipoprotein (HDL)
60 particles (11). ApoM has an important role in protecting the endothelial barrier function (8, 12) and affects
61 several potential anti-atherogenic pathways, such as reverse cholesterol transport (10, 19), formation of pre β -
62 HDL (10, 34, 47), and removal of reactive oxygen species (10, 18). Moreover, variations in the apoM gene
63 are associated with risk of cardiovascular disease (CVD) and altered plasma lipids (5). Further investigations
64 are however needed to clarify the exact role of apoM in atherosclerosis and dyslipidemia.

65 The bioactive lipid sphingosine-1-phosphate (S1P) is carried by apoM (12). ApoM-containing HDL
66 carries ~65 % of plasma S1P, while albumin carries ~35 % (27, 35). Hence, apoM is a chaperone, and its
67 biological effects are likely provided by S1P (36, 40). S1P acts through five G-protein-coupled receptors
68 (S1P_{r1-5}) (24), affecting diverse processes such as protection of the endothelial barrier (8, 12, 45), regulation
69 of angiogenesis (16), promotion of lymphocyte trafficking (39), and activation of endothelial nitric oxide
70 synthase (eNOS) (23). Accordingly, S1P has been implicated in several diseases, including inflammatory
71 diseases and atherosclerosis (30).

72 Exercise training decreases CVD risk by effects on endothelial function (21) and plasma lipids,
73 including in postmenopausal women (4, 28, 42). This finding is of particular interest as the menopausal
74 transition is accompanied by increased prevalence of dyslipidemia (7, 14, 15, 25), and endothelial
75 dysfunction (33). The apoM/S1P axis is associated with endothelial function (8, 12) and plasma lipids (5),
76 which are all influenced negatively by the menopausal transition and can be improved with exercise training.
77 The effect of menopause and exercise training on the apoM/S1P axis itself is unknown, and further
78 investigation is needed in order to understand if apoM and S1P are involved in menopausal and exercise-
79 induced changes. This has prompted the present study on how plasma apoM and S1P concentrations are

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.

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84 **Materials and methods**

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

90 *Participants* - In the present study, we used samples from 38 premenopausal women and 37 postmenopausal
91 women from the Copenhagen Women Study (31, 37). All included participants were healthy non-smokers
92 with no excessive alcohol intake and a body mass index of 18.5-30 kg/m². The premenopausal women had
93 regular menstrual cycles and were not using hormonal contraceptives. The postmenopausal women had not
94 experienced a menstrual cycle for at least 1 year and were not receiving hormone therapy. All women were
95 physically active less than 2 hours per week prior to the training intervention.

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

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

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 \pm 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

127 **Results**

128 *Characteristics of the participants* - Before the training intervention, the premenopausal women were 49.2±2
129 years old. The postmenopausal women were 53.3±3 years old and 3.1±1 years past their final menstruation.

130 *Effects of menopausal status* - Before training, the plasma apoM concentration was 32 % higher in the
131 postmenopausal than in the premenopausal women (1.08±0.2 µmol/l and 0.82±0.2 µmol/l, respectively)
132 (p<0.0001) (Figure 1A). The plasma S1P concentration was similar in the two groups (p>0.05) (Figure 1B).
133 Hence, the S1P/apoM ratio was 26 % lower in postmenopausal than premenopausal women before training
134 (0.45±0.1 and 0.61±0.2, respectively) (p<0.001) (Figure 2A). In accordance with previous findings from the
135 Copenhagen Women Study (40), plasma TC (p<0.0001), HDL-C (p<0.001), and LDL-C (p<0.01)
136 concentrations were higher in this subset of postmenopausal compared with premenopausal women, while
137 the plasma triglyceride concentration was similar in the two groups (p>0.05) (Table 1). The plasma
138 apoM/HDL-C ratio was also similar in the two groups (p>0.05) (Figure 2B).

139 *Effects of exercise training* - The three-month high-intensity training intervention increased the plasma apoM
140 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
141 µmol/l in postmenopausal women (p<0.05) (Figure 1A). The plasma S1P concentration increased from
142 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
143 postmenopausal women (p<0.05) (Figure 1B). The plasma S1P/apoM ratio was not affected by training
144 (p>0.05) (Figure 2A). As previously reported (40), plasma TC (p=0.01) and LDL-C (p<0.01) concentrations
145 decreased after the training period, whereas plasma HDL-C and triglyceride concentrations did not change
146 (p>0.05) (Table 1). The plasma apoM/HDL-C ratio was increased from 0.50±0.1 to 0.54±0.2 in
147 premenopausal women and from 0.56±0.2 to 0.60±0.2 in postmenopausal women, which was borderline
148 statistically significant (p=0.05) (Figure 2B).

149 *Correlations between apoM and lipids in plasma* - The plasma apoM concentration correlated positively
150 with plasma TC, HDL-C, and LDL-C concentrations both before and after the training intervention, while no
151 correlation was found between the plasma apoM and triglyceride concentration (Figure 3). Also, there was

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).

155 **Discussion**

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

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

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.

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

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.

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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, Charnas 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.

- 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.

- 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.

- 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.

- 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.

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378 **Figure legends**

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

380 (A) Plasma apoM concentrations in pre- (n=36) and postmenopausal (n=37) women. Changes between
381 groups were analyzed using Two-way repeated measures ANOVA: menopause*exercise, $P>0.05$; effect of
382 menopause, $P<0.0001$; effect of training, $P<0.05$. (B) Plasma S1P concentration in pre- (n=30) and
383 postmenopausal (n=30) women. Changes between groups were analyzed using Two-way repeated measures
384 ANOVA: menopause*exercise, $P>0.05$; effect of menopause, $P>0.05$; effect of training, $P<0.04$. Only
385 women with measurements before and after training have been included. * $P<0.05$: significantly different
386 from premenopausal. # $P<0.05$: significantly different from before training. ApoM, apolipoprotein M; S1P,
387 sphingosine-1-phosphate.

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

389 **ratios.** (A) Plasma S1P/apoM ratio in pre- (n=30) and postmenopausal (n=30) women. Changes between
390 groups were analyzed using Two-way repeated measures ANOVA: menopause*exercise, $P>0.05$; effect of
391 menopause, $P<0.001$, effect of training $P>0.05$. (B) Plasma apoM/HDL-C ratio in pre- (n=34) and
392 postmenopausal women (n=36). Changes between groups were analyzed using Two-way repeated measures
393 ANOVA: menopause*exercise, $P>0.05$; effect of menopause, $P>0.05$, effect of training $P=0.05$. Only
394 women with measures before and after training have been included. * $P<0.05$: significantly different from
395 premenopausal. ApoM, apolipoprotein; S1P, sphingosine-1-phosphate; HDL-C, high-density lipoprotein
396 cholesterol.

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

398 plasma apoM and TC (A, B), HDL-C (C, D), LDL-C (E, F), and triglyceride (G, H) concentrations in all
399 women before training (n=73) and after training (n=72). Data were evaluated by Pearson's correlation.
400 ApoM, apolipoprotein; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-
401 density lipoprotein cholesterol.

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.

405 **Tables**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.

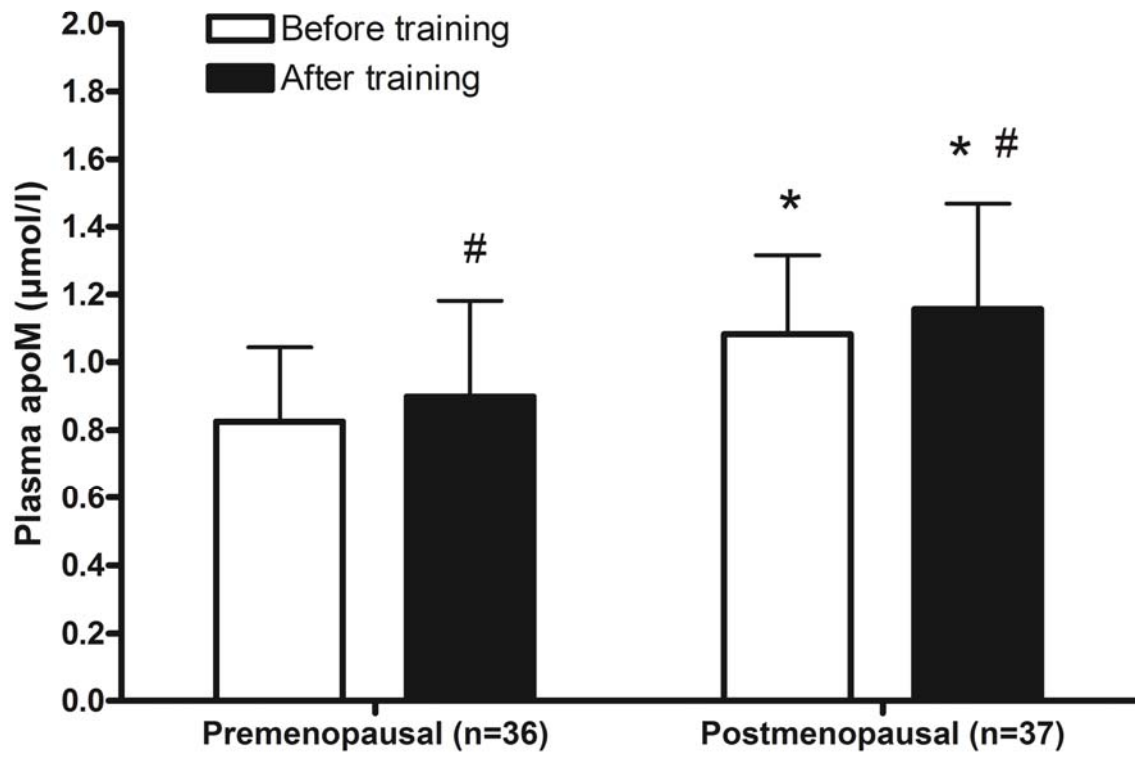
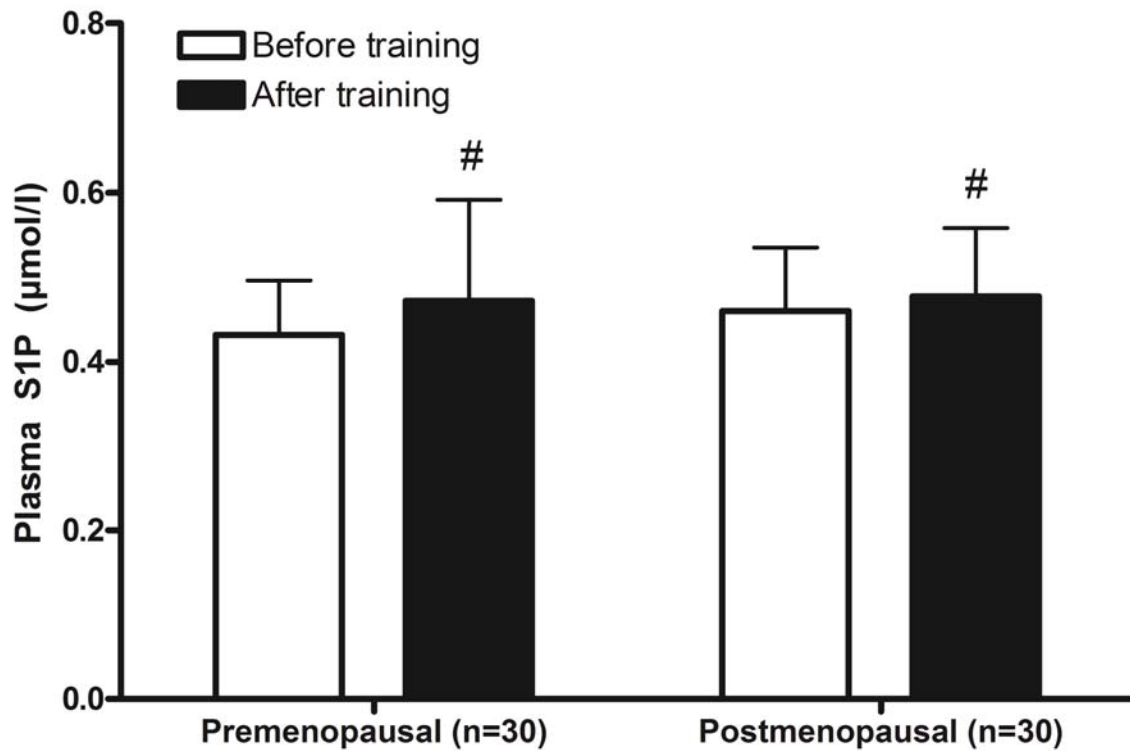
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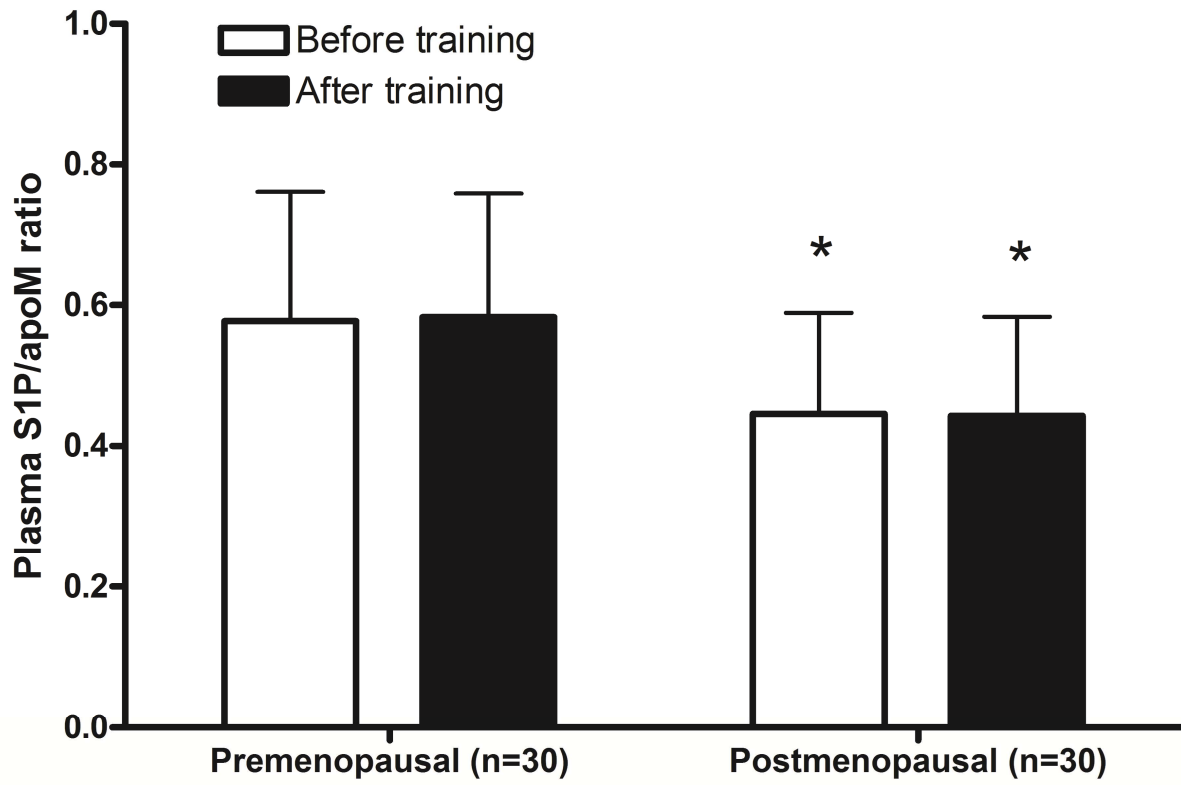
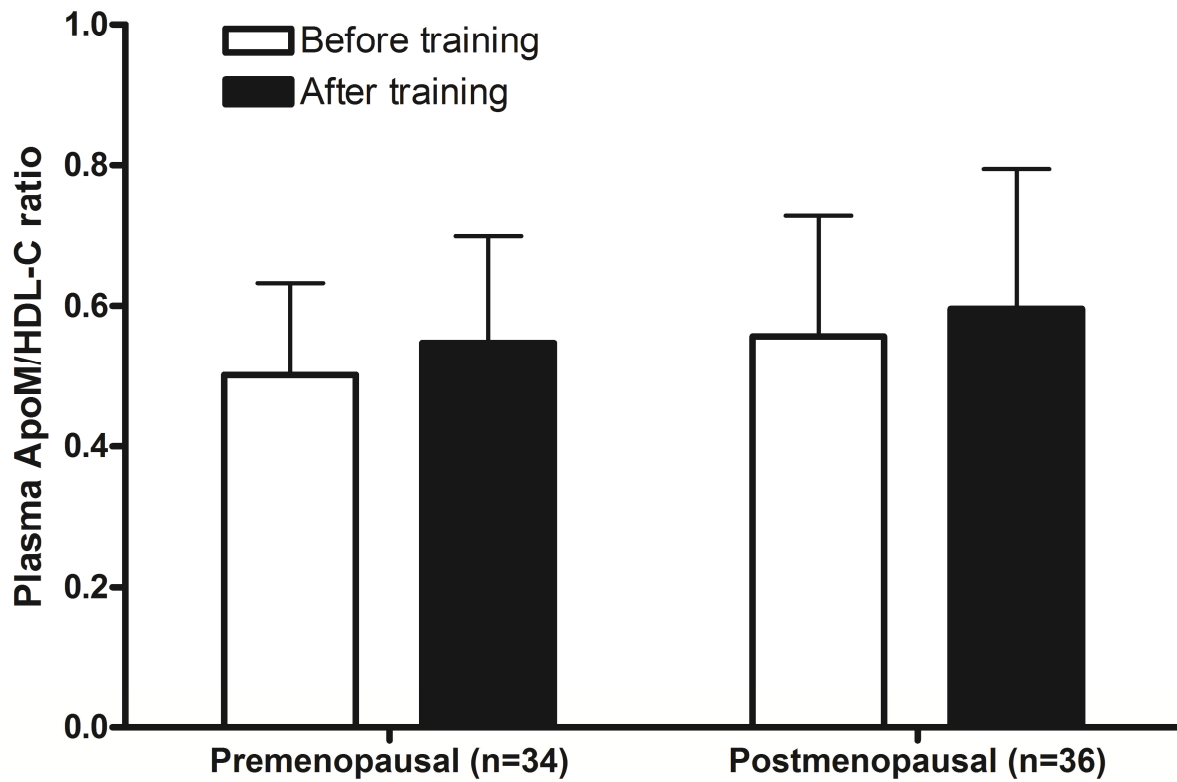
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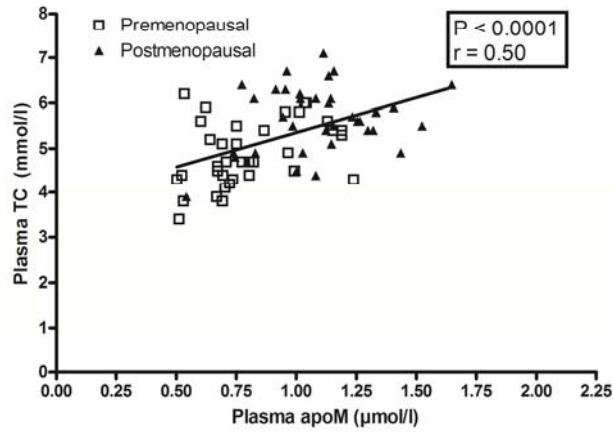
A**B**

A**B**

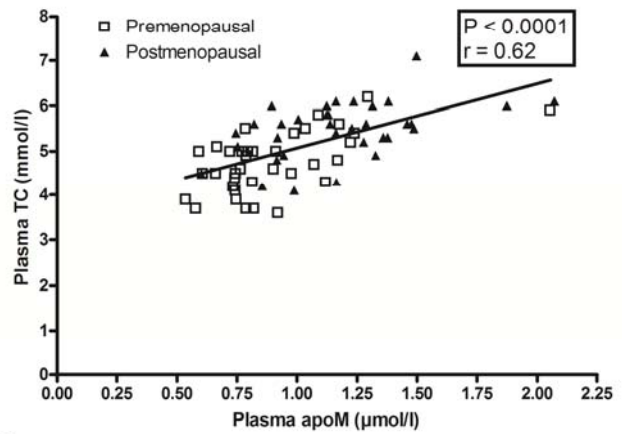
Before training (n=73)

After training (n=72)

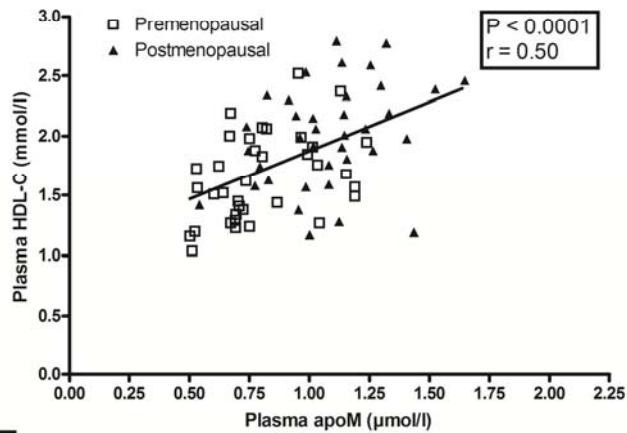
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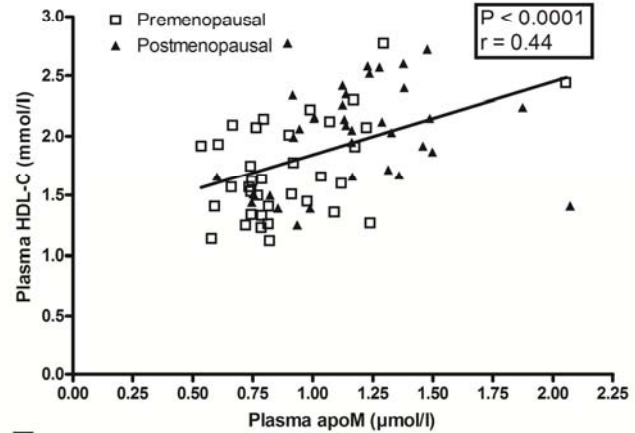
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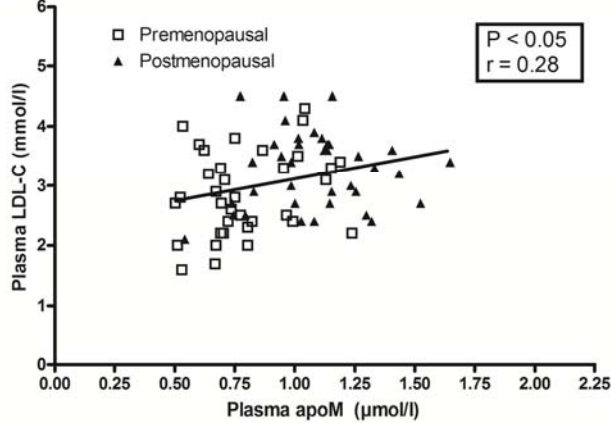
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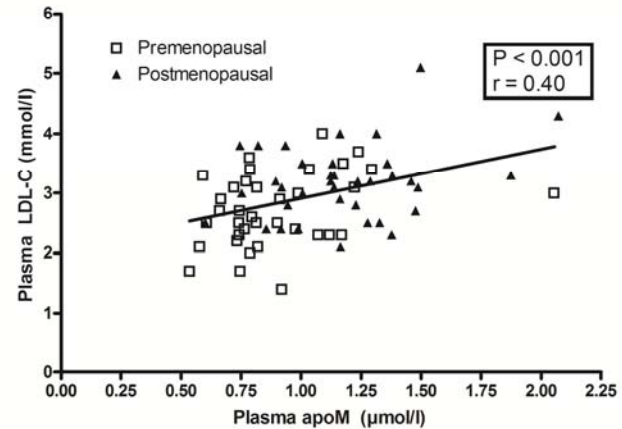
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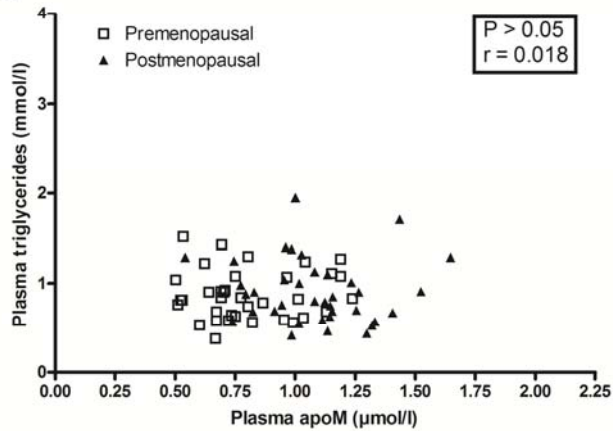
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F



G



H

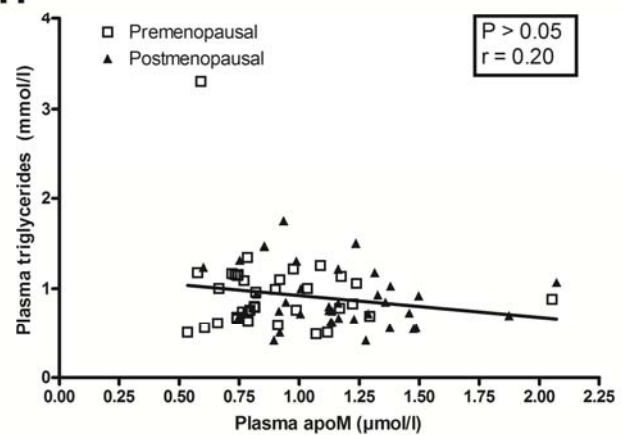
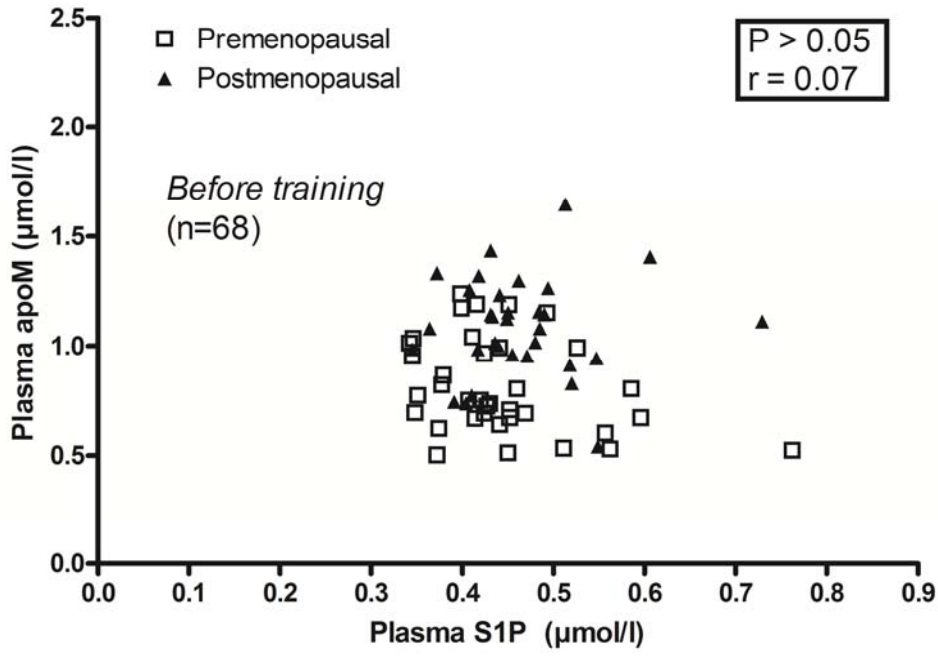


Figure 3, Yafasova et al

A



B

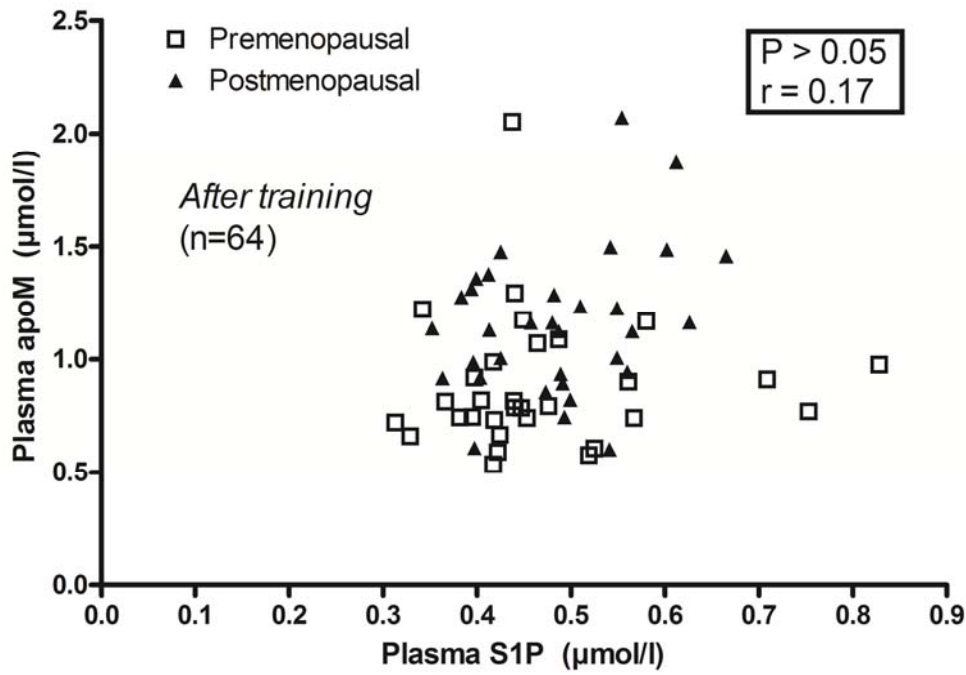


Figure 4. Yafasova et al.