

# *Impact of meal fatty acid composition on postprandial lipaemia, vascular function and blood pressure in postmenopausal women*

Article

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1 **Impact of meal fatty acid composition on postprandial lipaemia, vascular function and blood**  
2 **pressure in postmenopausal women**

3  
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22  
23 Running title: Meal fat, lipaemia and vascular function

24  
25 **Abbreviations in the text**

26 ACE: angiotensin-converting enzyme; Apo: apolipoprotein; AUC: area under the curve; BMI: body  
27 mass index; CETP: cholesteryl ester transfer protein; CM: chylomicron; CMR: CM remnants;  
28 CVD: cardiovascular disease; FMD: flow-mediated dilatation; HDL: high density lipoprotein;  
29 HDL-C: HDL cholesterol; LDL: low density lipoprotein; LDL-C: LDL cholesterol; LPL:  
30 lipoprotein lipase; MUFA: monounsaturated fatty acid; NEFA: non-esterified fatty acids; NO: nitric  
31 oxide; PUFA: polyunsaturated fatty acid; RAS: renin-angiotensin system; RCT: randomised clinical  
32 trial; RE: Retinyl esters; ROS: reactive oxygen species; Sf: Svedberg flotation rate; SFA: saturated  
33 fatty acid; TAG: triacylglycerol; TC: total cholesterol; TRL: TAG-rich lipoprotein; VLDL: very  
34 low density lipoprotein.

35

## 36 **Abstract**

37 Cardiovascular diseases (CVD) are the leading cause of death in women globally, with aging  
38 associated with progressive endothelial dysfunction and increased CVD risk. Natural menopause is  
39 characterised by raised non-fasting triacylglycerol (TAG) concentrations and impairment of  
40 vascular function compared with premenopausal women. However, the mechanisms underlying the  
41 increase in CVD risk after women have transitioned through the menopause are unclear. Dietary fat  
42 is an important modifiable risk factor in relation to both postprandial lipaemia and vascular  
43 reactivity. Meals rich in saturated and monounsaturated fatty acids are often associated with greater  
44 postprandial TAG responses compared with those containing n-6 polyunsaturated fatty acids, but  
45 studies comparing the effects of these fatty acids on vascular function during the postprandial phase  
46 are limited, particularly in postmenopausal women. A systematic search of the literature identified  
47 778 publications describing acute postprandial test meal studies including postmenopausal women.  
48 The impact of fat-rich meals on postprandial lipaemia was reported in 7 relevant studies, of which  
49 meal fat composition was compared in one study described by three papers. An additional study  
50 determined the impact of a high fat meal on vascular reactivity. Although there is moderately  
51 consistent evidence to suggest detrimental effects of high fat meals on postprandial lipaemia in  
52 postmenopausal women (compared with premenopausal women), there is insufficient evidence to  
53 establish the impact of meals of differing fat composition. Furthermore, there is no robust evidence  
54 to conclude the impact of meal fatty acids on vascular function or blood pressure. In conclusion,  
55 there is an urgent requirement for suitably powered robust randomised controlled trials to  
56 investigate the impact of meal fat composition on postprandial novel and established CVD risk  
57 markers in postmenopausal women, an understudied population at increased cardiometabolic risk.  
58

## 59 **Introduction**

60 Cardiovascular diseases (CVD) which include coronary heart disease (myocardial infarction and  
61 angina), stroke and peripheral vascular disease <sup>(1)</sup> are a key contributor to the burden of disease  
62 globally <sup>(2)</sup>. Over the past 50 years, the prevalence of CVD has fallen in Western populations,  
63 however, CVD are currently the major cause of death in women in the UK, accounting for 32% of  
64 all deaths <sup>(3)</sup>. Furthermore, the prevalence of CVD is dramatically increasing in other areas,  
65 including Eastern Europe, Asia and the Indian subcontinent <sup>(4)</sup>.

66 The aetiology for CVD is multifactorial and includes several modifiable risk factors, such as  
67 cigarette smoking, a sedentary lifestyle, obesity, elevated blood pressure, dyslipidaemia, type 2  
68 diabetes mellitus, and non-modifiable factors, such as advancing ageing, sex, family history of heart  
69 disease and ethnicity <sup>(5; 6)</sup>. Among the non-modifiable risk factors, ageing is associated with

70 progressive endothelial dysfunction (characterised by a loss of vascular wall homeostasis leading to  
71 a decrease in vascular reactivity and raised blood pressure) in both sexes, although it appears to  
72 occur earlier in men than women <sup>(7)</sup>. The most prominent sex related difference in physiological  
73 ageing is the menopause (cessation of menstruation) in women, which usually occurs between the  
74 ages of 45 and 55 y, with 51 y being the average age of menopause in the UK <sup>(8)</sup>. This natural part  
75 of aging in women contributes a significant cardiovascular milestone in terms of both physiology  
76 and pathology since oestrogen deficiency is known to impair lipid metabolism and endothelial  
77 function, and the menopause is a recognised risk factor for CVD <sup>(9)</sup>. It has further been shown by  
78 Schouw *et al.* <sup>(10)</sup> that for each year of delay in the age of onset of the natural menopause, CVD risk  
79 falls by 2%.

80

### 81 **Postprandial lipaemia**

82 Kolovou *et al.* defined postprandial lipaemia as a complex syndrome characterised by non-fasting  
83 hypertriacylglycerolaemia and its augmentation is associated with increased risk of cardiovascular  
84 events <sup>(11)</sup>. Following a fat containing meal, there is a transient rise in circulating triacylglycerol  
85 (TAG) rich lipoproteins (TRL), such as chylomicrons (CM) and very low density lipoprotein  
86 (VLDL). After entering the circulation, the CM TAG is hydrolysed into non-esterified fatty acids  
87 (NEFA) by lipoprotein lipase (LPL) forming cholesterol ester rich CM remnants, which are cleared  
88 by the liver by receptor-mediated uptake. VLDL follows a similar route of metabolism in the  
89 circulation as CM particles, but VLDL are hydrolysed at a slower rate, as the larger CM are  
90 preferential substrate for LPL. VLDL TAG depletion produce smaller VLDL (intermediate density  
91 lipoprotein or VLDL remnants), ultimately a proportion of which will be metabolised to low density  
92 lipoprotein (LDL). LDL particles are cleared from the bloodstream via the hepatic LDL receptor  
93 using apoB-100 as a ligand. During the postprandial period, there is an accumulation of TRL in the  
94 circulation due to competition between intestinal and hepatic TRL for the same lipolytic and  
95 receptor mediated uptake <sup>(12)</sup>. A delayed clearance of TRLs in the circulation enhances the  
96 accumulation of TRL particles carrying acceptor sites for the cholesteryl ester transfer protein  
97 (CETP) which transfers TAG from TRLs (CM and VLDL) and exchanges it with cholesteryl esters  
98 from high density lipoprotein (HDL) and LDL. Remodelling of the lipid content of the LDL and  
99 HDL particles make them suitable substrates for LPL and hepatic lipase, leading to the formation of  
100 smaller denser LDL (LDL<sub>3</sub>) and HDL (HDL<sub>3</sub>) particles <sup>(13)</sup>. HDL<sub>3</sub> is rapidly removed from the  
101 circulation decreasing circulating HDL cholesterol (HDL-C) concentrations, which is one proposed  
102 mechanism for the inverse association between exaggerated postprandial lipaemia and CVD risk  
103 <sup>(14)</sup>. Another possible mechanism is that LDL<sub>3</sub> has a lower binding affinity to the LDL-receptor,

104 reducing their rate of clearance from the circulation and enabling them to infiltrate the arterial wall  
105 <sup>(13)</sup>.

106 Since atherosclerosis is now also considered to be a postprandial phenomenon, three large  
107 prospective cohort studies aimed to determine the link between cardiovascular events and non-  
108 fasting TAG <sup>(15; 16; 17)</sup>. In the Norwegian Counties Study, hazard ratios of 1.2 and 1.03 for deaths  
109 from CVD per 1 mmol/l increase in non-fasting TAG were reported in women and men,  
110 respectively, after 27 years of follow up in a total of 86,261 participants <sup>(17)</sup>. Furthermore, the  
111 Copenhagen City Heart Study that followed 7581 women and 6391 men for 31 years showed that  
112 relative to women with non-fasting TAG of <1 mmol/L, hazard ratios for myocardial infarction  
113 ranged from 1.5 for women with TAG between 1.0-1.99 mmol/L rising to 4.2 for those with TAG  
114  $\geq 5$  mmol/L <sup>(16)</sup>. However, the corresponding hazard ratios for men were 1.3 and 2.1, respectively. In  
115 the Women's Health Study, fasting (n=20,118) and non-fasting (n=6391) TAG predicted  
116 cardiovascular events after 11.4 years of follow up after adjusting for age, blood pressure, smoking  
117 status and hormone therapy. The authors also reported that the strongest association between  
118 cardiovascular events and non-fasting TAG occurred 2-4 h after the last meal, with the association  
119 declining as the fasting time increased <sup>(15)</sup>. These studies have demonstrated the greater importance  
120 of non-fasting than fasting TAG concentrations as a predictor of CVD risk in women than men.

121 The relationship between postprandial lipaemia and CVD according to menopausal status is  
122 a topic of current interest. The impact of menopausal status on the variability of the postprandial  
123 lipaemic responses have been reported in a number of studies <sup>(18; 19; 20; 21)</sup> (Supplemental material 1).  
124 In general, premenopausal women have lower postprandial triacylglycerol (TAG) responses than  
125 men <sup>(22; 23; 24; 25)</sup>, which is in contrast to the higher reported responses observed in postmenopausal  
126 women compared with men of a similar age <sup>(26)</sup>. In response to a single oral vitamin A fat loading  
127 test, van Beek *et al.* <sup>(18)</sup> investigated whether a natural menopause was associated with reduced  
128 protection from exaggerated postprandial lipaemia. Higher concentrations of postprandial plasma  
129 TAG and retinyl palmitate (an indirect marker of CM) were observed in postmenopausal women  
130 compared with premenopausal women of similar age, BMI, daily energy and fat intake, *APOE*  
131 genotype, LPL activity, and HDL-C concentration, even after adjusting for the confounding effect  
132 of fasting TAG. Relative to premenopausal women, Masding *et al.* <sup>(20)</sup>, Schoppen *et al.* <sup>(19)</sup> and  
133 Jackson *et al.* <sup>(21)</sup> also reported significantly higher postprandial TAG responses after single and  
134 sequential fat-rich test meals in healthy postmenopausal women. Although raised LDL cholesterol  
135 (LDL-C) is an established risk factor for CVD, large prospective studies have shown non-fasting  
136 TAG to be a better predictor of CVD risk in women than fasting LDL-C <sup>(27; 28; 29)</sup>. Post-hoc analysis  
137 of the The Dietary Studies: Reading Unilever Postprandial Trials (DISRUPT) menopausal groups  
138 according to age also revealed a greater increase in non-fasting TAG than fasting LDL-C during the

139 late premenopausal period suggesting that age and the menopause have a differential impact on  
140 these two lipid CVD risk biomarkers <sup>(21)</sup>.

141 A major biochemical change that occurs in women after the menopause is a reduction in the  
142 secretion of endogenous oestrogen and progesterone <sup>(30)</sup>. These hormones not only play a major role  
143 in sexual physiology, but are also involved in various physiological processes associated with the  
144 vasculature and lipid metabolism. A reduction in oestrogen following the menopause has been  
145 shown to have a detrimental impact on lipoprotein metabolism, vascular reactivity and blood  
146 pressure (Figure 1). For example, there is much evidence to suggest that oestrogen (endogenous and  
147 exogenous) lowers fasting plasma concentrations of total and LDL-C, lipoprotein (a) and  
148 apolipoprotein B, whilst elevating HDL-C and apolipoproteins AI and AII <sup>(31; 32; 33)</sup>. The impact of  
149 oestradiol (the predominant type of oestrogen) on lipid metabolism is reported to contribute 25% of  
150 its protective effects for fasting lipid profile <sup>(34)</sup>. One possible mechanism to explain this effect, that  
151 was identified in *in vitro* animal studies, was an increase in the number of high affinity LDL  
152 receptors on liver cell membranes that enhance LDL uptake by the liver <sup>(33)</sup>. Exaggerated  
153 postprandial lipaemia is observed after the menopause <sup>(18)</sup> but the administration of even short term  
154 (two to six weeks) oestradiol therapy reduces the menopause-related rise in postprandial TAG in  
155 postmenopausal women <sup>(35; 36)</sup>. These findings indicate that 17 $\beta$ -estradiol may accelerate the  
156 postprandial clearance of TRL and have a beneficial effect on postprandial lipaemia.

157

## 158 **Vascular function and blood pressure**

159 Vascular function is a measure of cardiovascular health. The components of impaired vascular  
160 function, including hypertension <sup>(37; 38)</sup>, arterial stiffness <sup>(39)</sup> and impaired endothelial dependent  
161 vasodilation (endothelial dysfunction) <sup>(40; 41)</sup>, are all associated with cardiovascular mortality. In a  
162 healthy blood vessel, the endothelium, which is comprised of a monolayer of endothelial cells that  
163 lines the blood vessel walls, regulates vascular wall homeostasis by immediately responding to  
164 blood-borne and locally produced stimuli to regulate blood flow, blood pressure and vascular tone.  
165 It does so by maintaining a precise balance between the release of endothelium-derived vasodilators  
166 (such as nitric oxide (NO)), and vasoconstrictors (such as endothelin-I), which actively regulates  
167 vascular permeability to plasma constituents, platelets and leukocyte adhesion molecules <sup>(42)</sup> as well  
168 as aggregation and thrombosis <sup>(43)</sup>. However, when the production or bioavailability of NO is  
169 reduced, the resulting imbalance of these vasoactive substances disrupts vascular homeostasis. This  
170 ‘endothelial dysfunction’ is characterised by vasoconstriction, increased expression of adhesion  
171 molecules and pro-inflammatory cytokines, platelet activation and increased oxidative stress <sup>(44)</sup>,  
172 and is becoming increasingly recognised as an important step for the initiation of coronary  
173 atherosclerosis <sup>(45)</sup> and CVD risk in postmenopausal women <sup>(46)</sup>. There is supporting evidence of

174 impaired endothelial function after the menopause, which has been associated with a lack of  
175 endogenous oestrogen <sup>(7; 47)</sup>.

176 There are a number of non-invasive methods which are used to evaluate endothelial function  
177 <sup>(48)</sup>. Flow-mediated dilatation (FMD) is the gold standard technique that uses ultrasound to assess  
178 endothelium-dependent vasodilation in the conduit arteries in the peripheral circulation and is used  
179 as a surrogate measure of NO production <sup>(49)</sup>. It is now recognised as a screening tool to assess  
180 future CVD risk <sup>(40; 46; 50; 51)</sup>. Rossi *et al.* reported that postmenopausal women in the lowest tertile of  
181 % FMD response (reflective of impaired vascular reactivity) had the greatest relative risk of  
182 cardiovascular events. Furthermore, it has been shown that endothelial function is impaired across  
183 the stages of the menopause transition in healthy women with the highest % FMD response reported  
184 in premenopausal women, with a progressive decline in perimenopausal and postmenopausal  
185 women, respectively <sup>(52)</sup>. This suggests the perimenopausal stage (the transition towards the  
186 menopause where oestrogen production starts to fall) is a crucial turning point in women where  
187 changes in CVD risk commence.

188 Majmudar *et al.* <sup>(53)</sup> revealed that menopausal status is associated with reduced NO activity,  
189 which is restored with oestrogen replacement therapy and may be an important mechanism  
190 facilitating the detrimental effect of the menopause on CVD risk and mortality. Another study that  
191 acutely administered oestrogen (17 $\beta$ -oestradiol) to postmenopausal women demonstrated protective  
192 effects on forearm microvascular responses to both endothelium-dependent (acetylcholine) and -  
193 independent vasodilation (sodium nitroprusside) via improvements in NO activity <sup>(54)</sup>. Impaired  
194 blood flow in the microcirculation has been proposed to be an indicator of initial endothelial  
195 damage in subjects at risk of CVD <sup>(55)</sup>. Furthermore, it has been repeatedly shown that 17 $\beta$ -  
196 oestradiol stimulates the production of vasodilatory prostaglandins, such as prostacyclin (PGI<sub>2</sub>) <sup>(56;</sup>  
197 <sup>57)</sup>. These vascular effects are believed to be partly responsible for the long-term benefit of  
198 oestrogen therapy on cardiovascular risk in postmenopausal women. However, findings from the  
199 Women's Health Initiative study have questioned the benefits of oestrogen therapy, reporting that  
200 oestrogen therapy did not protect against myocardial infarction or coronary death after a short (6.8  
201 y) or longer-term (18 y) follow-up relative to a placebo, although the findings did show a lower risk  
202 of coronary heart disease among the younger postmenopausal women (50 to 59 y) <sup>(58; 59)</sup> <sup>(59)</sup>. More  
203 recently, a systematic review involving 43,637 women reported the number of cardiovascular  
204 events to increase following the long-term (>1 y) use of oestrogen therapy <sup>(60)</sup>. In contrast, there is  
205 much evidence to suggest that oestrogens (endogenous and exogenous) have several cardio-  
206 protective effects (Figure 1) <sup>(32; 61; 62)</sup>. These include reductions in plasma markers of endothelial  
207 activation (E-selectin) and increased fibrinolytic activity (increased factor VII; reduced fibrinogen,



plasminogen activator inhibitor type 1 and tissue plasminogen activator)<sup>(32; 63)</sup>. However, increased markers of inflammation (C-reactive protein) and hypercoagulability have also been reported<sup>(32; 61)</sup>.

Hypertension (high blood pressure) is one of the main age-related disorders in postmenopausal women<sup>(64; 65)</sup>, which has been identified as a leading risk factor for myocardial infarction and stroke in women<sup>(66)</sup>. The renin-angiotensin system (RAS) is a hormonal cascade, which plays a key role in the regulation of fluid and electrolyte balance, and arterial blood pressure. Upon activation of the RAS cascade, angiotensin II is produced in the liver by angiotensin-converting enzyme (ACE) following conversion of angiotensin I to angiotensin II<sup>(67)</sup>. Angiotensin II is a potent vasoconstrictor which degrades bradykinin (a vasodilator) causing arterioles to constrict, resulting in increased blood pressure<sup>(68)</sup>. It is well documented in the literature that oestrogen acts on RAS at different points of the cascade including the inhibition of ACE activity. *In vitro* and *in vivo* animal studies have also demonstrated the potential effects of oestrogen on the endothelial-dependent vasodilator response to acetylcholine due to oestrogen induced sensitisation measured in coronary and uterine arteries<sup>(69; 70; 71)</sup>. Loss of oestrogen-dependent cardiovascular protection induces endothelial dysfunction, and may also be involved in the activation of the RAS cascade. Evidence from both clinical and animal studies have shown an inverse association between oestrogen and the activation of RAS<sup>(72; 73; 74; 75)</sup>. This has been proposed to occur due to oestrogen induced downregulation of angiotensin receptor I expression leading to an augmented level of angiotensin II<sup>(73)</sup> (which is a major component of the RAS system) and has several harmful effects on the vascular wall including vasoconstriction, vascular smooth muscle cell proliferation, reactive oxygen species (ROS) generation, and endothelial cell apoptosis<sup>(76; 77; 78)</sup>. Oestrogen deficiency has also been reported to lead to an upregulation of ACE activity causing an accumulation of angiotensin II<sup>(79)</sup>.

### **Impact of meal fat composition on postprandial lipaemia and vascular function**

Diet is one of the most important modifiable risk factors in relation to CVD<sup>(80)</sup>. As a strategy to reduce the incidence of CVD, public health policy makers recommend that intakes of dietary saturated fatty acids (SFA) are reduced to <10% total energy in the UK<sup>(81)</sup>. Substituting SFA with unsaturated fatty acids may provide additional benefits in relation to CVD risk factors, including reductions in the fasting lipid profile and improvements in endothelial function. A systematic review proposed that lowering dietary SFA intake by modifying dietary fat composition rather than reduction in total fat intake, may reduce cardiovascular events by 14%<sup>(82)</sup>. Since individuals spend a large proportion of the day in the fed (postprandial) state, modifications to the fatty acid composition of our meals that are repeated on a daily basis may have a significant impact on postprandial lipaemia and vascular health, which over time could affect CVD risk.

243 The chronic effects of substitution of SFA with polyunsaturated fat (PUFA) on fasting lipid  
244 levels have been extensively studied <sup>(83)</sup>, however, the acute affects are less well known. One  
245 systematic review and meta-analysis of RCT compared the effects of oral fat tolerance tests with  
246 differing fatty acid compositions on postprandial TAG responses in men and women <sup>(84)</sup>. Relative to  
247 a single SFA-rich meal challenge, a PUFA-rich meal significantly reduced the postprandial  
248 lipaemic response over 8 h, whereas a trend for a reduced response was identified following a  
249 monounsaturated (MUFA) rich meal challenge. However, differences were not evident at 4 h  
250 suggesting that a longer follow-up time after the test meal (i.e. 8 h) is required to observe the acute  
251 effects of meal fat composition on postprandial lipaemia. Of the 18 studies included in the review  
252 by Monfort-Pires *et al.* <sup>(84)</sup> none of the studies included postmenopausal women which reflects the  
253 paucity of postprandial data in this population subgroup.

254 With regards to vascular function, West and colleagues <sup>(85)</sup> reported that consumption of a  
255 single high fat meal (50-105 g of fat) can impair postprandial FMD by 45% to 80% with  
256 observations of impaired FMD within 2 to 5 h after a high fat meal <sup>(86; 87; 88; 89)</sup>. Prolonged  
257 postprandial lipaemia is known to induce endothelial dysfunction by promoting the formation of  
258 free radicals by accelerating the rate of  $\beta$ -oxidation of free fatty acids (e.g. superoxide radicals).  
259 Increased production of ROS or free radicals reduce the amount of bioactive NO by chemical  
260 inactivation to form toxic peroxynitrite <sup>(90)</sup>. In addition, it has been shown that persisting oxidative  
261 stress will render endothelial nitric oxide synthase dysfunctional, markedly reducing NO production  
262 <sup>(91)</sup>. Indeed, high concentrations of TRLs during the postprandial state enhance inflammation by  
263 inducing the secretion of pro-inflammatory cytokines <sup>(92)</sup> and expression of soluble cell adhesion  
264 molecules <sup>(93)</sup>.

265 Reviews by Hall <sup>(94)</sup> and Vafeiadou *et al.* <sup>(95)</sup> stated that the acute effects of dietary fats on  
266 vascular function is less researched. The authors concluded that high fat meals have a detrimental  
267 effect on postprandial vascular function and that there is limited and inconclusive evidence for the  
268 comparative effects of test meals rich in MUFA or n-6 PUFA with SFA. Of note, the data derived  
269 from these reviews were mainly from studies where the effects of a single high fat meal on  
270 postprandial vascular function in different subject groups were determined; however, none of the  
271 studies identified in these reviews included only postmenopausal women only.

272 Therefore, we aimed to systematically review and critically evaluate the existing evidence  
273 from acute studies comparing meals rich in SFA, MUFA and n-6 PUFA on postprandial lipaemia,  
274 vascular reactivity, blood pressure and biomarkers of vascular function and inflammation in  
275 postmenopausal women. It is very timely to focus on postmenopausal women since they represent  
276 an understudied group within the population at increased CVD risk.

277

## 278 **Subjects and methods**

279 A systematic approach was used to identify all relevant published literature according to the method  
280 used by Vafeiadou *et al.* <sup>(95)</sup>. The PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>) database was  
281 used to perform the literature search, which included all studies published in English until October  
282 2016. A protocol that included search terms to conduct the literature search was prepared by two  
283 authors (KMR and MW) and then agreed by all authors. Three categories of search terms were  
284 identified: i) study group search term (postmenopausal or post-menopausal or post menopause or  
285 menopause or menopausal); ii) exposure search terms (which included descriptors of SFA, MUFA  
286 and n-6 PUFA, and relevant food sources, e.g. butter, safflower oil and olive oil); iii) outcomes  
287 (which included descriptors of vascular function, blood pressure, biomarkers of vascular function  
288 and inflammation, and plasma lipids) (Supplementary Information). The Medical Subject Heading  
289 Browser (<http://www.nlm.nih.gov/mesh/MBrowser.html>) was used to identify relevant exposures  
290 and outcomes. Additional studies (n=2) were identified through hand searching of original articles  
291 found using the PubMed search. The titles and abstracts of every paper was assessed for relevance  
292 at the initial stage by one author (KMR) and any uncertainties were discussed with other members  
293 of the review team until a consensus was reached. This review was restricted to epidemiological  
294 studies (cross-sectional, case-control and cohort) and RCT in postmenopausal women with respect  
295 to test meals rich in SFA, MUFA and/or n-6 PUFA. Only published peer-reviewed literature was  
296 considered (i.e. ‘grey’ literature, such as dissertations, conference proceedings, reports, letters to  
297 editors and other non-peer-reviewed research were excluded). Although Hall <sup>(94)</sup> and Vafeiadou *et*  
298 *al.* <sup>(95)</sup> previously reviewed the chronic and acute studies on vascular function, they did not  
299 specifically address the acute effects in postmenopausal women. In this present review, we only  
300 considered acute studies as our objectives were to determine the impact of meal fatty acids on non-  
301 fasting TAG responses, vascular function and blood pressure as important CVD risk factor in  
302 postmenopausal women. Figure 2 presents a summary of the literature search and reasons for  
303 exclusion of the studies.

304

## 305 **Results and Discussion**

306 This systematic search identified 778 publications in total. Of these, there were nine relevant  
307 articles describing seven independent studies in postmenopausal women that examined the acute  
308 effects of meals enriched in SFA and/or MUFA and/or n-6 PUFA on postprandial lipaemia <sup>(96; 97; 98;</sup>  
309 <sup>99; 100; 101; 102; 103; 104)</sup>. One of these studies also determined the impact of a single fat containing meal  
310 with a low PUFA:SFA ratio on vascular function <sup>(101)</sup> (Table 1). No studies were identified that  
311 reported the acute impact of meal fatty acids on postprandial blood pressure, or biomarkers of  
312 vascular function and inflammation in postmenopausal women. Only one single-blind RCT

313 compared the effects of meal fat composition on postprandial lipaemia using a sequential meal  
314 protocol, the results of which were presented in three publications <sup>(97; 98; 100)</sup>. As opposed to a single  
315 meal protocol, the use of a multiple meal design by the researchers is considered superior because it  
316 more closely mimics the eating pattern of free-living individuals, particularly in Westernised  
317 societies, and provokes a sustained lipaemic response. Five publications described cross-sectional  
318 epidemiological studies, which were single arm studies that did not have comparator meals and  
319 whose fatty acid compositions varied <sup>(96; 101; 102; 103; 104)</sup>. Among these postprandial studies with  
320 blood samples collected between 6 to 10 hours after the test meal, two studies <sup>(96; 102)</sup> used a  
321 sequential two meal protocol, whereas the other three studies <sup>(101; 103; 104)</sup> incorporated a single meal  
322 approach. In addition, one case-control study was identified that considered the responses of  
323 normolipaemic, hypercholesterolaemic and mixed hyperlipidaemic postmenopausal women to a  
324 single high fat meal <sup>(99)</sup>.

325 Data on these human studies will be presented in two sections that address the effects of  
326 total fat or fatty acid composition on i) postprandial lipaemia and ii) postprandial vascular function  
327 in postmenopausal women.

328

#### 329 **Acute effects of meal fat composition on postprandial lipaemia**

330 The five cross-sectional studies, investigating both single and sequential meals, provided consistent  
331 evidence that fat-rich loads, irrespective of fatty acid composition, augment postprandial lipaemia in  
332 postmenopausal women, with an increase in TAG being observed in all five studies during the  
333 postprandial period relative to baseline <sup>(96; 101; 102; 103; 104)</sup> (Table 1). Furthermore, Pirro *et al.* <sup>(99)</sup>  
334 investigated the changes in postprandial TAG concentrations after a standardised oral fat load (65g  
335 of fat) at baseline, 4, 6 and 8 h in postmenopausal women with hypercholesterolemia and mixed  
336 hyperlipidaemia and compared them with a control group of normolipidaemic postmenopausal  
337 women. A significantly greater postprandial TAG response was found in the mixed hyperlipidaemic  
338 women than in the hypercholesterolaemic and normolipidaemic women which may reflect their  
339 higher baseline TAG concentrations. As expected, other factors involved in lipid metabolism,  
340 including increases in apo B-48 <sup>(102)</sup>, glucose <sup>(103)</sup>, and insulin <sup>(103)</sup> as well as reductions in HDL <sup>(99;</sup>  
341 <sup>103; 104)</sup>, glutathione <sup>(101)</sup> and NEFA <sup>(103)</sup> were also observed postprandially compared with fasting  
342 values. However, comparison of the findings from the different studies are challenging due to  
343 differences in the nature of the fats and oils used in the test meal, the amount and composition of  
344 fat, and postprandial follow up times, as well as the use of both single and sequential test meal  
345 protocols. They are also limited in their cross-sectional design in that the lack of comparator meals  
346 prevents any conclusions from being made regarding the impact of meal fat composition on  
347 postprandial lipaemia. Among all nine articles (seven independent studies) reported in Table 1, only

one study that was described in three publications compared the postprandial lipaemic responses to test meals containing oils rich in SFA (palm oil), MUFA (olive oil), n-6 PUFA (safflower oil) and a mixture of n-6 PUFA and n-3 PUFA (safflower and fish oils)<sup>(97; 98; 100)</sup>. In this study, 10 postmenopausal women ingested a high fat breakfast containing 40 g of the assigned test fat followed by a low fat, high carbohydrate lunch (5.4 g total fat) given 5 h later. The authors observed significantly higher levels of plasma NEFA and lower insulin sensitivity following the SFA meal compared with the other test oils. During the postprandial state it has been shown that up to 50% of the liberated NEFA is dietary-derived CM-TAG due to the action of LPL upon TAG to release NEFA<sup>(100)</sup>. Although Robertson *et al.*<sup>(100)</sup> did not determine the specific fatty acid composition of the circulating NEFA after consumption of the meals, a similar study reported the postprandial change in the plasma NEFA profile to represent the fatty acid composition of the test meals<sup>(105)</sup>. Based on the same sequential meal study, Jackson *et al.* further examined the postprandial TAG and apo B-48 (the apolipoprotein specifically associated with CM) responses, including the responses in three distinct TRL subfractions, and reported significant differences in the apo B-48 time course profiles between the four different test oils<sup>(98)</sup>. In particular, the MUFA meal resulted in the formation of a greater number of both large (Svedberg flotation rate ( $S_f$ )>400 fraction) and moderately ( $S_f$  60-400 fraction) sized apo B-48 particles compared with the other three study meals. The findings from this study suggested that olive oil may enhance CM formation and Jackson *et al.*<sup>(97)</sup> hypothesised that MUFA may modify the activity or expression of intestinal microsomal TAG transfer protein, which is involved with TRL lipoprotein assembly.

### **Acute effects of meal fat composition on vascular function**

Only one study has also examined the acute impact of total fat and/or SFA and/or MUFA and/or n-6 PUFA on vascular reactivity in postmenopausal women. A significant decrease in the %FMD response at 2 h ( $2.3 \pm 2.6\%$ ) compared with baseline ( $7.7 \pm 2.8\%$ ,  $p < 0.05$ ) was observed in healthy postmenopausal women after a 65 g oral fat load with a PUFA:SFA ratio of 0.06<sup>(101)</sup> (Table 1). Since a comparator meal of a different fatty acid composition was not included in this study, conclusions regarding the impact of fatty acid composition on vascular function in postmenopausal women cannot be determined.

### **Summary**

A systematic approach was used to review the literature on the impact of meal fat composition (SFA, MUFA and n-6 PUFA) on postprandial lipaemia, blood pressure, vascular function and biomarkers of vascular function and inflammation in postmenopausal women. However, there is at present, an extremely limited number of RCT that have investigated the impact of meal fatty acid

383 composition on measures of postprandial lipaemia and vascular function in this population sub-  
384 group. Furthermore, differences in study designs (such as the absence of a comparator test meal,  
385 and differences in meal fat composition, study duration and outcome measures) prevent any firm  
386 conclusions being drawn from this literature review.

387

## 388 **Conclusions**

389 In conclusion, there is an urgent requirement for suitably powered RCT to investigate the effects of  
390 meal fat composition on postprandial lipaemia and vascular function in postmenopausal women.  
391 With the increased prevalence of non-communicable diseases in women, especially after the  
392 menopause, future studies should consider both healthy postmenopausal women and those at  
393 increased cardiometabolic risk using well-standardised measures of vascular function. Since non-  
394 fasting TAG is an important CVD risk factor for women, it is essential to use robust test meal  
395 protocols that are more reflective of habitual eating patterns to gain a greater understanding of the  
396 day-long postprandial handling of different dietary fats.

397

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400 conception of the literature search strategy. KMR undertook the literature search, extracted and  
401 interpreted the data from the literature and wrote the manuscript. MW, KGJ and JAL critically  
402 appraised the document at all stages. KGJ and JAL critically appraised the final manuscript. JAL  
403 was responsible for the final content. None of the authors have any conflicts of interest.

404

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**Table 1 Acute test meal studies investigating the effects of meal fat content and composition on postprandial lipaemia and vascular function in postmenopausal women**

| Reference  | Subject group, age (mean) and n  | Study design     | Meal type  | Amount of fat (% meal fat if available)                                | Fatty acid composition   | Time of postprandial data | Postprandial measurements (plasma/serum)  | Significant outcomes compared to baseline, unless otherwise stated  |
|--|--|------------------|------------|--|--|---------------------------|---|---|
| <b>Postprandial lipaemia</b>                     |  |                  |            |  |  |                           |   |   |
| Westerveld <i>et al.</i> (1996) <sup>(104)</sup> | 59 y n 16 normolipidaemic  | Cross sectional* | Single     | 50 g (40%)   | PUFA: SFA 0.06   | 8 h                       | TAG, HDL-C and HDL-Apo A-1  | ↓HDL-C at 3 to 8 h (p<0.05),<br>↓HDL-Apo A-1 at 3 and 6 h (p<0.05)<br>↑TAG at 8 h (p<0.05)  |
| Pirro <i>et al.</i> (2001) <sup>(99)</sup>       | 57 y n 17 normolipidaemic, 54 y n 17 hypercholesterolaemia and 55 y n 16 mixed hyperlipaemia | Case control     | Single     | 65 g (83%)   | PUFA: SFA 0.06   | 8 h                       | TC, TAG, HDL-C, HDL <sub>2</sub> , HDL <sub>3</sub> , LDL, LDL particle size, and Lp(a) | ↑TAG at 4,6 and 8 h, ↓HDL-C at 6 h and ↓Lp(a) at 4 and 6 h in normolipidaemic PoM (p<0.05)<br><br>↑TAG at 4, 6 and 8 h, ↓HDL-C at 4 and 6 h, ↓HDL <sub>2</sub> at 4 h and ↓Lp(a) at 4 h in hypercholesterolaemia PoM (p<0.05)<br><br>↑TAG at 4, 6 and 8 h, ↓LDL size at 4 and 6 h, ↓HDL-C at 4, 6 and 8 h, ↓HDL <sub>2</sub> at 6 h and ↓Lp(a) at 4 and 6 h in mixed hyperlipaemia PoM (p<0.05) |
| Silva <i>et al.</i> (2005) <sup>(102)</sup>      | 52-76 y (62 y) n 17  | Cross sectional* | Sequential | Breakfast: 30 g (46%)<br>Lunch: 44 g (52%)                             | Breakfast (27 %E SFA, 12 %E MUFA, 5 %E PUFA and 2 %E Trans)<br>Lunch (27 %E SFA, 18 %E MUFA, 5 %E PUFA and 2 %E Trans) | 10 h                      | TAG, and apo B-48   | ↑TAG at 210 min after breakfast and 60 min after lunch<br>↑Apo B-48 at 150 min after breakfast and 60 min after lunch   |
| Alssema <i>et al.</i> (2008) <sup>(96)</sup>     | 60.1 y n 76  | Cross sectional* | Sequential | Both breakfast and lunch compositions:<br><br>Fat rich meal: 50 g fat, | No information   | 8 h                       | TAG, HDL-C and CETP   | ↑TAG at 8 h (p<0.05), ↓HDL-C at 8 h (p<0.05) in fat rich meal<br>↑TAG at 8 h (p<0.05), ↓HDL-C at 8 h and ↑CETP in CHO rich meal (p<0.05)  |

|  |                                  |   |                |  |   |   |                                      |  |  |
|--|----------------------------------|---|----------------|--|---|---|--------------------------------------|--|--|
|  |                                  |   |                |  | 56 g CHO,<br>28 g protein   |   |                                      |  |  |
|  |                                  |   |                |  | CHO rich<br>meal:<br>4 g fat,<br>162 g CHO,<br>22 g protein                   |   |                                      |  |  |
| Wassef <i>et al.</i><br>(2012) <sup>(103)</sup>  | 58 y (45-74 y)<br>n 19 obese PoM | Cross<br>sectional*                         | Single         | <sup>13</sup> C-labeled<br>breakfast 80 g<br>fat (68%) +<br>0.017 g<br><sup>13</sup> C-triolein/g<br>fat | 25 %E SFA, 26 %E MUFA,<br>10 %E PUFA and 6 %E other<br>sources                | 6 h   | TAG, glucose,<br>NEFA and<br>Insulin | ↑TAG after meal<br>↓NEFA between 1 to 2 h<br>↑Glucose at 1 h<br>↑Insulin AUC at 1 h  |  |
| Robertson <i>et al.</i> (2002) <sup>(100)</sup>  | 50-63 y (56 y)<br>n 10           | Single-<br>blind<br>randomised<br>crossover | Seque<br>ntial | Breakfast:<br>41 g <sup>†</sup><br>Lunch:<br>6 g   | High SFA (g/100 g):<br>10 g n-6 PUFA, 0 g n-3 PUFA,<br>40 g MUFA and 50 g SFA | 8 h   | Glucose,<br>NEFA and<br>insulin      | High insulin response: SFA > n-6<br>PUFA > n-3 PUFA > MUFA<br>(p<0.006)<br>Glucose: No significant effect<br>↑NEFA at 5 h following high SFA<br>breakfast and 30 min after low-fat<br>high-CHO meal<br>↓insulin sensitivity: SFA < n-6 PUFA<br>< n-3 PUFA < MUFA |  |
| Jackson <i>et al.</i><br>(2002a) <sup>(98)</sup> |                                  |   |                | High MUFA (g/100 g):<br>11 g n-6 PUFA, 0 g n-3 PUFA,<br>72 g MUFA and 17 g SFA                           |   |   |                                      |  |  |
| Jackson <i>et al.</i><br>(2002b) <sup>(97)</sup> |                                  |   |                | High n-6 PUFA (g/100 g):<br>74 g n-6PUFA, 0 g n-3 PUFA,<br>15 g MUFA and 11 g SFA                        | TAG and<br>apo B-48   | ↑apo B-48 in MUFA than SFA, n-6<br>PUFA and n-3/n-6 PUFA meals<br>(p≤0.009)   |                                      |  |  |
|  |                                  |   |                | High n-3/n-6 PUFA (g/100 g):<br>39 g n-6 PUFA, 22 g n-3<br>PUFA, 22 g MUFA and 19 g<br>SFA               | TAG, apo B-48,<br>and in three<br>TAG-rich<br>lipoprotein<br>subfractions     | apo B-48 IAUC in the S <sub>F</sub> 60-400<br>fraction greater than in the S <sub>F</sub> > 400<br>fraction for the SFA, n-6 PUFA and<br>MUFA meals (p<0.04)<br>↑apo B-48 IAUC in the S <sub>F</sub> > 400<br>fraction in MUFA than SFA, n-6<br>PUFA and n-3/n-6 PUFA meals<br>(p<0.02) |                                      |  |  |
| Postprandial lipaemia and vascular function      |                                  |   |                |  |   |   |                                      |  |  |
| Siepi <i>et al.</i><br>(2002) <sup>(101)</sup>   | 57 y n 10                        | Cross<br>sectional*                         | Single         | 65 g   | PUFA: SFA 0.06  | 6 h   | TAG and GSH                          | ↑TAG at 4 and 6 h (p<0.05)<br>↓GSH at 2 h (p<0.05)   |  |
|  |                                  |   |                |  |   |   | Brachial FMD                         | ↓FMD at 2 h (p<0.05)   |  |

\* No comparator group.



† Values given per 100 g of test oil of which 41 g was included in the breakfast.

Arrows refer to the direction of change over time relative to baseline (fasting), unless otherwise stated.

Abbreviations: AUC; area under the curve, CETP; cholesteryl ester transfer protein, CHO; carbohydrate, E; energy, FMD; flow-mediated dilatation, GSH; glutathione, HDL-C; high density lipoprotein cholesterol, IAUC; incremental area under the curve, LDL; low density lipoprotein, Lp (a); lipoprotein (a), MaxC; maximum concentration, MUFA; monounsaturated fat, NEFA; non-esterified fatty acid, PoM; postmenopausal women, PrM; premenopausal women, PUFA; polyunsaturated fat, RE; Retinyl esters, SFA; saturated fat, TAG; triacylglycerol, TC; total cholesterol.

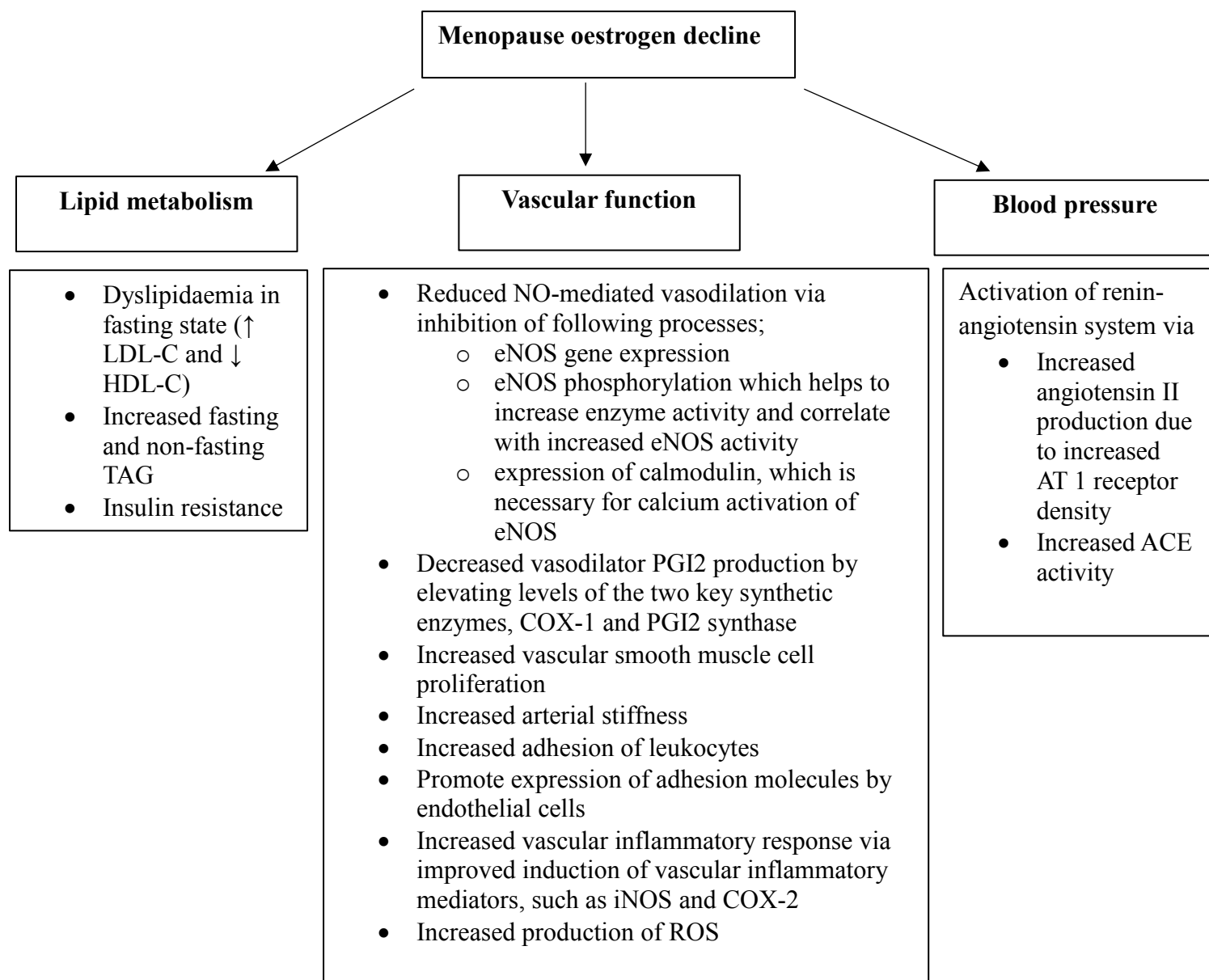
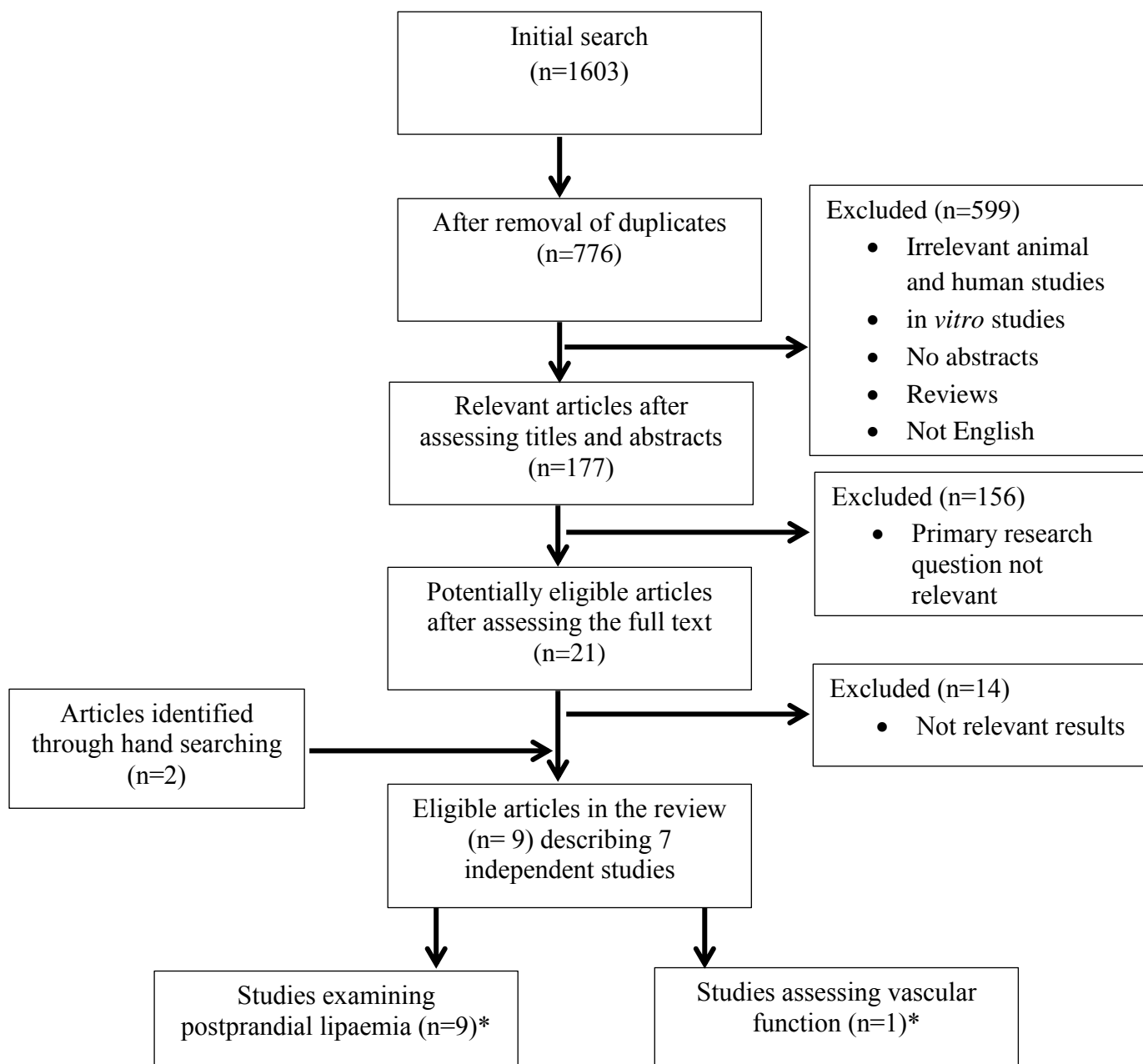


Figure 1: Consequences of the decline in oestrogen during the menopause on the lipid profile, endothelial function and blood pressure. Adapted from Davis *et al.*<sup>(106)</sup> Abbreviations: ACE; angiotensin converting enzyme, AT-1; angiotensin I receptor, COX; cyclooxygenase, eNOS; endothelial nitric oxide synthase, HDL-C; high density lipoprotein cholesterol, iNOS; inducible NO synthase, LDL-C; low density lipoprotein cholesterol, NO; nitric oxide, PGI; prostaglandin, ROS; reactive oxygen species, TAG; Triacylglycerol



**Figure 2: Flow of information through the different phases of the review**

\*Of the studies included in the review, one publication reported both postprandial lipaemia and vascular function.

**Supplementary Table 1 Acute test meal studies investigating the impact of menopausal status on the variability of the postprandial lipaemic responses**

\* No comparator group.

† Values given per 100 g of test oil of which 41 g was included in the breakfast.

| Reference                                     | Subject group, age (mean) and n   | Study design     | Meal type  | Amount of fat (% meal fat if available) | Fatty acid composition                      | Time of postprandial data | Postprandial measurements                             | Significant outcomes   |
|---|---|------------------|------------|---|---|---------------------------|---|--|
| van Beek <i>et al.</i> (1999) <sup>(18)</sup> | 47-52 y (50 y) n 23 PoM women and 47-52 y (49 y) n 21 PrM women   | Case control     | Single     | 50 g (40%)                              | PUFA: SFA 0.06                              | 12 h                      | TAG and Vitamin A/retinyl palmitate                   | ↑TAG AUC at 0-8 h (p=0.024)<br>↑TAG ΔAUC (p=0.020) in PoM compared to PrM at 0-8 h<br>↑Vitamin A AUC (p=0.001) in PoM compared to PrM at 0-8 h |
| Masding <i>et al.</i> (2006) <sup>(20)</sup>  | 34-56 y (42 y) n 8 PrM and 46-68 y (58 y) n 8 PoM healthy<br><br>32-54 y (39 y) n 8 PrM and 53-70 y (61y) n 8 PoM type 2 diabetic | Case and control | Single     | 45 g                                    | No information                              | 6 h                       | TAG, NEFA, Glucose, and <sup>13</sup> C-palmitic acid | ↑TAG AUC in healthy PoM than PrM (p<0.05)<br>↑ <sup>13</sup> C-palmitic acid in healthy PoM than PrM (p<0.01)                                  |
| Schoppen <i>et al.</i> (2010) <sup>(19)</sup> | 18-36 y (20.9 y) n 20 PrM and 51-59 y (55.7 y) n 18 PoM   | Case and control | Single     | Breakfast: 75.3 g (62.3%)               | 11.8 %E SFA, 39.7 %E MUFA and 6.6 %E PUFA   | 7 h                       | TAG and TC  | ↑TAG and TC in PoM than PrM (p<0.0001)<br>Peak TAG at 240 min in PoM and 120 min PrM (p<0.0001)  |
| Jackson <i>et al.</i> (2010) <sup>(21)</sup>  | 42 y n 37 PrM and 60 y n 61 PoM   | Case and control | Sequential | Breakfast: 51 g<br>Lunch: 31 g          | 29 g SFA at breakfast and 14 g SFA at lunch | 8 h                       | TAG   | ↑TAG IAUC (p=0.002), MaxC (p=0.037) and time to reach MaxC (p=0.009) in PoM than PrM   |

Arrows refer to the direction of change over time compared with premenopausal women.

Abbreviations: AUC; area under the curve, HDL; high density lipoprotein, IAUC; incremental area under the curve, LDL; low density lipoprotein, Lp (a); lipoprotein (a), MaxC; maximum concentration, MUFA; monounsaturated fat, NEFA; non-esterified fatty acid, PoM; postmenopausal women, PrM; premenopausal women, PUFA; polyunsaturated fat, SFA; saturated fat, TAG; triacylglycerol, TC; total cholesterol.

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