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

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Abbreviations in the text

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

Abstract

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

Introduction

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

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

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

Postprandial lipaemia

Kolovou *et al*. defined postprandial lipaemia as a complex syndrome characterised by non-fasting hypertriacylglycerolaemia and its augmentation is associated with increased risk of cardiovascular events [11]. Following a fat containing meal, there is a transient rise in circulating triacylglycerol (TAG) rich lipoproteins (TRL), such as chylomicrons (CM) and very low density lipoprotein (VLDL). After entering the circulation, the CM TAG is hydrolysed into non-esterified fatty acids (NEFA) by lipoprotein lipase (LPL) forming cholesterol ester rich CM remnants, which are cleared by the liver by receptor-mediated uptake. VLDL follows a similar route of metabolism in the circulation as CM particles, but VLDL are hydrolysed at a slower rate, as the larger CM are preferential substrate for LPL. VLDL TAG depletion produce smaller VLDL (intermediate density lipoprotein or VLDL remnants), ultimately a proportion of which will be metabolised to low density lipoprotein (LDL). LDL particles are cleared from the bloodstream via the hepatic LDL receptor using apoB-100 as a ligand. During the postprandial period, there is an accumulation of TRL in the circulation due to competition between intestinal and hepatic TRL for the same lipolytic and receptor mediated uptake [12]. A delayed clearance of TRLs in the circulation enhances the accumulation of TRL particles carrying acceptor sites for the cholesteryl ester transfer protein (CETP) which transfers TAG from TRLs (CM and VLDL) and exchanges it with cholesteryl esters from high density lipoprotein (HDL) and LDL. Remodelling of the lipid content of the LDL and HDL particles make them suitable substrates for LPL and hepatic lipase, leading to the formation of smaller denser LDL (LDL_3) and HDL (HDL_3) particles [13]. HDL_3 is rapidly removed from the circulation decreasing circulating HDL cholesterol (HDL-C) concentrations, which is one proposed mechanism for the inverse association between exaggerated postprandial lipaemia and CVD risk [14]. Another possible mechanism is that LDL_3 has a lower binding affinity to the LDL-receptor,

reducing their rate of clearance from the circulation and enabling them to infiltrate the arterial wall [13].

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

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

late premenopausal period suggesting that age and the menopause have a differential impact on these two lipid CVD risk biomarkers [21].

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

Vascular function and blood pressure

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

impaired endothelial function after the menopause, which has been associated with a lack of endogenous oestrogen [7; 47].

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

Majmudar *et al.* [53] revealed that menopausal status is associated with reduced NO activity, which is restored with oestrogen replacement therapy and may be an important mechanism facilitating the detrimental effect of the menopause on CVD risk and mortality. Another study that acutely administered oestrogen (17β-oestradiol) to postmenopausal women demonstrated protective effects on forearm microvascular responses to both endothelium-dependent (acetylcholine) and -independent vasodilation (sodium nitroprusside) via improvements in NO activity [54]. Impaired blood flow in the microcirculation has been proposed to be an indicator of initial endothelial damage in subjects at risk of CVD [55]. Furthermore, it has been repeatedly shown that 17β-oestradiol stimulates the production of vasodilatory prostaglandins, such as prostacyclin (PGI_2) [56; 57]. These vascular effects are believed to be partly responsible for the long-term benefit of oestrogen therapy on cardiovascular risk in postmenopausal women. However, findings from the Women's Health Initiative study have questioned the benefits of oestrogen therapy, reporting that oestrogen therapy did not protect against myocardial infarction or coronary death after a short (6.8 y) or longer-term (18 y) follow-up relative to a placebo, although the findings did show a lower risk of coronary heart disease among the younger postmenopausal women (50 to 59 y) [58; 59] [59]. More recently, a systematic review involving 43,637 women reported the number of cardiovascular events to increase following the long-term (>1 y) use of oestrogen therapy [60]. In contrast, there is much evidence to suggest that oestrogens (endogenous and exogenous) have several cardio-protective effects (Figure 1) [32; 61; 62]. These include reductions in plasma markers of endothelial activation (E-selectin) and increased fibrinolytic activity (increased factor VII; reduced fibrinogen,

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

Hypertension (high blood pressure) is one of the main age-related disorders in postmenopausal women [64; 65], which has been identified as a leading risk factor for myocardial infarction and stroke in women [66]. 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 [67]. Angiotensin II is a potent vasoconstrictor which degrades bradykinin (a vasodilator) causing arterioles to constrict, resulting in increased blood pressure [68]. 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[69; 70; 71]. 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 [72; 73; 74; 75]. This has been proposed to occur due to oestrogen induced downregulation of angiotensin receptor I expression leading to an augmented level of angiotensin II [73] (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 [76; 77; 78]. Oestrogen deficiency has also been reported to lead to an upregulation of ACE activity causing an accumulation of angiotensin II [79].

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 [80]. 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 [81]. 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% [82]. 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.

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

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

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

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

Subjects and methods

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

Results and Discussion

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

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

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

Acute effects of meal fat composition on postprandial lipaemia

The five cross-sectional studies, investigating both single and sequential meals, provided consistent evidence that fat-rich loads, irrespective of fatty acid composition, augment postprandial lipaemia in postmenopausal women, with an increase in TAG being observed in all five studies during the postprandial period relative to baseline [96; 101; 102; 103; 104] (Table 1). Furthermore, Pirro *et al.* [99] investigated the changes in postprandial TAG concentrations after a standardised oral fat load (65g of fat) at baseline, 4, 6 and 8 h in postmenopausal women with hypercholesterolemia and mixed hyperlipidaemia and compared them with a control group of normolipidaemic postmenopausal women. A significantly greater postprandial TAG response was found in the mixed hyperlipidaemic women than in the hypercholesterolaemic and normolipidaemic women which may reflect their higher baseline TAG concentrations. As expected, other factors involved in lipid metabolism, including increases in apo B-48 [102], glucose [103], and insulin [103] as well as reductions in HDL [99; 103; 104], glutathione [101] and NEFA [103] were also observed postprandially compared with fasting values. However, comparison of the findings from the different studies are challenging due to differences in the nature of the fats and oils used in the test meal, the amount and composition of fat, and postprandial follow up times, as well as the use of both single and sequential test meal protocols. They are also limited in their cross-sectional design in that the lack of comparator meals prevents any conclusions from being made regarding the impact of meal fat composition on 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) [97; 98; 100]. 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 [100]. Although Robertson *et al.* [100] 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 [105]. 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 [98]. 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.* [97] 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 ± 2.6%) compared with baseline (7.7 ± 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 [101] (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

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

Conclusions

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

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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
Postprandial lipaemia								
Westerveld *et al.* (1996)[104]	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 ($p<0.05$), ↓HDL-Apo A-1 at 3 and 6 h ($p<0.05$) ↑TAG at 8 h ($p<0.05$)
Pirro *et al.* (2001)[99]	57 y n 17 normolipaemic, 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_2 HDL_3, 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 normolipaemic PoM ($p<0.05$) ↑TAG at 4, 6 and 8 h, ↓HDL-C at 4 and 6 h, ↓HDL_2 at 4 h and ↓Lp(a) at 4 h in hypercholesterolaemia PoM ($p<0.05$) ↑TAG at 4, 6 and 8 h, ↓LDL size at 4 and 6 h, ↓HDL-C at 4, 6 and 8 h, ↓HDL_2 at 6 h and ↓Lp(a) at 4 and 6 h in mixed hyperlipaemia PoM ($p<0.05$)
Silva *et al.* (2005)[102]	52-76 y (62 y) n 17	Cross sectional*	Sequential	Breakfast: 30 g (46%) Lunch: 44 g (52%)	Breakfast (27 %E SFA, 12 %E MUFA, 5 %E PUFA and 2 %E Trans) 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 ↑Apo B-48 at 150 min after breakfast and 60 min after lunch
Alssema *et al.* (2008)[96]	60.1 y n 76	Cross sectional*	Sequential	Both breakfast and lunch compositions: 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 ↑TAG at 8 h ($p<0.05$), ↓HDL-C at 8 h and ↑CETP in CHO rich meal ($p<0.05$)

				56 g CHO, 28 g protein CHO rich meal: 4 g fat, 162 g CHO, 22 g protein				
Wassef *et al.* (2012)[103]	58 y (45-74 y) n 19 obese PoM	Cross sectional*	Single	^{13}C-labeled breakfast 80 g fat (68%) + 0.017 g ^{13}C-triolein/g fat	25 %E SFA, 26 %E MUFA, 10 %E PUFA and 6 %E other sources	6 h	TAG, glucose, NEFA and Insulin	↑TAG after meal ↓NEFA between 1 to 2 h ↑Glucose at 1 h ↑Insulin AUC at 1 h
Robertson *et al.* (2002)[100] Jackson *et al.* (2002a)[98] Jackson *et al.* (2002b)[97]	50-63 y (56 y) n 10	Single-blind randomised crossover	Sequential	Breakfast: 41 g† Lunch: 6 g	High SFA (g/100 g): 10 g n-6 PUFA, 0 g n-3 PUFA, 40 g MUFA and 50 g SFA High MUFA (g/100 g): 11 g n-6 PUFA, 0 g n-3 PUFA, 72 g MUFA and 17 g SFA High n-6 PUFA (g/100 g): 74 g n-6PUFA, 0 g n-3 PUFA, 15 g MUFA and 11 g SFA High n-3/n-6 PUFA (g/100 g): 39 g n-6 PUFA, 22 g n-3 PUFA, 22 g MUFA and 19 g SFA	8 h	Glucose, NEFA and insulin	High insulin response: SFA > n-6 PUFA > n-3 PUFA > MUFA ($p<0.006$) Glucose: No significant effect ↑NEFA at 5 h following high SFA breakfast and 30 min after low-fat high-CHO meal ↓insulin sensitivity: SFA < n-6 PUFA < n-3 PUFA < MUFA
							TAG and apo B-48	↑apo B-48 in MUFA than SFA, n-6 PUFA and n-3/n-6 PUFA meals ($p\leq0.009$)
							TAG, apo B-48, and in three TAG-rich lipoprotein subfractions	apo B-48 IAUC in the S_f 60-400 fraction greater than in the S_f> 400 fraction for the SFA, n-6 PUFA and MUFA meals ($p<0.04$) ↑apo B-48 IAUC in the Sf> 400 fraction in MUFA than SFA, n-6 PUFA and n-3/n-6 PUFA meals ($p<0.02$)
Postprandial lipaemia and vascular function								
Siepi *et al.* (2002)[101]	57 y n 10	Cross sectional*	Single	65 g	PUFA: SFA 0.06	6 h	TAG and GSH	↑TAG at 4 and 6 h ($p<0.05$) ↓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.

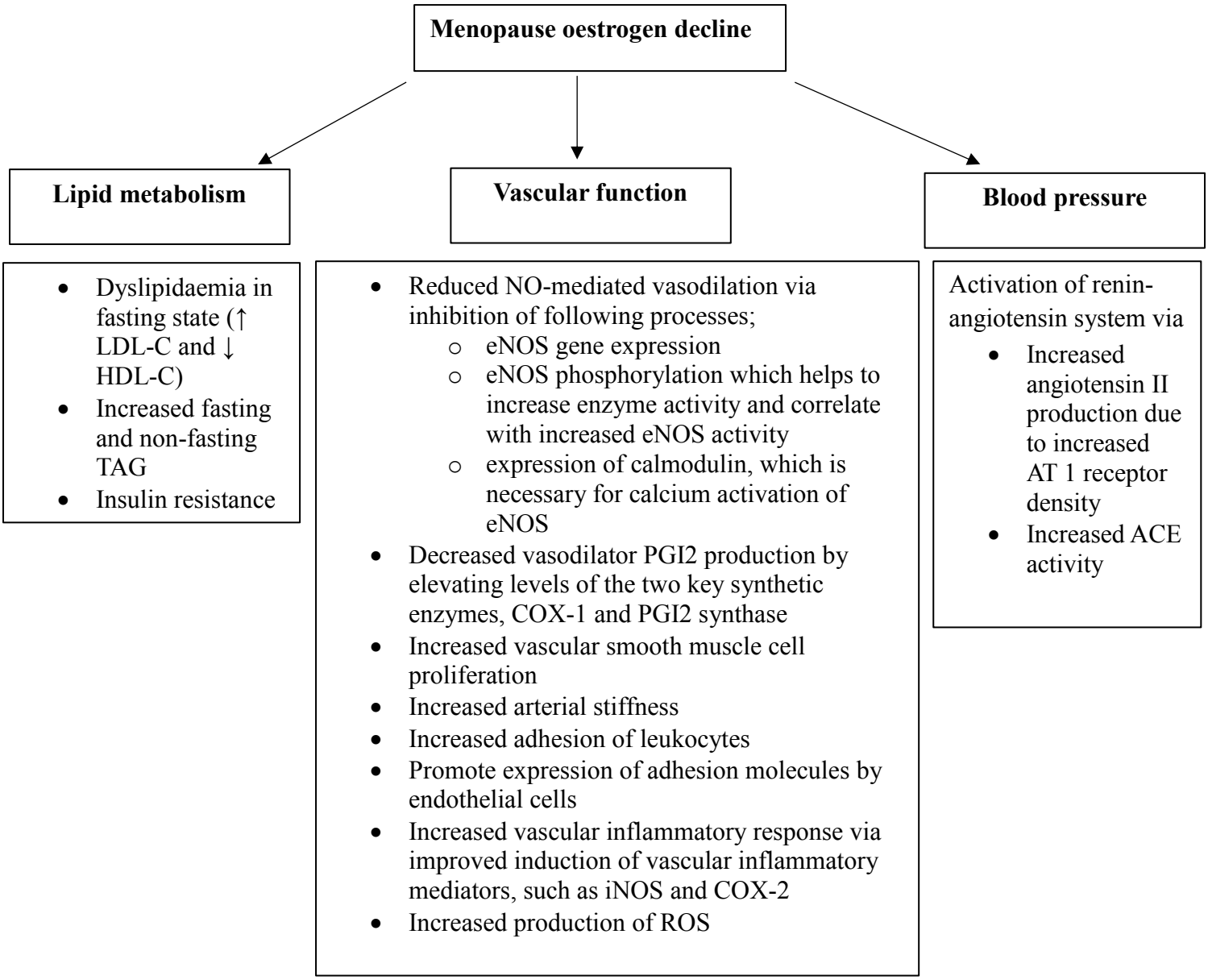

Figure 1: Consequences of the decline in oestrogen during the menopause on the lipid profile, endothelial function and blood pressure. Adapted from Davis *et al.*[106] Abbreviations: ACE; angiotensin converting enzyme, AT-1; angiotensin I receptor, COX; cyclooxygenase, eNOS; endothelial nitric oxide 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

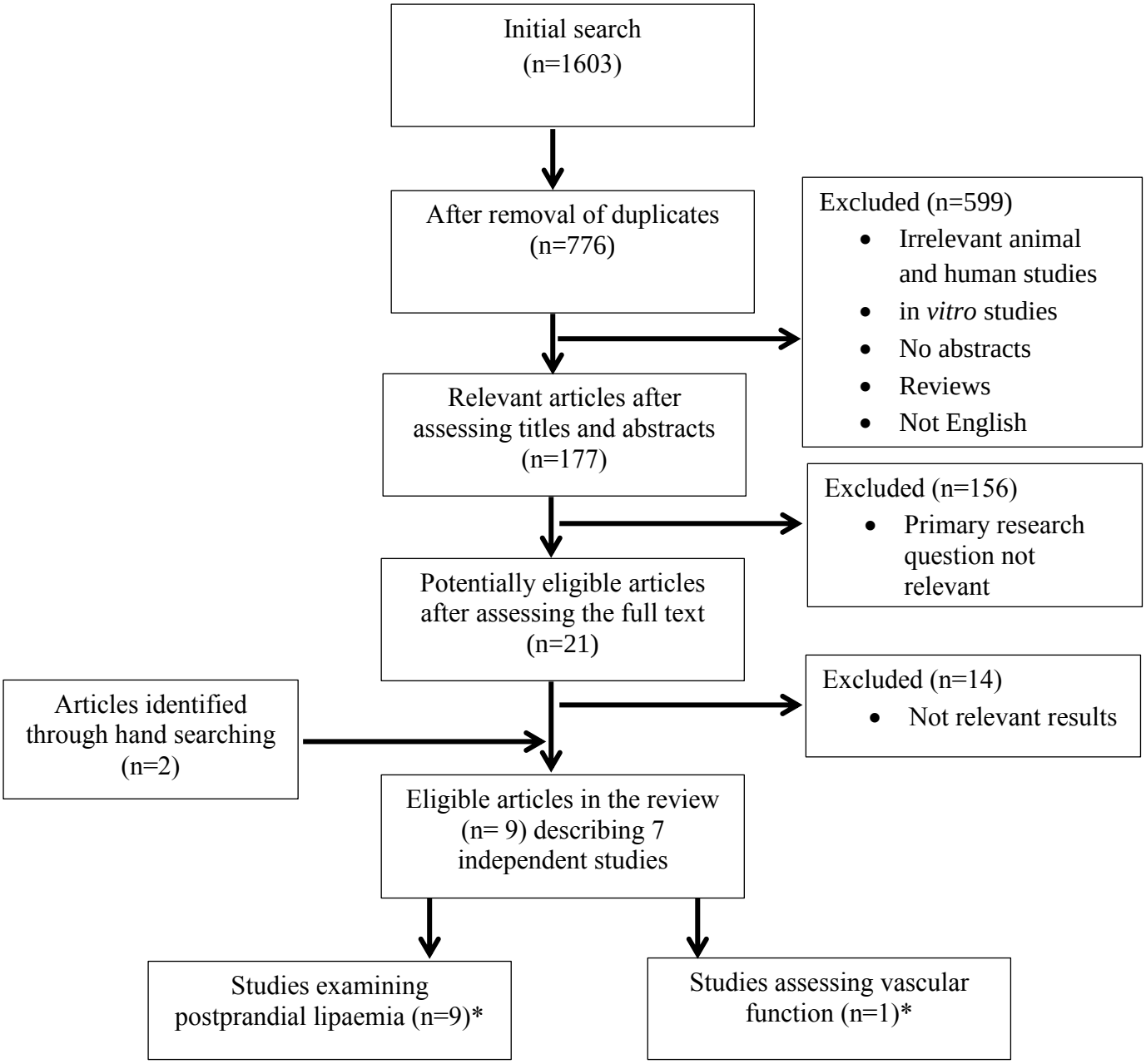

Figure 2: Flow of information through the different phases of the review

*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 *et al.* (1999)[18]	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) ↑TAG ΔAUC (p=0.020) in PoM compared to PrM at 0-8 h ↑Vitamin A AUC (p=0.001) in PoM compared to PrM at 0-8 h
Masding *et al.* (2006)[20]	34-56 y (42 y) n 8 PrM and 46-68 y (58 y) n 8 PoM healthy 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 ^{13}C-palmitic acid	↑TAG AUC in healthy PoM than PrM (p<0.05) ↑^{13}C-palmitic acid in healthy PoM than PrM (p<0.01)
Schoppen *et al.* (2010)[19]	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) Peak TAG at 240 min in PoM and 120 min PrM (p<0.0001)
Jackson *et al.*(2010)[21]	42 y n 37 PrM and 60 y n 61 PoM	Case and control	Sequential	Breakfast: 51 g 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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