

A prospective study of the relationships between change in body composition and cardiovascular risk factors across the menopause.

Short running title: Menopause: body composition and CVD risk.

Aaron Dehghan¹ (MBBS), Senthil K Vasan¹ (PhD), Barbara A Fielding² (PhD), Fredrik Karpe^{1,3} (MD, PhD)

1. Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, UK
2. Department of Nutritional Sciences, University of Surrey, Guildford, GU2 7WG, UK.
3. NIHR Oxford Biomedical Centre, Oxford University Hospital Trust and University of Oxford, Oxford, UK.

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Correspondence: Prof F Karpe, Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, Oxfordshire OX3 7LE, UK.

E-mail: Fredrik.Karpe@ocdem.ox.ac.uk

Telephone: +44-1865-857222

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Abstract

Objective: Menopause increases the risk of cardiovascular disease (CVD) which in part has been attributed to the rises in cholesterol and blood pressure (BP). This study examined the hypothesis that menopausal changes in body composition and regional fat depots relate to the change in CVD risk factors.

Methods: A prospective recall study was designed to capture premenopausal women to be re-examined soon after menopause. A total of 97 women from the Oxford Biobank underwent dual x-ray absorptiometry, blood biochemistry and BP readings pre- and post-menopause.

Results: Despite minimal changes in body weight over the 5.1 ± 0.9 year follow-up period, there was an increase in total fat mass and a decline in lean mass, where the proportional change of regional fat mass was the greatest for the visceral fat depot (+22%, $p < 0.01$).

Plasma ApoB (+12%, $p < 0.01$) and CRP (+45%, $p < 0.01$) increased as did systolic (+7%, $p < 0.001$) and diastolic BP (+5%, $p < 0.001$). Plasma NEFA decreased (-20%, $p < 0.05$) which may reflect on a change in adipose tissue function across the menopause. PCSK-9 decreased (-26%, $p < 0.01$) which suggests a compensation for the postmenopausal reduction in LDL receptor activity.

Using a multilinear regression analyses the changes in ApoB and DBP were associated with visceral fat mass change, but this association was lost when adjusted for total fat mass change.

Conclusion: The increase in CVD risk factor burden across menopause may not be driven by changes in body composition, rather by functional changes in end organs such as adipose tissue and liver.

Keywords: menopause, cardiovascular disease, lipids, fat distribution, DEXA

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in women¹. The incidence of CVD in women before 50 years of age appears to lag behind that of men by approximately 10 years². After the age of 50, which tends to coincide with the menopause transition, the sex differences in incidence rapidly level out³. This is explained by an accelerated increase in the incidence of female CVD^{1,2} around menopause, with post-menopausal women having twice the rate of cardiovascular events compared to aged matched pre-menopausal women⁴. The increase seen across the menopausal transition is significantly higher than that seen at the same ages in men, suggesting menopause as a potential risk factor for CVD independent of ageing⁵.

During menopausal transition, women show numerous metabolic changes, independent of ageing, which could account for the rise in cardiovascular events associated with menopause. Menopause has been shown to be associated with a rise in blood pressure (BP) with both cross-sectional and longitudinal studies showing an increase in systolic and/or diastolic BP⁶. However, in the longitudinal studies the findings are inconsistent with some studies showing no BP changes^{7,8} or even a reduction in BP in the years following the menopause⁹. Other metabolic changes seen with the menopause include an adverse effect on the lipid profile and glucose metabolism^{10,11} including increments in apolipoprotein B (ApoB), triglycerides, total cholesterol and LDL-Cholesterol (LDL-C) across this period. Longitudinal studies have shown that changes in the lipid profile including LDL-C, ApoB and triglycerides tend to primarily occur in the two years surrounding the final menstrual period (FMP)⁵. In some studies, these changes have stretched further into the early postmenopausal period (24 months from the FMP) and up to 6 years after¹².

The menopause is also known to be associated with increases in total fat mass and changes in body fat distribution towards a more android phenotype¹⁰. The majority of evidence to support these fat distribution changes come from cross sectional studies comparing pre-menopausal to post-menopausal women using dual energy X-ray absorptiometry (DEXA) measurements^{13,14}. However, fewer studies have examined longitudinally using CT imaging of abdominal fat distribution alongside DEXA for other parameters¹⁵⁻¹⁷. Although CT is well validated for this purpose a wider use is restricted by the radiation dose, variability in image analysis and cost. In contrast, DEXA scans involves less radiation exposure, is less expensive and is now validated against CT measurements to give accurate estimates of both VAT and subcutaneous fat¹⁸. We have recently shown in a large general population that DEXA-measured VAT and android fat masses are associated with a CVD and type 2 diabetes risk factors whereas the abdominal subcutaneous fat alongside gynoid and leg show protective associations¹⁹, and the protective association is stronger in women.

The aims of this study were to prospectively measure changes in metabolic parameters and body fat distribution across the menopause including markers NEFA and PCSK-9 reflecting metabolic tissue function. We then aimed to answer a specific question as to whether, within a large population-based cohort of apparently healthy British women, their individual changes in body fat depots were associated with the worsening of CVD risk factors seen across the same time period.

Methods

Participants: We initially identified 150 white women aged between 44 – 48 years from the Oxford Biobank²⁰, who had undergone a dual x-ray absorptiometry (DEXA) scan and were premenopausal at their initial screening visit, and at subsequent follow-up were aged 50 years

or above. Of these, a total of 103 women were successfully recalled for a second visit ranging between 3 – 7 years from their initial visit. To define menopausal status, we used the Stages of Reproductive Aging Workshop (STRAW) +10 staging criteria²¹. Any women self-identifying as peri- or postmenopausal, based on symptoms and/or menstrual cycle, were included in the study. If the menopausal status was uncertain then FSH levels were used to determine their menopausal status and a level of >25 IU/L was used as a cut off for inclusion into the post-menopausal group. We excluded two women who had a FSH <25 IU/L and 4 women who were on hormone therapy, leaving a total sample size of 97 women for analysis. All participants gave informed, written consent and the study was approved by the Oxfordshire Clinical Research Ethics Committee.

Anthropometric and biochemical measurements: The pre and postmenopausal visits had identical measurements which included conventional anthropometric measurements (height, weight and BMI), a DEXA scan, blood pressure and a fasting venous blood sample using standard protocols by trained research nurses²⁰. Plasma was analysed for triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), ApoB, non-esterified fatty acids (NEFA), C-reactive protein (CRP), insulin and glucose concentrations as previously described²⁰. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula. Plasma FSH and PCSK-9 levels were determined using AlphaLISA (PerkinElmer, Waltham, Massachusetts, USA) assays as per the manufacturer's protocols. The pre- and post-menopausal samples were assayed at different time points and different batches but were done using the same analytical platforms. Our assays undergo periodic quality assessment against national calibration standards (UK-NEQAS). The DEXA was performed on GE Lunar iDXA which included quantification of total body lean and fat mass and regional android, visceral, subcutaneous, gynoid and leg fat and lean masses²⁰.

Statistical analysis: Data are presented as mean values $\pm$ standard deviations or when appropriate median + interquartile range (IQR). The measurements at the two time points were compared using paired t-tests for normally distributed and log-transformed variables and Wilcoxon signed-rank test was used as appropriate.

The value for change (Δ -change) was calculated as the mean difference between pre- and postmenopausal measurements for clinical measurements, biochemical analyses and body composition. To normalise any skewed data and allow comparison between the DXA and metabolic measurements, which are recorded in different units, all data (pre + postmenopausal values and delta values) were transformed into z-scores using Fisher Yates transformation. This allows direct comparison of magnitude of association per 1 SD change¹⁹. To evaluate the correlation between body compartments, blood pressure and the lipid profile, Pearson's coefficients were estimated using the z-scores in both the pre- and postmenopausal groups.

Univariate and multivariate (adjusted for total fat mass, time between visits, premenopausal age and baseline outcome value) linear regression models were used initially to examine the association of z-transformed premenopausal fat and lean compartments (independent variables) on the change in systolic blood pressure, diastolic blood pressure and ApoB levels (dependent variables) across the menopause. Similar analyses (adjusting for same confounders as above) were used to examine the association of z-transformed changes in fat and lean compartments on z-transformed changes in systolic blood pressure, diastolic blood pressure and ApoB levels across the menopause and results are presented as estimate (β) and 95% confidence intervals (CI).

Statistical analysis was performed using SPSS Statistics version 24 (IBM, SPSS products, Chertsey, UK). Two-tailed P values of 0.05 or less were considered significant.

Results

Changes in body composition, blood pressure and lipid risk factors across menopause: There were 5.1 ± 0.9 years between the pre and postmenopausal visits in the 97 women in this study (Table 1). In this time period, the overall change in body weight was not statistically significant but there were significant changes in body composition. The total fat mass increased by 1.3 kg ($p < 0.01$); of the fat depots measured, the major contributor to this was leg fat (0.4 kg, $p < 0.01$). The total lean mass decreased by 0.4 kg ($p < 0.05$), including a reduction in skeletal muscle mass of 0.2 kg ($p < 0.01$) measured as the (appendicular) lean mass of the legs. The relative increase in different fat depots varied with the lowest being leg fat mass at 5% ($p < 0.01$), followed by subcutaneous abdominal fat at 13% ($p < 0.05$), and lastly the greatest proportional increase being in VAT mass at 22% ($p < 0.01$) (Figure 1).

Both systolic and diastolic BP increased substantially across the menopause (Table 1). The changes of conventional lipids were small (Table 1): total cholesterol and triglycerides were largely unchanged together with HDL-cholesterol whereas there was a non-significant rise in LDL-C (+5%, $p = 0.16$). A significant increase in ApoB (+12%, $p < 0.001$), in the absence of changes in triglycerides reflects increase in the LDL particle concentration in plasma across menopause. In terms of regulators of LDL particle numbers in plasma, lower plasma PCSK9 concentrations (-21%, $p < 0.01$) were observed after the menopause transition. There were no changes in fasting plasma glucose and insulin concentrations. There was however a reduction

(-20%, $p < 0.05$) in plasma non-esterified fatty acids (NEFA). There was a small but statistically significant increase in the plasma concentration of CRP ($p < 0.01$).

Correlations between DEXA body compartments and BP and lipid CVD risk factors: Total and regional abdominal fat masses showed positive associations with BP and ApoB in both pre- and postmenopausal state (Suppl. Table 1 and 2). The associations were weak or absent with LDL-cholesterol but robustly negative with HDL-cholesterol. The associations between leg fat mass and ApoB and LDL-C were weak and statistically non-significant.

Associations between the premenopausal DEXA body compartments and the change in BP and ApoB across the menopause: The premenopausal fat or lean mass compartment did not correlate with BP or ApoB in an unadjusted model whereas adjustment for total fat mass brought out significant negative association between VAT and systolic BP and positive association between subcutaneous fat and diastolic BP suggesting that regional fat masses rather than total fat mass are drivers for these CVD risk factors (Table 2).

Associations between change in DEXA body compartments and change in BP and ApoB across menopause: To analyse whether parameters changing across menopause did so in a coordinated way we selected the established CVD risk factors that showed a robust increase across menopause, as above, and examined the relationship between the change of these factors with the change of the total and regional fat and lean masses.

Whereas the absolute change in systolic BP did not correlate significantly with change in total fat, regional fat and lean masses across menopause, the rise in diastolic BP was significantly associated with the absolute increments in android and VAT fat masses. The

decrease in lean mass across menopause was also unrelated to the increase in diastolic BP.

The increase of ApoB across menopause was associated with increase in android, VAT and total fat mass, but not with change in leg fat mass or lean mass.

We further investigated if the changes in the regional fat mass were independent of the total fat mass change, premenopausal age, time between visits and baseline outcome value (Table 3). Following adjustments for the above confounders, the relationship between the increase in diastolic BP and android fat remained significant, but other associations were lost. Similarly, the relationships seen between regional fat compartments and ApoB levels were also lost when adjusted for change in total fat mass suggesting that any of the changes attributed to the regional compartments in the adjusted model are probably mediated by changes in total fat mass across the menopause.

Discussion

This study was designed to examine the longitudinal associations between body composition (fat and lean mass) with known CVD risk factors across the menopause transition. Despite a small and statistically non-significant increase in body weight there was an increase in total fat mass seemingly counterbalanced by a decrease in lean mass which appears to be driven by a reduction in skeletal muscle mass since the appendicular (leg lean mass) was proportionally most reduced. Greatest proportional increase across the menopausal transition was observed with VAT. These findings are in agreement with previous longitudinal CT-based studies¹⁵⁻¹⁷ and larger cross-sectional studies^{13,14}.

There was an expected rise in ApoB without statistically significant changes in total cholesterol, triglycerides, HDL cholesterol and calculated-LDL, which aligns with previous studies showing that the pre to post-menopausal transition is associated with raised LDL-C and that postmenopausal women tend to have a predominance of small, dense LDL compared with pre-menopausal²²⁻²⁴. This could add to an increased CVD risk beyond the measurement of conventional lipoproteins²²⁻²⁴.

Although the menopausal rise in ApoB was associated with change in visceral and abdominal fat mass, it was also associated with the change in total fat mass. When, however, the associations for regional fat depots were adjusted for other potential confounders there was no statistically significant association suggesting that the rise in ApoB is not independently associated with fat distribution changes across menopause. It is possible that other factors, presumably operating in the liver like LDL receptor activity, are likely to determine the ApoB concentration. The modest rise in ApoB with a largely unchanged LDL-C as we observe may relate to the decline in estrogen levels associated with menopause. Estrogen suppresses hepatic lipase that is involved in the transitioning of intermediate to LDLs²⁵ and therefore an increase in its activity through the menopause is a possible explanation.

To our knowledge this is the first study to report longitudinal changes in PCSK-9 across the menopause and our findings contrasts to previous cross-sectional studies that have found the opposite association comparing groups of pre and postmenopausal women^{26,27}. This reduction in PCSK-9 may signal a liver-specific adaptation to menopause with possible implications for hepatic cholesterol homeostasis and is likely to be related to pre- to postmenopausal partitioning of hepatic lipids leading to distinct patterns of lipoprotein secretions²⁸. A significant reduction in the fasting plasma NEFA concentration across menopause may

implicate a menopause-related functional change in the control of adipose tissue lipolysis and therefore indirectly also in fat storage. It is noted that the drop in fasting NEFA across menopause is of the same order of magnitude as difference between the pre-menopausal female NEFA concentrations compared with age-equivalent men²⁰. It could be that menopause alters the tissue response away from a particularly efficient fat storing organ as in the premenopausal state²⁹⁻³¹ to the male fat storage (android fat accumulation) pattern. It has also been shown that longitudinally women transitioning from pre-menopause to peri / post menopause have a decrease in subcutaneous adipose tissue lipolysis, although this was thought to be related to ageing rather than menopause³². These suggest that impaired adipose tissue and liver function across menopause could contribute to the worsening of the CVD risk factor profile.

When examining the remaining biochemical tests, we found no changes in fasting glucose or insulin levels, which is consistent with existing data showing that the menopause transition does not negatively affect glucose metabolism^{7,33,34}. As expected we also found that CRP increased across the menopause transition which is a well-recognised inflammatory risk marker for CVD in postmenopausal women³⁵.

Previous studies across the menopause have shown inconsistent effect of menopause on systolic and diastolic BP⁶. This could possibly relate to differing study designs, sample size, ethnic background, age-range of participants, menopause classification, years of follow-up, and different methods of BP monitoring as well as inclusion of both natural and surgically induced menopausal women, who have differing hormonal and cardiovascular outcomes³⁶. Change in android fat across menopause with an increase in diastolic BP is consistent with other observations that intrabdominal fat accumulation could be an important risk factor for

high BP³⁷. Although not directly investigated in this study the most probable explanation for the rise in BP seen independent of fat accumulation is the rapid decline in endothelial vasodilator function related to oestrogen deficiency across the menopause transition³⁸. Again, it seems the menopausal effect on blood pressure is only modestly linked to a change in fat mass and more likely regulated at the end organ, i.e. vascular bed.

We recognise some of the strengths and limitations of our study. The strength is the longitudinal design of the study that included homogeneous, apparently healthy, white women, with well-defined menopausal follow-up period and identical measurements taken at both time points using same platforms thus eliminating variability in measurements. Although apparently healthy, due to non-availability of data, we have not accounted for lifestyle factors such as diet, exercise, alcohol intake or smoking status which can clearly impact on metabolic health. Our results are also limited to the UK population and therefore generalizability of our finding among other ethnic groups is limited, due to inherent differences in body composition. The protocol involved re-examination of women within only ~5y gap. It could well be that some anthropometric adaptations associated with menopause take longer to have their full effect on metabolic homeostasis.

Conclusion

Our study provides evidence that across the menopause transition there are changes seen in body fat distribution towards a more android like distribution alongside a worsening in cardiovascular risk factors across this same time period. However, in this study it appears that the changes in established CVD risk factors across the menopause are not attributed to any significant degree to body composition changes, or perhaps in particular the distinct increase

in VAT. Although leg fat mass has been shown to have a protective association on CVD risk markers, the increase across menopause observed in this study was in line with a total fat mass increase and without a re-distribution towards gynoid adiposity, any positive association of leg fat mass may have been counterbalanced by increments in other fat depots. We also observe a number of effects suggesting that qualitative and functional changes in metabolic organs occur across menopause and these effects might have a stronger impact on CVD risk profile than changes in body composition.

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Figure 1. Showing the percentage (%) change across the menopause of different fat depots and lean tissue measured by DEXA.