Changes in abdominal subcutaneous adipose tissue phenotype following menopause is associated with increased visceral fat mass

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Menopause is associated with a redistribution of adipose tissue towards central adiposity, known to cause insulin resistance. In this cross-sectional study of 33 women between 45 and 60 years, we assessed adipose tissue inflammation and morphology in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) across menopause and related this to menopausal differences in adipose tissue distribution and insulin resistance. We collected paired SAT and VAT biopsies from all women and combined this with anthropometric measurements and estimated whole-body insulin sensitivity. We found that menopause was associated with changes in adipose tissue phenotype related to metabolic dysfunction. In SAT, postmenopausal women showed adipocyte hypertrophy, increased inflammation, hypoxia and fibrosis. The postmenopausal changes in SAT was associated with increased visceral fat accumulation. In VAT, menopause was associated with adipocyte hypertrophy, immune cell infiltration and fibrosis. The postmenopausal changes in VAT phenotype was associated with decreased insulin sensitivity. Based on these findings we suggest, that menopause is associated with changes in adipose tissue phenotype related to metabolic dysfunction in both SAT and VAT. Whereas increased SAT inflammation in the context of menopause is associated with VAT accumulation, VAT morphology is related to insulin resistance.

Loss of ovarian function leads to an increased risk of metabolic disease including metabolic syndrome, type 2 diabetes, and cardiovascular disease1–3. The mechanisms are poorly understood but is believed to be related to the increased visceral adipose tissue (VAT) deposition seen in relation to menopause4–6. For a given total fat mass, postmenopausal women store significantly more VAT than premenopausal women4. The mechanisms behind the redistribution of fat with menopause are largely unknown. However, it is generally believed that subcutaneous adipose tissue (SAT) dysfunction, e.g. as seen with lipodystrophy7, cause lipid spill over from SAT, whereby lipids will be redirected and stored in less favorable anatomic locations such as the VAT (and other ectopic sites)8,9.

We previously showed, that an increase in both total fat mass and VAT mass were associated with a more pronounced increase in ectopic lipid deposition and insulin resistance (IR) in postmenopausal women compared with premenopausal women4. These findings indicate that the health-related consequences linked to expanding

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the fat mass might increase after menopause and suggest that postmenopause could be related to AT dysfunction, both in SAT and VAT, with a lower threshold for lipid spillover to peripheral organs, in postmenopausal women. The manner in which the adipose tissue (AT) expands and remodels, directly impacts the risk of metabolic disease. Pathologic AT remodeling is characterized by adipocyte hypertrophy, pericellular fibrosis (PCF), and chronic inflammation all contributing to IR. Studies in rodents show that oophorectomy leads to an increased adipocyte size, infiltration of immune cells, and a higher expression of inflammatory cytokines in both inguinal- and gonadal adipose depots (equivalent to SAT and VAT in humans). In humans, postmenopause is associated with systemic low-grade inflammation. However, little is known about the relationship between menopause, changes in AT phenotype and metabolic disease. We collected paired SAT and VAT biopsies from pre-, peri-, and postmenopausal women. We hypothesized, that menopause was associated with changes in AT phenotype, and that this could be related to postmenopausal AT redistribution and IR.

**Results**

**Subject characteristics.** Subject characteristics are shown in Table 1. Thirteen women were defined as premenopausal, five as perimenopausal, and fifteen as postmenopausal. Postmenopausal women had a median time since menopause of 4 (interquartile range, IQR 3–7) years. The groups showed no significant differences in body weight (p = 0.55) or total fat mass (p = 0.12). Postmenopausal women had a 123% (95% CI 16–231%) bigger VAT mass compared to premenopausal women and for a given total fat mass, postmenopausal women stored 18% (95% CI 2–33%) more VAT.

Peri- and postmenopausal women had higher plasma HbA1c (peri: 12%, 95% CI 1–23% and post: 11%, 95% CI 3–19%) and a lower estimated insulin sensitivity (IS) (peri: 42%, 95% CI 7–77% and post: 29%, 95% CI 3–55%) compared to premenopausal women. Menopausal status did not affect total number of adipocytes in SAT (p = 0.31) or VAT (p = 0.86) (Fig. 1E). Adipocyte number per kg SAT was only borderline associated with menopausal status with a tendency to fewer adipocytes after menopause (p = 0.07). Postmenopausal women had 15% (95% CI 3–24%) fewer adipocytes per kg VAT compared to premenopausal women (Fig. 1F).

**Adipocyte size and number.** Adipocyte size distribution differed significantly between pre- and postmenopausal women in both SAT (p < 0.01) and VAT (p < 0.01) with a higher frequency of smaller adipocytes in postmenopausal women and of bigger adipocytes in postmenopausal women. Adipocyte size distribution in perimenopausal women was not significantly different from either pre- or postmenopausal women (Fig. 1A–D). Menopausal status did not affect total number of adipocytes in SAT (p = 0.31) or VAT (p = 0.86) (Fig. 1E). Adipocyte number per kg SAT was only borderline associated with menopausal status with a tendency to fewer adipocytes after menopause (p = 0.07). Postmenopausal women had 15% (95% CI 3–24%) fewer adipocytes per kg VAT compared to premenopausal women (Fig. 1F).

Messenger-RNA (mRNA) expression of the adipogenic marker Peroxisome proliferator activated receptor (PPAR)-γ in SAT was not associated with menopausal status (p = 0.60). PPAR-γ in VAT was increased (83%, 95% CI 21–144%) in postmenopausal women compared to perimenopausal women. Fatty acid synthase (FAS), a marker of lipogenesis, was decreased by 61% (95% CI 16–106%) in SAT in postmenopausal women compared to premenopausal women. In SAT, hypoxia inducible factor (HIF)-1α expression was significantly increased (255%, 95% CI 3–40%) in postmenopausal women compared to premenopausal women (p = 0.026). Stearoyl-CoA desaturase (SCD)1, reflecting mono-unsaturated lipidogenesis, was decreased by 61% (95% CI 16–106%) in SAT in postmenopausal women compared to perimenopausal women. Fatty acid synthase (FAS), a marker of lipogenesis, was decreased by 61% (95% CI 16–106%) in SAT in postmenopausal women compared to premenopausal women.

**Adipose tissue immune cell infiltration.** CD163+ cell infiltration in SAT was not significantly associated with menopausal status (p = 0.11). In VAT, number of CD163+ cells were increased by 111% (95% CI 26–195%) in postmenopausal women compared to premenopausal women (Fig. 2A). AT CD68+ cell infiltration was comparable across menopausal status in both SAT (p = 0.66) and VAT (p = 0.40), as was CD3+ cell infiltration (SAT: p = 0.22 and VAT: p = 0.26) (Fig. 2B,C). CD20+ cell infiltration in SAT was not significantly associated with menopausal status (p = 0.63). Postmenopausal women had a 169% (95% CI 59–278%) increase in CD20+ cells in VAT compared to premenopausal women (Fig. 2D).

**Adipose tissue fibrosis.** Postmenopausal women had increased PcF in both SAT (21%, 95% CI 3–40%) and VAT (130%, 95% CI 54–206%) compared to premenopausal women (Fig. 2E,F).

**Adipose tissue inflammatory markers.** mRNA expression of interleukin (IL)-6 in SAT was increased by 152% (95% CI 35–267%) in postmenopausal women compared to premenopausal women. VAT IL-6 mRNA expression was not associated with menopausal status (p = 0.34). IL-18 expression in SAT was increased by 41% (95% CI 21–60%) in postmenopausal women compared to premenopausal women. VAT IL-18 mRNA expression was not associated with menopausal status (p = 0.76). IL-18 mRNA expression was 7.6 (95% CI 6.7–8.5) times higher in VAT compared to SAT (p < 0.0001). Tumor necrosis factor (TNF)-α expression in AT was comparable across menopausal status (SAT, p = 0.25 and VAT, p = 0.23). Monocyte Chemoattractant Protein (MCP)-1 expression was increased by 67% (95% CI 20–114%) in SAT in postmenopausal women compared to premenopausal women. VAT MCP-1 mRNA expression was not significantly associated with menopausal status (p = 0.09) (Fig. 2G).
Subcutaneous adipose tissue phenotype visceral fat accumulation. In this cohort, an increased VAT mass was associated with low SAT FAS ($\beta = -1.33$, $p < 0.001$), high HIF-1α ($\beta = 0.52$, $p = 0.01$), CD163+.

Table 1. Subject characteristics. Data are presented as median (interquartile range). $E_2$, estradiol, $FSH$, follicle stimulating hormone, $IU$, international units, $AU$, arbitrary units, $BMI$, body mass index, $SAT$, subcutaneous adipose tissue, $VAT$, Viseral adipose tissue, $L$, liter, $VO_2$ max maximum oxygen uptake, $MET$, Metabolic equivalents, $LDL$, low-density lipoprotein, $HDL$, high-density lipoprotein, $FFA$, free fatty acids, $AUC$, area under the curve. *Significantly different from premenopausal, $p < 0.05$. b Self-reported. c Estimated insulin sensitivity calculated through Composite Matsuda Index.
Figure 1. Adipocyte size and mRNA expression in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) in pre- (SAT, n = 10; VAT, n = 12), peri- (SAT and VAT, n = 5), and postmenopausal women (SAT, n = 12; VAT, n = 14). (A) Adipocyte size distribution in SAT in pre-, peri-, and postmenopausal women, (B) Adipocyte size distribution in VAT, (C) Representative HE-section of SAT and VAT, (D) Adipocyte size in SAT and VAT, (E) Adipocyte number in SAT and VAT, (F) Adipocyte number per kg fat in SAT and VAT, (G) mRNA expression of Peroxisome Proliferator-Activated Receptor (PPAR)-γ, Fatty Acid Synthase (FAS), Steraoyl-CoA Desaturase (SCD)1, Hypoxia-inducible factor (HIF)-1α, Vascular Endothelial Growth Factor A (VEGFA), Estrogen Receptor 1 (ESR1), and Adiponectin (AdipoQ) in both SAT and VAT. Data are presented as median (interquartile range). *Significant difference, p < 0.05.
displayed increased PcF in both SAT and VAT. A previous study showed that increasing AT PcF was associated with increased VAT fibrosis ($R = 0.429$, $p = 0.02$), CD163+ cell infiltration ($R = 0.504$, $p = 0.005$), IL-18 production of reactive oxygen species (inducers of HIF-1α expression), deteriorate mitochondrial function, and the ability to lose weight, e.g. through exercise, following menopause. Estrogen has been suggested to prevent the development of obesity24, and we observed decreased expression of FAS in SAT were all associated with increased VAT deposition, even after correcting for total fat mass (Table 2).

**Visceral adipose tissue phenotype and estimated insulin sensitivity.** In VAT, increased HIF-1α and MCP-1 expression was associated with decreased estimated IS even after correcting for VAT mass ($\beta_1 = -0.37$, $p = 0.01$ and $\beta_2 = -0.25$, $p = 0.03$), whereas the associations between increased adipocyte size and PcF and estimated IS were only borderline significant after adjusting for VAT mass ($\beta_1 = -0.14$, $p = 0.06$ and $\beta_2 = -0.36$, $p = 0.06$) (Table 3). The regression analyses revealed that the association between VAT adipocyte size, PcF, and HIF-1α and estimated IS were stronger than the association between estimated IS and VAT mass itself ($\beta_3$ adipocyte size: $-0.415$ vs. $\beta_1$ VAT mass: $-0.227$; $\beta_3$ PcF: $-0.377$ vs. $\beta_2$ VAT mass: $-0.349$; $\beta_3$ HIF-1α: $-0.516$ vs. $\beta_2$ VAT mass: $-0.504$). Increased VAT IL-6 and TNF-α expression were also associated with decreased estimated IS, however, these associations were diminished after correcting for VAT mass ($\beta_1 = -0.34$, $p = 0.16$ and $\beta_2 = -0.27$, $p = 0.08$).

Apart from a negative association between SAT CD3+ cell infiltration and estimated IS ($\beta = -0.08$, $p = 0.01$), SAT phenotype was not associated with estimated IS (Supplementary Information 1).

**Aging.** SAT inflammation and -morphology was not correlated to age. Increasing age was significantly associated with increased VAT fibrosis ($R = 0.429$, $p = 0.02$), CD163+ cell infiltration ($R = 0.504$, $p = 0.005$), IL-18 ($R = 0.755$, $p = 0.005$) and TNF-α expression ($R = 0.463$, $p = 0.02$) (Supplementary Information 2).

**Discussion**

The key findings of this study were that postmenopause is associated with changes in AT phenotype related to metabolic dysfunction in both SAT and VAT. Whereas increased SAT inflammation in the context of menopause is associated with VAT accumulation, changes in VAT morphology is associated with decreased estimated IS.

Adipocyte hypertrophy was evident in both SAT and VAT of postmenopausal women and the number of adipocytes per kg fat was reduced with menopause. Even the calculated total number of adipocytes seemed to be 5–15% lower in the postmenopausal women, although this difference did not reach statistical significance. The fact that loss of endogenous sex hormone production is associated with adipocyte hypertrophy is in accordance with studies in rodents, where oophorectomy leads to an increase in adipocyte size in both inguinal and gonadal adipose depots18–20. The lower number of adipocytes in postmenopausal women indicate that a number of adipocytes might perish in relation to menopause. Estrogen has been shown to regulate cell death in several cell types21,22,23. However, whether the lower adipocyte number after menopause in this study reflects adipocyte apoptosis induced by estrogen withdrawal remains speculative. What drives the adipocyte hypertrophy with menopause is yet to be understood. In this study, we investigated the mRNA expression of a subset of adipogenic and lipogenic markers. We found the key rate-limiting enzyme in de novo lipogenesis, FAS, to be decreased in SAT of postmenopausal women. FAS expression has previously been shown to be lower with obesity24, and we speculate that the downregulation of FAS in this study could reflect a compensatory attempt to avoid further lipid deposition in the adipocyte. Inability to store excess lipids in SAT is known to cause lipid overflow and ectopic lipid deposition. In accordance with this, we found the low FAS expression in SAT to be strongly associated with increased VAT deposition. VAT PPAR-γ expression was increased in postmenopausal women which could reflect an attempt to overcome the increased need for lipid storage in this depot. However, this alone is unlikely to explain the substantial differences in VAT mass across menopause. Studies in rodents generally show increased mRNA expression of lipogenic and adipogenic markers in both inguinal and gonadal depots following oophorectomy25,26 but functional and mechanistic studies are highly warranted to understand how adipogenesis and lipogenesis are regulated with loss of ovarian function.

Adipocyte hypertrophy is believed to cause hypoxia contributing to the induction of AT inflammation27,28. In relation to this, we found that increased adipocyte size was associated with increased HIF-1α expression (the major mediator of the hypoxic response29) in both SAT and VAT. Furthermore, the HIF-1α promoter region is known to contain estrogen response elements and estrogen has been shown to ameliorate HIF-1α signaling to prevent AT hypoxia and fibrosis30. Consequently, both the increased adipocyte size and estrogen withdrawal could contribute to the vastly increased expression of HIF-1α in SAT after menopause. Postmenopausal women displayed increased PcF in both SAT and VAT. A previous study showed that increasing AT PcF was associated with a decreased ability to lose fat25. Thus, we speculate, that the pathologic AT remodeling could compromise the ability to lose weight, e.g. through exercise, following menopause. Estrogen has been suggested to prevent the production of reactive oxygen species (inducers of HIF-1α expression), deteriorate mitochondrial function, and counteract healthy adipose tissue expansion25,32–34. Thus, taking this into consideration, future studies should investigate the possible role of reactive oxygen species in the relationship between loss of ovarian function and changes in adipose tissue phenotype.

Estrogen stimulates the expression of VEGFA (inducing neovascularization in the tissue to avoid hypoxia)34. Paradoxically, we showed that VEGFA expression in VAT was increased with menopause and propose that this could be a compensatory reaction to a hypoxic environment in the AT25. Adiponectin improves oxidative conditions and enhance insulin sensitivity36,37. Thus, we speculated that an increased expression of adiponectin in premenopausal adipose tissue could contribute to the beneficial metabolic phenotype in this group of women. However, we saw no differential expression of adiponectin in either SAT or VAT across menopause. This is in accordance with a previous study investigating circulating adiponectin levels in both humans and mice38.

The mechanisms underlying the redistribution of fat towards increased VAT deposition with menopause are unknown. In this study, increased expression of inflammatory markers, immune cell infiltration, hypoxia, and decreased expression of FAS in SAT were all associated with increased VAT deposition, even after correcting for
total fat mass. This finding could suggest that subcutaneous AT dysfunction might contribute to VAT deposition following menopause. It is widely debated whether subcutaneous AT serves a protective role or contributes to metabolic deterioration and this is likely to depend on the anatomic location— as well as the morphologic phenotype of the subcutaneous adipose depot. In relation to this, immune cell infiltration in the subcutaneous fat compartment have previously been associated with increased VAT mass in both humans and rodents and genetically modified rodent models where immune cell infiltration in AT are depleted are protected against IR.

Low ESR1 expression in adipose tissue has been related to adiposity in humans and studies in rodents show that AT depletion of ESR1 leads to mitochondrial dysfunction, adipocyte hypertrophy and IR. In this study, we found no differences in expression of ESR1 across menopausal status in either SAT or VAT indicating that it is not a differential expression of ESR1 per se that drives the menopausal differences in AT phenotype. As expected, serum follicle stimulating hormone (FSH) increased across menopause. A recent study in rodents showed that aging is associated with increased inflammation in gonadal AT. In accordance with this, we showed that aging is associated with increased VAT inflammation in postmenopausal women and the causal line of these actions should be investigated in future longitudinal studies.

Menopause was associated with increased CD163+ cell infiltration in VAT. CD163+ cells, originally referred to as M2 macrophages, are believed to play a role in tissue repair and matrix remodeling. Total number of CD163+ cells in AT is known to increase with obesity and has been associated with IR. Whether the increased CD163+ cell number is compensatory or contributes to VAT inflammation remains debated. In this study, we found no association between number of CD163+ cells, in either SAT or VAT, and estimated IS suggesting that it is unlikely to be VAT inflammation per se that drives the IR following menopause. Opposite to this, markers of VAT morphology (increased adipocyte size, PCF, and HIF-1α) tended to be associated with decreased estimated IS and the association between all three and estimated IS were stronger than the association between estimated IS and VAT mass itself. Neither SAT inflammation nor morphology was related to estimated IS. These findings are in accordance with a previous study in males, that found increased VAT adipocyte size, but not inflammation, to predict IR and further showed that SAT phenotype was unrelated to IR. The fact that VAT, but not SAT, phenotype was associated with estimated IS might be explained by the anatomic differences in location of adipose depots, with the close proximity between the VAT depot and the liver, where lipid overflow is believed to cause hepatic lipotoxicity and thereby IR. IR itself has also been shown to cause adipose tissue inflammation. Thus, we cannot exclude that the IR could precede and promote the observed adipose tissue inflammation in postmenopausal women and the causal line of these actions should be investigated in future longitudinal studies.

Menopause was associated with increased CD163+ cell infiltration in VAT. CD163+ cells, originally referred to as M2 macrophages, are believed to play a role in tissue repair and matrix remodeling. Total number of CD163+ cells in AT is known to increase with obesity and has been associated with IR. Whether the increased CD163+ cell number is compensatory or contributes to VAT inflammation remains debated. In this study, we found no association between number of CD163+ cells, in either SAT or VAT, and estimated IS suggesting that the link between the two might be subsidiary. CD20+ cell number was also increased in VAT of postmenopausal women, (B) Number of CD68+ cells/100 adipocytes in SAT and VAT, (C) Number of CD3+ cells/100 adipocytes in SAT and VAT, (D) Number of CD20+ cells/100 adipocytes in SAT and VAT, (E) Pericellular fibrosis (PCF)/100 adipocytes in SAT and VAT, (F) Representative immunohistochemistry- and counter stainings of SAT and VAT, (G) mRNA expression of interleukin (IL)-6, IL-18, Tumor Necrosis Factor (TNF)-α, and Monocyte Chemotactic Protein (MCP)-1, in SAT and VAT. Data are presented as median (interquartile range).
Table 2. Subcutaneous adipose tissue inflammation and morphology associated with visceral adipose tissue mass. SAT: subcutaneous adipose tissue, VAT: visceral adipose tissue, PPAR: Peroxisome proliferator activated receptor, FAS: fatty-acid synthase, SCD: Stearoyl-CoA desaturase, HIF: Hypoxia-inducible factor, VEGFA: vascular endothelial growth factor, ESR1: estrogen receptor 1, AdipoQ: adiponectin, CD: cluster of differentiation, PcF: pericellular fibrosis, IL: interleukin, TNF: tumor necrosis factor, MCP: Monocyte Chemoattractant Protein. N = 26. *Model corrected for total fat mass.

| VAT mass, L | SAT Adipocyte size (β (95% CI)) | p-value | VAT mass, L* | SAT Adipocyte size (β (95% CI)) | p-value |
|------------|---------------------------------|---------|--------------|---------------------------------|---------|
| β          | 0.20 (−0.76 to 1.15)            | 0.67    | −0.27 (−1.17 to 0.62) | 0.53    |
| SAT PPAR-γ | −0.29 (−0.81 to 0.22)           | 0.24    | 0.04 (−0.65 to 0.72)  | 0.90    |
| SAT FAS    | −1.23 (−1.67 to −0.80)          | <0.001  | −1.33 (−1.93 to −0.72)| <0.001  |
| SAT SCD1   | −0.86 (−1.56 to −0.17)          | 0.02    | −0.78 (−1.75 to 0.20)| 0.11    |
| SAT HIF-1α | 0.77 (0.34 to 1.21)             | 0.002   | 0.52 (0.13 to 0.92)  | 0.01    |
| SAT VEGFA  | −0.40 (−0.88 to 0.08)           | 0.10    | −0.06 (−0.50 to 0.39)| 0.80    |
| SAT ESR1   | −0.37 (−0.80 to 0.07)           | 0.09    | −0.03 (−0.44 to 0.38)| 0.88    |
| SAT AdipoQ | −0.52 (−0.92 to −0.12)          | 0.01    | −0.19 (−0.61 to 0.24)| 0.36    |
| SAT CD163* cells | 0.13 (0.07 to 0.20) | <0.001 | 0.09 (0.00 to 0.17) | 0.04    |
| SAT CD68* cells | 0.16 (−0.04 to 0.35) | 0.10    | 0.14 (−0.03 to 0.30)| 0.10    |
| SAT CD3* cells | 0.65 (0.15 to 1.15) | 0.01    | 0.45 (0.03 to 0.86) | 0.04    |
| SAT CD20* cells | 0.86 (0.28 to 1.44) | 0.005   | 0.65 (0.11 to 1.19)| 0.02    |
| SAT PcF    | 0.17 (0.04 to 0.30)             | 0.01    | 0.10 (−0.04 to 0.24)| 0.14    |
| SAT IL-6 mRNA | 0.75 (0.19 to 1.30) | 0.01    | 0.50 (0.03 to 0.96) | 0.04    |
| SAT IL-18 mRNA | 4.34 (2.71 to 5.98) | <0.001  | 3.28 (1.23 to 5.33)| 0.003   |
| SAT TNF-a mRNA | 0.59 (0.15 to 1.04) | 0.01    | 0.40 (0.03 to 0.77)| 0.03    |
| SAT MCP-1 mRNA | 1.24 (0.09 to 2.39) | 0.04    | 0.93 (0.04 to 1.81)| 0.04    |

Table 3. Visceral adipose tissue inflammation and morphology associated with estimated insulin sensitivity. VAT: visceral adipose tissue, AU: arbitrary units, PPAR: Peroxisome proliferator activated receptor, FAS: fatty-acid synthase, SCD: Stearoyl-CoA desaturase, HIF: hypoxia-inducible factor, VEGFA: vascular endothelial growth factor, ESR1: estrogen receptor 1, AdipoQ: adiponectin, CD: cluster of differentiation, PcF: pericellular fibrosis, IL: interleukin, TNF: tumor necrosis factor, MCP: Monocyte Chemoattractant Protein. N = 25. *Estimated insulin sensitivity calculated through Composite Matsuda Index. **Model corrected for VAT mass.

| Estimated insulin sensitivity, AU | β (95% CI) | p-value | Estimated insulin sensitivity, AU* | β (95% CI) | p-value |
|---------------------------------|------------|---------|---------------------------------|------------|---------|
| VAT adipocyte size              | −0.19 (−0.31 to −0.07) | 0.004   | −0.14 (−0.30 to 0.01) | 0.06    |
| VAT PPAR-γ                      | 0.05 (−0.57 to 0.67) | 0.88    | 0.03 (−0.71 to 0.78) | 0.93    |
| VAT FAS                         | 0.49 (−0.02 to 1.00) | 0.06    | 0.10 (−0.39 to 0.60) | 0.67    |
| VAT SCD1                        | 0.16 (−1.06 to 1.37) | 0.79    | 0.23 (−1.23 to 1.69) | 0.75    |
| VAT HIF-1α                      | −0.32 (−0.67 to 0.03) | 0.07    | −0.37 (−0.66 to −0.09)| 0.01    |
| VAT VEGFA                       | 0.03 (−0.27 to 0.32) | 0.86    | 0.03 (−0.23 to 0.29) | 0.82    |
| VAT ESR1                        | 0.05 (−0.38 to 0.48) | 0.80    | −0.09 (−0.48 to 0.31)| 0.65    |
| VAT AdipoQ                      | 0.06 (−0.24 to 0.36) | 0.69    | 0.06 (−0.21 to 0.34)| 0.63    |
| VAT CD163* cells               | −0.37 (−1.01 to 0.28) | 0.25    | 0.05 (−0.67 to 0.77) | 0.89    |
| VAT CD68* cells                | 0.25 (−0.06 to 0.57) | 0.11    | 0.24 (−0.04 to 0.52) | 0.09    |
| VAT CD3* cells                 | −0.31 (−0.84 to 0.22) | 0.24    | −0.21 (−0.71 to 0.28)| 0.38    |
| VAT CD20* cells                | −0.33 (−0.69 to 0.04) | 0.08    | −0.17 (−0.56 to 0.23)| 0.39    |
| VAT PcF                        | −0.46 (−0.82 to −0.1) | 0.02    | −0.36 (−0.72 to 0.01)| 0.06    |
| VAT IL-6 mRNA                  | −0.52 (−0.96 to −0.09) | 0.02    | −0.34 (−0.81 to 0.14)| 0.16    |
| VAT IL-18 mRNA                 | −0.17 (−0.35 to 0.01) | 0.07    | −0.13 (−0.29 to 0.04)| 0.13    |
| VAT TNF-a mRNA                 | −0.35 (−0.67 to −0.03) | 0.03    | −0.27 (−0.57 to 0.04)| 0.08    |
| VAT MCP-1 mRNA                 | −0.22 (−0.59 to 0.15) | 0.20    | −0.25 (−0.47 to −0.03)| 0.03    |
narrow and the effects of age on adipose phenotype, especially in SAT, were sparse. Thus, the contribution of age to the overall findings are believed to be modest.

We conclude that menopause is associated with changes in AT phenotype related to metabolic dysfunction in both SAT and VAT. Whereas increased SAT inflammation in the context of menopause was associated with VAT accumulation, changes in VAT morphology was associated with decreased estimated IS. Based on these findings, we speculate that changes in SAT and VAT phenotype in relation to menopause could represent important mechanistic links between menopause, adipose redistribution and IR. Furthermore, the findings suggest a metabolically more detrimental effect of expanding the SAT and VAT depots after menopause. Thus, future studies should focus on efficient strategies such as diet or exercise to prevent postmenopausal metabolic deterioration and adipose tissue dysfunction.

Methods
Participants. Women between 45 and 60 years were recruited through the Department of Gynecology, Rigshospitalet, Denmark, and were all undergoing benign gynecological surgeries. Types of surgeries included hysterectomy, and uni- and bilateral oophorectomies. Included indications for hysterectomy were uterine fibroids and menstrual bleeding disorders. Indications for unilateral oophorectomies were ovarian cysts and bilateral oophorectomies were performed due to genetic predisposition to cancer.

The women were divided into premenopausal, perimenopausal and postmenopausal based on menstrual status and FSH. In accordance with international guidelines, a period of 12 months of amenorrhea is a prerequisite for the label of postmenopause. A cut-off of FSH ≥ 20 IU/l were chosen to distinguish a regularly ovulating ovary from an ovary with a gradually declining function, in accordance with larger population studies.

Premenopausal women were defined as having regular menstrual bleedings (10 menstrual bleedings within the last 12 months) and serum FSH < 20 IU/l. Postmenopausal as having amenorrhea for > 12 months and FSH ≥ 20 IU/l. Women who did not meet the subdivision criteria because of menstrual bleedings within the last 12 months despite serum FSH > 20 IU/l were defined as perimenopausal.

Exclusion criteria were: (1) chronic diseases (including endometriosis), (2) infections during the last 4 weeks, (3) more than 7 alcohol units/week, (4) premature menopause (before age 40 years), (5) hysterectomy or oophorectomy prior to study inclusion, (6) body mass index (BMI) > 35 kg/m² (as MRIs could not be performed with higher BMI).

Premenopausal women with regular menstrual bleedings went through all tests in the follicular phase of their menstrual cycle (day 1–8). Women with irregular bleedings were tested on a random cycle day, where serum FSH were obtained simultaneously. Postmenopausal women were also tested on a random day.

A subset of the women (n = 18) also participated in a sub-study investigating ectopic lipid deposition with menopause and systemic inflammation, published previously.

Informed consent was obtained in writing and verbally from all participants. The study was approved by the ethical committee of the Capital Region, Denmark (H-3-2014-096) and performed according to the declaration of Helsinki.

Study design. A maximum of 1 month prior to surgery, women reported to the laboratory in the morning, on two separate days, following an overnight fast. All women were instructed not to perform any vigorous exercise 48 h prior to the experiment and abstain from coffee, tea, or alcohol 24 h before. The first experimental day included (1) health exam, and (2) oral glucose tolerance test (OGTT). The second day included (1) whole-body dual-energy X-ray absorptiometry (DXA) scan, (2) biopsies from the SAT and skeletal muscle, and (3) fitness test (VO₂ max test).

On the day of surgery, a biopsy was collected from the omentum majus when surgery was initiated. All biopsies (including omental) were taken before noon, following an overnight fast.

Body composition. Fat- and lean body masses were assessed through a DXA scan (Lunar Prodigy Advance; GE Medical Systems Lunar, Milwaukee, WI). Software (Prodigy, enCORE 2004, version 8.8; GE Lunar Corp, Madison, WI) was used to estimate regional- and total fat, and fat-free tissue masses.

Magnetic resonance imaging (MRI) of the abdomen was performed using a Siemens Magnetom Prisma 3 Tesla matrix magnetic resonance scanner (Erlangen, Germany) at 3-mm intervals as described previously. All adipose tissue located from the diaphragm to pelvic floor inside the peritoneum was traced manually as the visceral fat region of interest. Multi-image analysis software (Mango; Research Imaging Institute, Houston, TX) was used to calculate the total volume of visceral fat from the T1-weighted MRI sequence. A single reader, who was blinded to the menopausal status of the subjects, performed all image analyses.

Estimated insulin sensitivity. Whole-body estimated IS was calculated from an OGGT using the Composite Matsuda IS Index. Blood samples were drawn at time points – 10, 0, 15, 30, 60, 90, and 120 min after drinking 83 g of glucose monohydrate (corresponding to 75 g anhydrous glucose) dissolved in 300 mL of water. Baseline values were calculated as a mean of the – 10- and 0-min samples.

Adipose tissue biopsies. Biopsies from abdominal SAT were obtained with a modified Bergström needle. VAT biopsies were collected as soon as surgery was initiated. All AT biopsies were divided in three; (1) frozen in liquid nitrogen and stored at – 80 °C, (2) fixed in 2% depolymerized paraformaldehyde plus 0.15% picric acid and embedded in paraffin, (3) isolation of adipocyte precursors.
Immunohistochemistry. Paraffin-embedded biopsies were sectioned (5 µm thick) in two depths at least 200 µm apart.

Details on immunohistochemistry staining and antibodies are shown in Supplementary Information 3. Stained cells were counted manually by a single person blinded to the menopausal status of the subjects. In order to get a representative sampling at least 100 adipocytes were counted for each ID. Cells of interest were counted in at least two different regions of the embedded tissue. Quantification of cells was performed using ImageJ software (NIH, Bethesda, MD, USA).

Median volume of adipocytes was estimated using the formula $V = \frac{4}{3} \pi r^3$, and total number was calculated dividing the volume of the fat depot with median volume of adipocytes.

RNA isolation and quantitative real-time PCR. Total RNA was extracted from AT using RNeasy lipid tissue mini kit (Qiagen, Hilden, Germany). RNA was reverse transcribed according to the manufacturer's protocol (Applied Biosystems, Foster City, CA, USA). The RNA levels were determined by quantitative real time PCR using a Viia7 7 sequence detector (Applied Biosystems, Foster City, CA, USA). A mean value of 18S rRNA and GAPDH mRNA levels were used as endogenous control. Primer sequences are shown in Supplementary Information 4.

Due to limited tissue, five pre- and five postmenopausal women were not included in SAT PCR analyses. This was the case for two pre- and two postmenopausal women in VAT PCR analyses.

VO2 max test. VO2 max was determined using a progressive exercise test on a bicycle ergometer (Monark 839E, Monark, Varberg, Sweden) with a 5-min warm up at 70 watts followed by a 20-W increase in workload each minute until exhaustion. VO2 was continuously measured by indirect calorimetry (Quark b2, Cosmed, Rome, Italy).

Questionnaires. Participants completed a Minnesota Leisure Time Physical Activity Questionnaire (MLT-PQ) to determine their physical activity levels over the last 4 months, estimated as an activity metabolic index. The daily energy intake was estimated from a comprehensive 3-day food diary before the first visit. Based on this, mean caloric intake was calculated.

Statistics. All variables were log-transformed if not normally distributed. Data are presented as median (IQR) unless otherwise stated. Variables of interest were compared using a one-way ANOVA with a Fisher's Least Significance Difference post-hoc test. Regression models were checked for assumptions of the linear model, including normal distribution of the residuals, homogeneity of variance, linearity, and independent observations. Standardized β ($\beta_s$) was used to estimate the contribution of each predictor to the dependent variable, within a multiple regression analysis. Distribution of adipocyte size was assessed using a mixed model. A random effect accounting for an individual specific level was included. Simple correlations between variables were investigated using Pearson correlation analyses.

Mixed model analyses were performed in SAS Enterprise Guide version 7.1. Other statistical analyses were performed using IBM SPSS statistics version 25. P < 0.05 was considered statistically significant.

Data availability
The datasets generated during and/or analyzed during the current study are not publicly available due to Danish legislation on data protection but are available from the corresponding author on reasonable request and with permission from the Ethical Committee of the Capital Region, Denmark.

Received: 14 April 2021; Accepted: 30 June 2021
Published online: 20 July 2021

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Acknowledgements
The Centre for Physical Activity Research (CFAS) is supported by TrygFonden (Grants ID 101390 and ID 20045).
During the study period, the Centre of Inflammation and Metabolism (CIM) was supported by a grant from the 
Danish National Research Foundation (DNRF55).

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J.A.: Conceptualization, investigation, writing, T.P.: conceptualization, writing and editing, E.A.S.: investiga-
tion, writing and editing, M.B.: methodology, writing and editing, T.W.: methodology, writing and editing, C.T.: 
methodology, writing and editing, C.E.: methodology, writing and editing, B.K.P.: conceptualization, writing 
and editing, A.T.P.: conceptualization, writing and editing, B.L.: conceptualization, writing.

Funding
Funding was provided by Augustinus Fonden (Grant no. 17-2354).

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/ 
10.1038/s41598-021-94189-2.

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