## Supplementary Information

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

Julie Abildgaard[1,2*], Thorkil Ploug[3], Elaf Al-Saoudi[1], Thomas Wagner[4], Carsten Thomsen[5], Caroline Ewertsen[6], Michael Bzorek[7], Bente Klarlund Pedersen[1], Anette Tønnes Pedersen[8], Birgitte Lindegaard[1,9]

[1] The Centre of Inflammation and Metabolism and the Centre for Physical Activity Research, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
[2] Department of Growth and Reproduction, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[3] Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[4] Department of Oncology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[5] Department of Radiology, Zealand University Hospital, Roskilde, Denmark
[6] Department of Radiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[7] Department of Pathology, Zealand University Hospital, Næstved, Denmark
[8] Department of Gynecology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[9] Department of Pulmonary and Infectious Diseases, Nordsjællands University Hospital, Hillerød, Denmark

**Supplementary 1**. Subcutaneous adipose tissue inflammation and morphology associated with estimated insulin sensitivity.

| | **Estimated insulin sensitivity, AU[a]** | | **Estimated insulin sensitivity, AU[a,b]** | |
|---|---|---|---|---|
| | β (95 % CI) | p-value | β (95 % CI) | p-value |
| SAT adipocyte size | -0.07 (-0.79 – 0.64) | 0.83 | 1.66 (-0.48 – 0.82) | 0.60 |
| SAT PPAR-γ | 0.46 (-0.41 – 1.33) | 0.28 | 0.16 (-0.76 – 1.08) | 0.72 |
| SAT FAS | 0.26 (-0.06 – 0.58) | 0.09 | 0.15 (-0.21 – 0.52) | 0.39 |
| SAT SCD1 | 0.26 (-0.31 – 0.82) | 0.35 | 0.13 (-0.43 – 0.68) | 0.64 |
| SAT HIF-1α | 0.06 (-0.45 – 0.57) | 0.81 | 0.25 (-0.24 – 0.74) | 0.30 |
| SAT VEGFA | 0.29 (-0.04 – 0.62) | 0.08 | 0.19 (-0.18 – 0.56) | 0.29 |
| SAT ESR1 | 0.22 (-0.10 – 0.53) | 0.16 | 0.10 (-0.26 – 0.46) | 0.56 |
| SAT AdipoQ | 0.26 (-0.04 – 0.55) | 0.08 | 0.14 (-0.24 – 0.53) | 0.44 |
| SAT $CD163^+$ cells | -0.32 (-0.67 – 0.02) | 0.06 | -0.05 (-0.53 – 0.43) | 0.83 |
| SAT $CD68^+$ cells | -0.31 (-0.71 – 0.10) | 0.13 | -0.27 (-0.62 – 0.09) | 0.13 |
| SAT $CD3^+$ cells | -0.62 (-1.00 – -0.25) | 0.003 | -0.51 (-0.86 – -0.16) | 0.007 |
| SAT $CD20^+$ cells | -0.27 (-0.65 – 0.10) | 0.14 | -0.12 (-0.49 – 0.26) | 0.52 |
| SAT PcF | -0.55 (-0.96 – -0.13) | 0.01 | -0.37 (-0.82 – 0.08) | 0.10 |
| SAT IL-6 mRNA | -0.20 (-0.71 – 0.31) | 0.42 | 0.01 (-0.54 – 0.56) | 0.98 |
| SAT IL-18 mRNA | -0.31 (-0.79 – 0.18) | 0.20 | -0.08 (-0.67 – 0.52) | 0.79 |
| SAT TNF-α mRNA | -0.05 (-0.50 – 0.41) | 0.83 | 0.05 (-0.38 – 0.48) | 0.81 |
| SAT MCP-1 mRNA | -0.04 (-1.02 – 0.94) | 0.93 | 0.12 (-0.80 – 1.04) | 0.79 |

[a] Estimated insulin sensitivity calculated through Composite Matsuda Index.

[b] Model corrected for total mass

SAT, subcutaneous adipose tissue. PPAR, Peroxisome proliferator activated receptor. FAS, fatty-acid synthase. SCD, Stearoyl-CoA desaturase. HIF, Hypoxia-inducible factor. VEGFA, Vascular endothelial growth factor A. ESR1, Estrogen receptor 1. AdipoQ, adiponectin. CD, cluster of differentiation. PcF, pericellular fibrosis. IL, interleukin. TNF, tumor necrosis factor. MCP, Monocyte Chemoattractant Protein.

**Supplementary 2**. Adipose tissue inflammation and morphology correlated to age

| | Age | |
|---|---|---|
| | R | p-value |
| SAT adipocyte size | -0.026 | 0.90 |
| VAT adipocyte size | 0.306 | 0.10 |
| SAT PPAR-γ | -0.038 | 0.87 |
| VAT PPAR-γ | 0.048 | 0.81 |
| SAT FAS | -0.283 | 0.21 |
| VAT FAS | -0.288 | 0.15 |
| SAT FAS | -0.276 | 0.23 |
| VAT FAS | 0.056 | 0.78 |
| SAT fibrosis | 0.330 | 0.10 |
| VAT fibrosis | 0.429 | 0.02 |
| SAT $CD163^+$ cell infiltration | 0.396 | 0.06 |
| VAT $CD163^+$ cell infiltration | 0.504 | 0.005 |
| SAT $CD68^+$ cell infiltration | 0.032 | 0.88 |
| VAT $CD68^+$ cell infiltration | -0.006 | 0.98 |
| SAT $CD3^+$ cell infiltration | 0.172 | 0.43 |
| VAT $CD3^+$ cell infiltration | 0.243 | 0.21 |
| SAT $CD20^+$ cell infiltration | 0.126 | 0.55 |
| VAT $CD20^+$ cell infiltration | 0.290 | 0.13 |
| SAT IL-6 | 0.348 | 0.12 |
| VAT IL-6 | 0.220 | 0.27 |
| SAT IL-18 | 0.533 | 0.17 |
| VAT IL-18 | 0.755 | 0.005 |
| SAT TNF-α | 0.012 | 0.96 |
| VAT TNF- α | 0.463 | 0.02 |
| SAT MCP-1 | 0.236 | 0.30 |
| VAT MCP-1 | 0.169 | 0.40 |
| SAT HIF-1α | 0.209 | 0.36 |
| VAT HIF-1α | 0.278 | 0.16 |

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 A. ESR1, Estrogen receptor 1. CD, cluster of differentiation. PcF, pericellular fibrosis. IL, interleukin. TNF, tumor necrosis factor. MCP, Monocyte Chemoattractant Protein.

**Supplementary 3.** Immunohistochemistry methods and antibody details

***Methods***

Dewaxing and antigen retrieval was performed by immersing slides in EnVision™ FLEX Target Retrieval Solution, High pH (Dako, Glostrup, cat.no K8004) and heated at 65ºC for 16 hours in the PT-module according to the manufacturer's instructions. After pre-treatment, slides were incubated with the primary antibodies for 30 min. The reactions were detected/visualized using the standard polymer technique EnVision™ FLEX /HRP Detection Reagent (Dako, cat. no K8000) and signal intensity was enhanced using EnVision™ FLEX+ Mouse (LINKER) (Dako, cat. no K8021). Finally, sections were counterstained with hematoxylin and mounted with pertex.

Hematoxylin-eosin staining was used as counter-staining and furthermore used to quantify adipocyte size.

| Antibody | Clone | Manufacturer | Catalogue no. | Dilution |
|---|---|---|---|---|
| CD163 | MRQ-26 | Cell Marque, Rocklin, CA, USA | 163M-16 | 1:25 |
| CD68 | PGM1 | DAKO, Glostrup, Denmark | GA613 | Ready-to-use |
| CD3 | LN10 | Leica Biosystem, Wetzlar, Germany | NCL-L-CD3-565 | 1:25 |
| CD20 | L26 | DAKO, Glostrup, Denmark | GA604 | Ready-to-use |

CD, cluster of differentiation.

**Supplementary 4.** Primer sequences

| | Primer sequence |
|---|---|
| **IL-6** | |
| *Forward* | 5’ -TTTTGTACTCATCTGCACAGC-3’ |
| *Reverse* | 5’ -GGATTCAATGAGGAGACTTGC-3’ |
| **IL-18** | |
| *Forward* | 5’ -CAACAAACTATTTGTCGCAGCA -3’ |
| *Reverse* | 5’ -TGCCACAAAGTTGATGCAAT -3’ |
| **TNF-α** | |
| *Forward* | 5’ -TTGAGGGTTTGCTACAACATGGG-3’ |
| *Reverse* | 5’ -GCTGCACTTTGGAGTGATCG-3’ |
| **MCP-1** | |
| *Forward* | 5’-GTCTTGAAGATCACAGCTTCTTTGG-3’ |
| *Reverse* | 5’-AGCCAGATGCAATCAATGCC-3’ |
| **HIF-1α** | |
| *Forward* | 5’-CCCCAGTCACCTGCTGTTAT-3’ |
| *Reverse* | 5’-AGATCTCCTTGGCCACAATG-3’ |
| **PPAR-γ** | |
| *Forward* | GAAACTTCAAGAGTACCAAAGTGCAA |
| *Reverse* | AGGCTTATTGTAGAGCTGAGTCTTCTC |
| *Probe* | FAM 5'-CAAAGTGGAGCCTGCATCTCCACCTTATT-3' Tamra |
| **FAS** | |
| *Forward* | 5’-TGGTCACGGACGATGACCGTC-3’ |
| *Reverse* | 5’-GGTTGATGCCTCCGTCCACGAT-3’ |
| **SCD1** | |
| *Forward* | 5’-CACCCAGCTGTCAAAGAGAAGG-3’ |
| *Reverse* | 5’-AGGACGATATCCGAAGAGGTGG-3’ |
| **VEGFA** | |
| *Forward* | 5’- ATCTGCATGGTGATGTTGGA -3’ |
| *Reverse* | 5’- GGGCAGAATCATCACGAAG -3’ |
| **ESR1** | |
| *Forward* | 5’- AGGTGGACCTGATCATGGAG -3’ |
| *Reverse* | 5’- AAGCTTCGATGATGGGCTTA -3’ |
| **AdipoQ** | |
| *Forward* | 5’- AAAACCTCCCCCAAGCAGAGCTTC -3’ |
| *Reverse* | 5’- TGAGGAACAGGGATGAGTTCAGCA -3’ |

PPAR, Peroxisome proliferator activated receptor. FAS, fatty-acid synthase. SCD, Stearoyl-CoA desaturase. HIF, Hypoxia-inducible factor. VEGFA, Vascular endothelial growth factor A. ESR1, Estrogen receptor 1. AdipoQ, Adiponectin. IL, interleukin. TNF, tumor necrosis factor. MCP, Monocyte Chemoattractant Protein.