Adipose Tissue biopsy

Adipose tissue biopsies were performed using local anesthesia and a small-bore liposuction approach. In the abdominal depot, samples were obtained lateral to the umbilicus, and in the femoral depot from the antero-lateral aspect of the mid-thigh. Approximately 600mg-2800 mg of adipose tissue were obtained with each biopsy.

To ensure we had enough adipose tissue for all required analysis, 2-3 AT biopsies per depot were performed on each participant in Study 1. Because Study 2 was a confirmatory cohort from a separate study protocol, participants had only 1 abdominal AT biopsy.

Immunohistochemical quantification of adipose tissue macrophage content

Adipose tissue samples (≈350-400mg) were fixed in 10% zinc formalin (Protocol, Kalamazoo, MI) for 24 h, paraffin-embedded and sequential 5-micron slide-mounted sections were stained with a total macrophage and monocyte marker antibody against CD68 (Clone PG-M1, Dako, Carpinteria, CA); a pro-inflammatory M1 macrophage marker CD14 antibody (Sigma-Aldrich, St. Louis, MO) and an M2 macrophage or anti-inflammatory macrophage marker antibody against CD206 (Clone 685645, R&D Systems, Minneapolis, MN). Ten to twelve randomly selected images per slide were taken at 40 × magnification, and the AMCounter automated image analysis program (Biomedical Imaging Resource) (Morgan-Bathke, Harteneck et al. 2017) was used to count adipocytes and positively stained cells as ATM if they displayed the known morphological characteristics of macrophages. From this, we derived the number of positively stained cells per 100 adipocytes and, using fat cell size and lipid content of tissue, we calculated ATM per milligram of tissue (Morgan-Bathke, Harteneck et al. 2017). All slides were labeled in

a blinded manner so that the participant, research protocol, and biopsy site were unknown to the reader.

mRNA cytokine expression

RNA was isolated from AT using the RNeasy Lipid Tissue mini kit . The isolated RNA was then reverse transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems #4368813) as described by the manufacturer into cDNA. RT-PCR was performed using Taqman Gene Expression assays (Applied Biosystems (IL-6= Hs00174131_m1, TNF = Hs00174128_m1, IL-10 = Hs00961622_m1, IL-1β = Hs01555410_m1and CYCA=Hs99999904_m1) and TaqMan Fast Advanced Master Mix (Applied Biosystems #4444964) on an ABI Quant thermocycler using "Fast" settings in duplicate. The ΔΔCt method was used to analyze the data and cyclophilin A was used to normalize samples.

Table. Relationship between IC_{50} and adipose tissue inflammatory markers

Study 2 (N=38)

Inflammatory markers	**IC_{50} r**	**p-value**	**Adjusted *p****
Macrophages in Abdominal fat depot (ATM/100 adipocytes)			
CD68	0.36	0.06	0.09
CD14	0.50	0.007	0.13
CD206	0.50	0.007	0.20
Macrophages in Abdominal fat depot (ATM/milligram tissue)			
CD68	-0.15	0.42	0.09
CD14	0.15	0.53	0.22
CD206	-0.03	0.84	0.35
Senescence associates β-Galactosidase staining cells (%)			
Abdominal depot	0.29	0.28	0.22

ATM, adipose tissue macrophages.

*Analysis adjusted for fat cell size, sex and peakVO_2 using multiple linear regression

p-values <0.05 were considered statistically significant for the predefined primary endpoints: association between IC_{50} and FCS; association between IC_{50} and total (CD68) and pro-inflammatory (CD14) ATM content; the association between IC_{50} and senescent cell content; association between IC_{50} and inflammatory cytokine gene expression (IL-6 and TNF-α).

p-values <0.01 were considered statistically significant for the post-hoc associations between IC_{50} and anti-inflammatory (CD206) ATM content, between IC_{50} and IL-1β, and between IC_{50} and IL-10.