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ORIGINAL ARTICLE

Expression of lipogenic markers is decreased in subcutaneous adipose tissue and adipocytes of older women and is negatively linked to GDF15 expression

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Abstract

In aging, the capacity of subcutaneous adipose tissue (SAT) to store lipids decreases and this results in metabolically unfavorable fat redistribution. Triggers of this age-related SAT dysfunction may include cellular senescence or endoplasmic reticulum (ER) stress. Therefore, we compared lipogenic capacity of SAT between young and older women and investigated its relation to senescence and ER stress markers. Samples of SAT and corresponding SAT-derived primary preadipocytes were obtained from two groups of women differing in age (36 vs. 72 years, $n = 15$ each) but matched for fat mass. mRNA levels of selected genes (lipogenesis: ACACA, FASN, SCD1, DGAT2, ELOVL6; senescence: p16, p21, NOX4, GDF15; ER stress-ATF4, XBP1s, PERK, HSPA5, GADD34, HYOU1, CHOP, EDEM1, DNAJC3) were assessed by qPCR, protein levels of GDF15 by ELISA, and mitochondrial function by the Seahorse Analyzer. Compared to the young, SAT and in vitro differentiated adipocytes from older women exhibited reduced mRNA expression of lipogenic enzymes. Out of analyzed senescence and ER stress markers, the only gene, whose expression correlated negatively with the expression of lipogenic enzymes in both SAT and adipocytes, was GDF15, a marker of not only senescence but also mitochondrial dysfunction. In line with this, inhibition of mitochondrial ATP synthase in adipocytes strongly upregulated GDF15 while reduced expression of lipogenic enzymes. Moreover, adipocytes from older women had a tendency for diminished mitochondrial capacity. Thus, a reduced lipogenic capacity of adipocytes in aged SAT appears to be linked to mitochondrial dysfunction rather than to ER stress or accumulation of senescent cells.

Keywords Subcutaneous adipose tissue · Lipogenesis · Aging · Senescence · Stress of endoplasmic reticulum · Mitochondrial dysfunction

Introduction

Subcutaneous adipose tissue (SAT) is an organ specialized for the synthesis and metabolically safe storage of lipids through process of lipogenesis and thus it is indispensable for the maintenance of whole-body energy homeostasis [24]. For lipogenesis, AT utilizes mainly dietary lipids (in a process referred to as reesterification

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of fatty acids) but it can synthetize fatty acids de novo from glucose or other acetyl/malonyl CoA sources in the processes referred to as de novo lipogenesis (DNL). Emerging data implicate DNL in the maintenance or improvement of insulin sensitivity, as DNL generates insulin sensitizing lipokines and enhances fluidity of membranes necessary for insulin signaling [8, 23, 28, 33]. Thus, DNL can be considered as one of the features of metabolically healthy adipocytes.

In aging, the capacity of SAT to synthetize and store lipids progressively decreases and this may contribute to metabolically unfavorable fat redistribution, dyslipidemia, insulin resistance, and metabolic syndrome [30]. Despite substantial health impact of this SAT dysfunction in older people, its cellular and molecular triggers remain rather unclear. It has been suggested that the aging-related dysfunction of various tissues can be partly related to the accumulation of senescent cells. Senescent cells cannot fulfill their original function, and moreover, they exert highly pro-inflammatory phenotype described as the senescence-associated secretory phenotype (SASP) that can profoundly affect the function of bystander cells [22].

Another possible inhibitor of lipogenesis in aging adipocytes can be stress of endoplasmic reticulum (ER), an organelle essential for lipid synthesis [15]. ER stress, the condition when ER folding or synthetic capacity becomes overwhelmed, leads to the activation of a signaling network known as the unfolded protein response (UPR). UPR has three arms that are dependent on ER-located transmembrane proteins: inositol-requiring protein 1 (IRE1), protein kinase RNA-like ER kinase (PERK), and activating transcription factor 6 (ATF6). Upon their activation, protein translation is transiently attenuated while expression of ER chaperones is stimulated by active forms of several transcription factors, i.e., spliced form of X-box binding protein (XBP1s; IRE1 arm), maturated ATF6 (ATF6 arm), and ATF4 (PERK arm) [11]. Thus, the general aim of the UPR is to restore the ER homeostasis mainly through the reinforcement of ER folding capacity. At the same time, IRE-1 branch of the UPR leads to the phosphorylation and activation of c-Jun-N-terminal kinase (JNK) [11]. JNK activity may lead to a variety of downstream effects depending on the cellular context, some of which include apoptosis, cell survival, insulin resistance and inflammation [12]. Indeed, the experiments on rodents established ER stress as a trigger of insulin resistance and other metabolic disturbances caused by obesity [9]. In line with this, we showed recently that ER stress impairs DNL in adipocytes and differentiation of preadipocytes [14]. In addition, ER stress appears to be higher in SAT from aged mice [7]. Therefore, our goal was to compare the lipogenic capacity in SAT of young and older women, in relation to senescence and ER stress markers.

Material and methods

Subjects and assessment of anthropometric and biochemical measures

The two groups of healthy women ($n = 15$ per group) differing in age (group of young and older) but matched for fat mass percentage were recruited at the Third Faculty of Medicine of Charles University and University Hospital Kralovske Vinohrady, Prague, Czech Republic. Exclusion criteria were diagnosed cancer, diabetes, cardiovascular diseases, liver and renal diseases, and long-term medications to lower inflammation (anti-rheumatics and analgesics affecting cyclooxygenases, 100 mg of anopyrin daily was acceptable). Subjects taking medication to lower cholesterol levels and blood pressure (representing 70–90% of older population) were admitted to the study.

Anthropometric measurements, blood sampling and SAT needle biopsy were performed after overnight fasting as previously described [4]. LDL cholesterol levels were calculated using Friedewald formula (total cholesterol minus high-density lipoprotein-cholesterol minus triglycerides/5 in mg/dl).

All procedures performed in studies involving human participants were in accordance with the ethical standards of the ethics committee of the Third Faculty of Medicine of Charles University and University Hospital Kralovske Vinohrady and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Chemicals

Culture media were from Lonza Std. (Switzerland) and FBS qualified for MSC was from ThermoFisher Scientific (USA). FGFβ and EGF were supplied by Immunotools (Germany); rosiglitazone was provided by Cayman (Estonia). Other chemicals were from Sigma-Aldrich (USA).

Isolation and culture of preadipocytes

Biopsy SAT sample was cleaned from blood vessels and fibrous material, minced into pieces, and digested in 1.5 volume of collagenase I (300 units/ml, Serva, Germany) for 50–60 min in 37 °C shaking water bath. Digested tissue was filtered through 250 μm strainer to remove undigested scraps, diluted with PBS/gentamycin, and centrifuged at 600*g* for 5 min. Pellet containing cells from the stromal vascular fraction was incubated in erythrocyte lysis buffer for 10 min at room temperature. Cells were centrifuged and resuspended in PM4 medium [27] with 132 nmol/l insulin. The further cultivation and differentiation of cells was carried out as described in [26]. Twelve-day differentiated cells were washed with PBS

and cultured in basal medium (DMEM/F12 supplemented with 0.1 μg/ml transferrin) for 24 h and then harvested for RNA isolation.

Gene expression analysis

Total RNA was isolated using RNeasy (cells) or Rneasy Lipid Tissue (SAT) Mini Kit (Qiagen, Germany). RNA concentration was measured using Nanodrop1000 (Thermo Fisher Scientific, USA). DNAse I (Thermo Fisher Scientific, USA) treatment was applied to remove genomic DNA. Total RNA was reverse transcribed using High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA). RT-qPCR was performed in duplicates on ABI PRISM 7500 using TaqMan gene expression assays (*ACACA*: Hs01046047_m1, *ATF4*: Hs00909569_g1, *DGAT2*: Hs01045913_m1, *DNAJC3*: Hs00534483_m1, *EDEM1*:Hs00976004_m1, *ELOVL6*: Hs00225412_m1, *FASN*: Hs01005622_m1, *GADD34*: Hs00169585_m1, *GDF15*: Hs01379108m1, *GUSB*: Hs00939627_m1, *HSPA5*: Hs99999174_m1, *HYOU1*: Hs00197328_m1, *CHOP*: Hs01090850_m1, *NOX4*: Hs00171132, $p16^{INK4a}$: Hs00923894_m1, *p21*: Hs00355782_m1; *PERK*: Hs00984006_m1, *RPS13*: Hs01011487_g1, *SCD1*: Hs01682761_m1; Thermo Fisher Scientific) and TaqMan Fast Advanced Master Mix (Thermo Fisher Scientific, USA) or specific primers (*XBP1*-total-forward: 5′-CGCTGAGGAGGAAACTGAA-3′, *XBP1*-total-reverse: 5′-CACTTGCTGTTCCAGCTCACTCAT-3′, *XBP1*-spliced-forward: 5′-GAGTCCGCAGCAGGTGCA-3′, *XBP1*-spliced reverse 5′- ACTGGGTCCAAGTTGTCCAG-3′) and Power Sybr Green PCR Master Mix (Thermo Fisher Scientific, USA). Data were normalized to geometric mean of two endogenous controls *RPS13* and *GUSB*, except for the experiment with oligomycin, where only RPS13 was used as endogenous control, as GUSB expression was affected by the treatment. Expression of XBP1s was normalized to total XBP1. Fold change of expression was calculated using ΔΔCt method.

The selection of analyzed markers was based on the previously reported importance of genes in either lipogenesis, ER stress, or senescence and the fact that mRNA levels of these genes are known to correlate with their protein levels or biological activity (referenced in the "Discussion" section). NOX4 and GDF15 markers were selected upon the suggestion by an expert in the field of cellular senescence, Zdenek Hodny, MD, PhD.

Analysis of plasma and conditioned media

Plasma samples were prepared from uncoagulated peripheral blood by centrifugation. Two times diluted plasma and undiluted conditioned media were used to quantify the level of GDF15 by DuoSet GDF15 ELISA kit (R&D Systems, USA) according to the manufacturer's recommendation.

Seahorse measurement

Cellular respiration of in vitro differentiated adipocytes was measured using the XF-24 analyzer (Seahorse Bioscience). Preadipocytes were seeded at a density of 2200 cells per well (XF24 Cell Culture Microplate) and allowed 7 days to reach confluence when the differentiation was started. At day 12, the culture medium was replaced with the XF assay medium supplemented with 2.5 mM L-glutamine, 1 mM pyruvate, and 17.5 mM glucose. Oxygen consumption rate measurements were obtained before and after sequential additions of 1 μM oligomycin, 1 μM FCCP, and 1 μM rotenone/antimycin A to the adipocytes. Data were normalized to total protein content.

Treatment with oligomycin

Twelve-day differentiated adipocytes were washed with PBS prior to the experiment and then treated with basal media supplemented with 100 nM oligomycin in DMSO or vehicle alone for 24 h (similarly as described by Montero et al. [20]). Then conditioned media were collected, centrifuged to remove cellular debris, and stored at −80 °C until use. Cells were harvested for RNA isolation.

Statistical analysis

The GraphPad Prism 6.0 software was used for data analysis. To analyze differences between groups or treatments, Mann–Whitney or unpaired *t* tests were performed as appropriate. Clinical data are presented as mean ± SD, other data as mean±SEM. Correlations of gene expression data (from both experimental groups together) were performed using Spearman's test. The level of significance was set at $p \leq 0.05$.

Results

Clinical characteristics

The clinical data of young and older subjects are depicted in Table 1. The two groups differed in age (36.6 ± 7.1 years for young and 72.1 ± 5.1 years for older), but were matched for the percentage of fat mass (FM; 37.2% and 38.9% for young and older, respectively). The matching for fat mass was selected since this relative value related to adipose tissue mass is less biased by aging-related sarcopenia than other general anthropometric measures as weight and BMI. Metabolically, both groups had similar insulin sensitivity

Table 1 Anthropometric and biochemical characteristics of young and older women

	Young $n = 15$	Older $n = 15$
Age (year)	36.6 ± 7.1	72.1 ± 5.1***
Weight (kg)	86.2 ± 8.23	70.4 ± 4.0***
Fat mass (%)	37.2 ± 4.0	38.9 ± 4.1
Fat mass (kg)	32.2 ± 5.8	27.5 ± 3.6*
Fat free mass (kg)	54.0 ± 4.4	43.0 ± 3.2***
Waist circumference (cm)	97.1 ± 9.0	84.9 ± 4.6***
Hip circumference (cm)	116.0 ± 6.6	104.7 ± 4.8***
Waist to hip ratio	0.8 ± 0.1	0.8 ± 0.1
BMI (kg/m^2)	31.0 ± 2.4	27.0 ± 1.8***
Cholesterol (mmol/l)	5.1 ± 0.8	4.9 ± 0.7
HDL cholesterol (mmol/l)	1.8 ± 0.4	1.7 ± 0.5
LDL cholesterol (mmol/l)	2.8 ± 0.6	2.7 ± 0.6
Triglycerides (mmol/l)	1.1 ± 0.4	0.9 ± 0.3
Fasting glucose (mmol/l)	5.3 ± 0.4	6.0 ± 0.7*
Fasting insulin (mU/l)	9.2 ± 3.0	6.8 ± 2.3*
HOMA-IR	2.2 ± 0.8	1.9 ± 0.8

BMI body mass index, *FM* fat mass, *HDL* high-density lipoprotein, *HOMA-IR* homeostasis model assessment of the insulin resistance index, *LDL* low-density lipoprotein

Values are presented as means ± SD, ***$p < 0.001$, *$p < 0.05$

calculated as HOMA-IR despite the differences in fasting glucose and insulin levels.

Expression of lipogenic genes in SAT decreases with age

To compare lipogenic potential of SAT of young and older, we analyzed mRNA expression of five major lipogenic genes. SAT mRNA transcripts of *FASN*, a rate limiting enzyme in DNL, and *DGAT2*, an enzyme catalyzing the final step of the triglyceride synthesis, were significantly less expressed in the group of older women ($p < 0.05$; Fig. 1a). Levels of mRNA for these two genes strongly correlated (Fig. 1d). The tendency to the lower expression was observed also for mRNA of *ACACA* and *SCD1*, even though the difference was not statistically significant. mRNA expression of *ELOVL6* did not differ between the two groups.

SAT from older women displays more senescent phenotype than SAT from young women

To analyze the level of senescence in the SAT samples, we measured expression of $p16^{INK4a}$, an inhibitor of cell cycle progression and well-established marker of senescence, and three other senescent markers in both groups. SAT from older women expressed more $p16^{INK4a}$, *p21*, and *NADPH oxidase 4* (*NOX4*) mRNA transcript compared to SAT from the young ($p < 0.05$; Fig. 1b). The expression level of an additional senescence marker, GDF15, was not different in SAT from the two groups of women, but the negative correlation between its expression and mRNA expression of all analyzed lipogenic markers was found (Fig. 1d and not shown). To further validate GDF15 as a general (not SAT-specific) marker of aging also in our cohorts of women, we analyzed its circulating levels and confirmed that GDF15 levels were significantly higher in older women compared to young ones (Fig. 1e).

ER chaperones are not elevated in SAT from older women, despite increased expression of XBP-1s and PERK

To determine the level of ER stress, we measured the mRNA expression of nine UPR markers involved in all three UPR arms. Despite higher expression of *XBP1s*, an essential transcription factor activated by IRE1-UPR branch, and *PERK*, one of the stress sensors, in SAT from older women, the expression of ER chaperones *HSPA5*, *DNAJC3*, and *HYOU3* and phosphatase *GADD34* were significantly less expressed in SAT of this group (Fig. 1c). Expression of *HSPA5* and other chaperones correlated well with that of *DGAT2* and *FASN* (Fig. 1d and not shown). mRNA expression of other ER genes involved in UPR, specifically *ATF4*, *CHOP*, and *EDEM1* was not different between the groups.

Lower lipogenic potential of older women is manifested also in in vitro differentiated adipocytes and is negatively linked to GDF15 expression

To assess adipocyte-specific aging-related differences in lipogenic, ER stress, and senescence markers, we used in vitro differentiated adipocytes. Adipose precursors were isolated from SAT biopsies in the subgroups of volunteers ($n = 11$ for each young and older women), subcultivated for three passages and then differentiated into adipocytes.

Similarly as seen in SAT, in vitro differentiated adipocytes from older women exerted a co-regulated reduction of mRNA level for lipogenic genes (*FASN*, *DGAT2*, *SCD1*) compared to the cells from young group (Fig. 2a, d and not shown) and the expression of all lipogenic markers was strongly correlated with the expression of *GDF15* (Fig. 2d and not shown). Also *HSPA5* and *DNAJC3* mRNA were less expressed in adipocytes from older women despite higher *XBP1s* expression. However, adipocyte mRNA

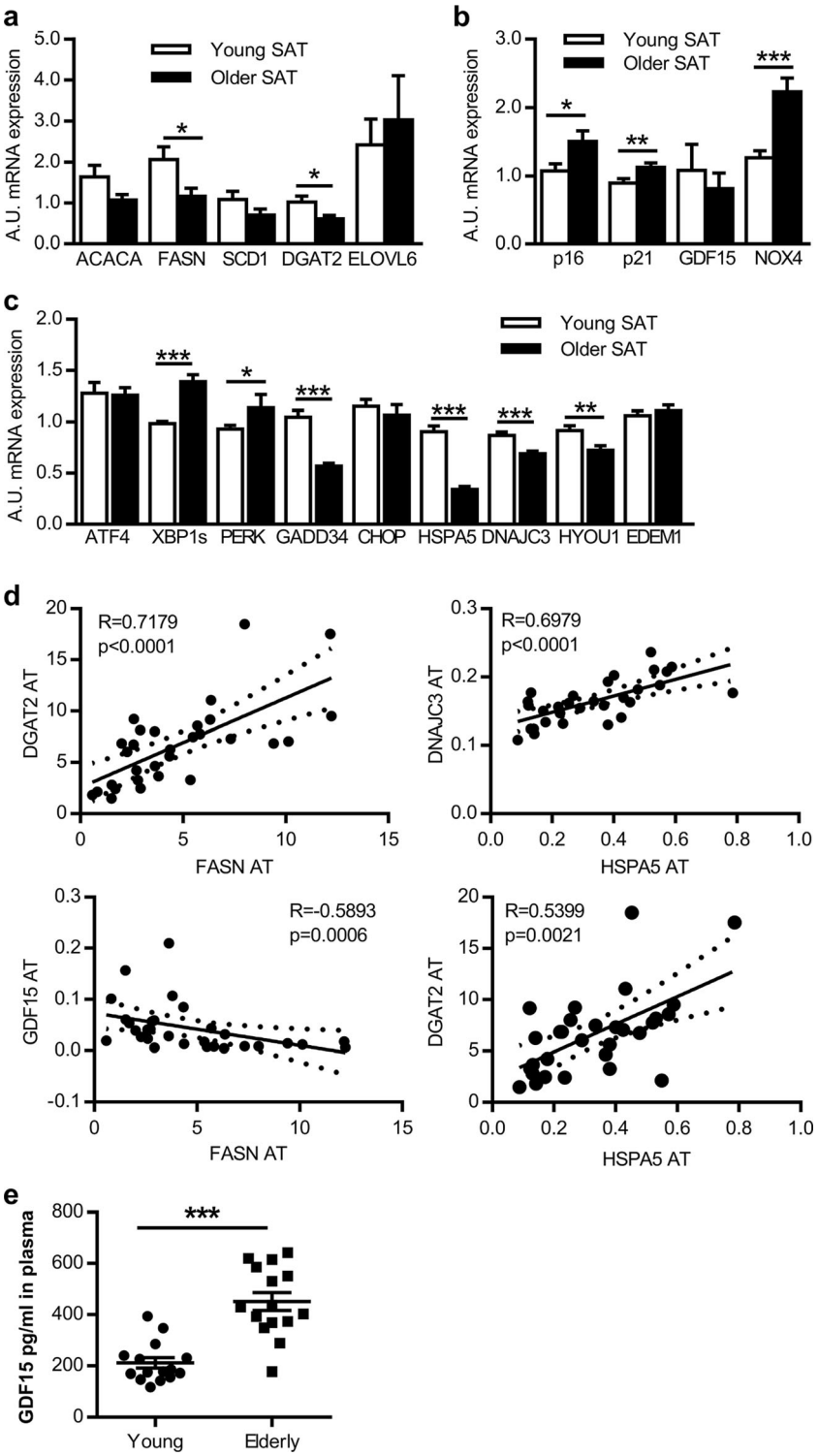

Fig. 1 Effect of aging on selected markers in SAT and plasma. mRNA expression of markers of lipogenesis (**a**), senescence (**b**), and ER stress (**c**) in groups of young and older women ($n = 15$ in each group) are depicted as a fold change related to the first subject in the group of young. **d** Correlation of selected markers. **e** Plasma levels of GDF15 in groups of young and older women ($n = 15$ in each group). Data are means ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

levels of chaperones did not correlate with the expression of lipogenic markers (not shown).

In contrast to SAT, the expression of senescent markers $p16^{INK4a}$, *p21*, and *NOX4* was not different between the cells from two age-differing groups, while adipocytes from older women expressed three times more of *GDF15 mRNA* compared to cells from the young (Fig. 2b). Another difference between expression patterns in SAT vs. adipocytes was higher expression of *ATF4*, a transcription factor important for the expression

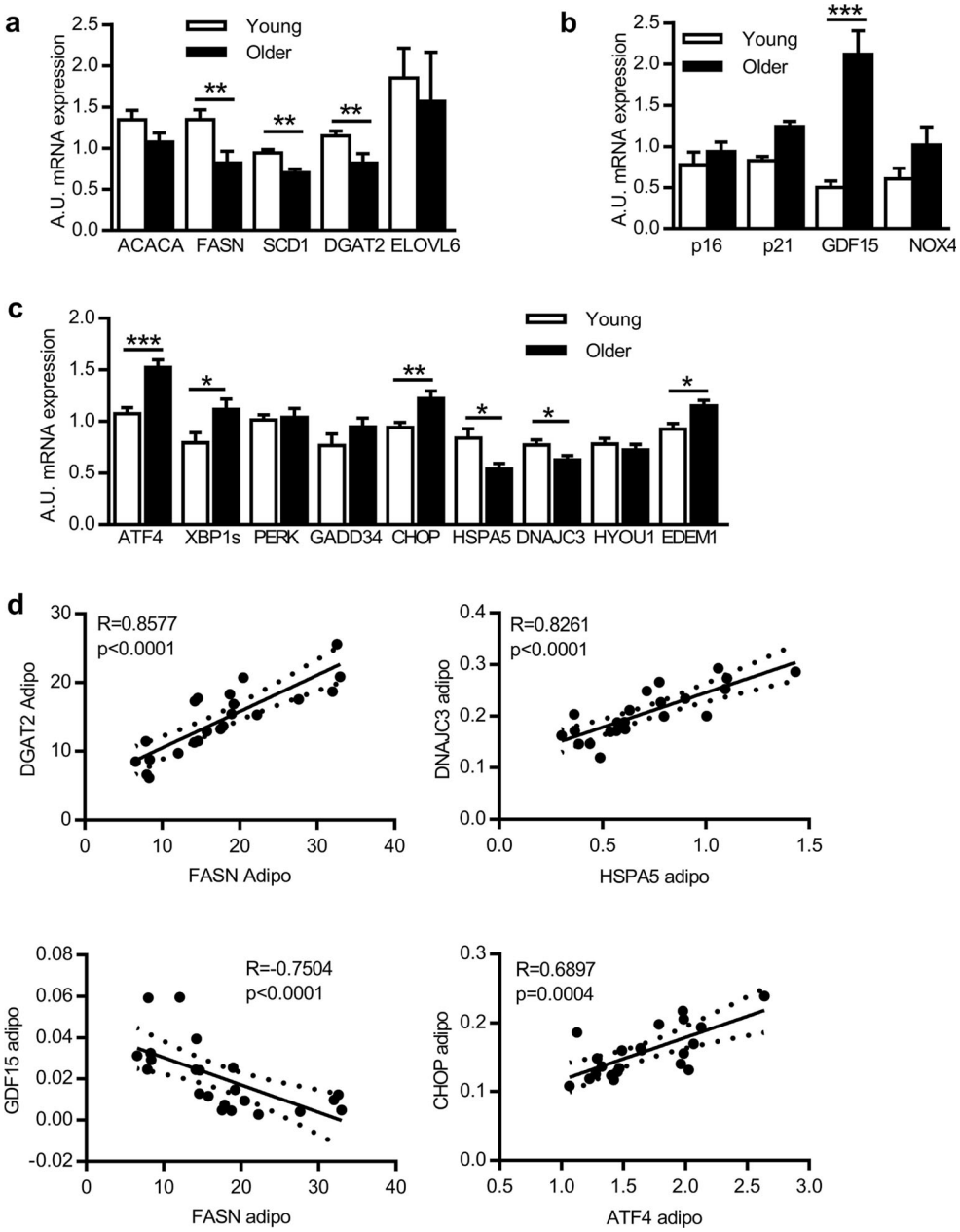

Fig. 2 Effect of aging on mRNA expression of selected markers in in vitro differentiated adipocytes. mRNA expression of markers of lipogenesis (**a**), senescence (**b**), and ER stress (**c**) in cells from young and older women ($n = 11$ in each group) are depicted as a fold change related to the first sample in the group of young. Data are means ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. **d** Correlation of selected markers

of *EDEM1*, a marker of ER-associated degradation of misfolded glycoproteins (*ERAD*), and a proapoptotic transcription factor *CHOP*, in adipocytes from older women compared to cells from the young (Fig. 2c). mRNA levels of these three genes were co-regulated (Fig. 2d and not shown).

GDF15 expression in adipocytes is linked with mitochondrial dysfunction

As *GDF15* is not only a marker of senescent tissue but also a marker of mitochondrial dysfunction, we evaluated the effect of mitochondrial stress induced by oligomycin treatment of adipocytes on *GDF15* mRNA levels and protein secretion together with lipogenic markers expression. Oligomycin treatment led to more than 100-fold upregulation of *GDF15* mRNA levels as well as it enhanced its secretion by adipocytes (Fig. 3a, b). Concomitantly, the same treatment decreased the expression of all analyzed lipogenic enzymes (Fig. 3c).

These results prompted us to compare mitochondrial functions in a subgroup of adipocytes from young and older women using the Seahorse Analyzer. Mitochondrial stress test revealed that adipocytes from older women had a tendency for lower respiration (both basal and maximal) and ATP production (Fig. 3d).

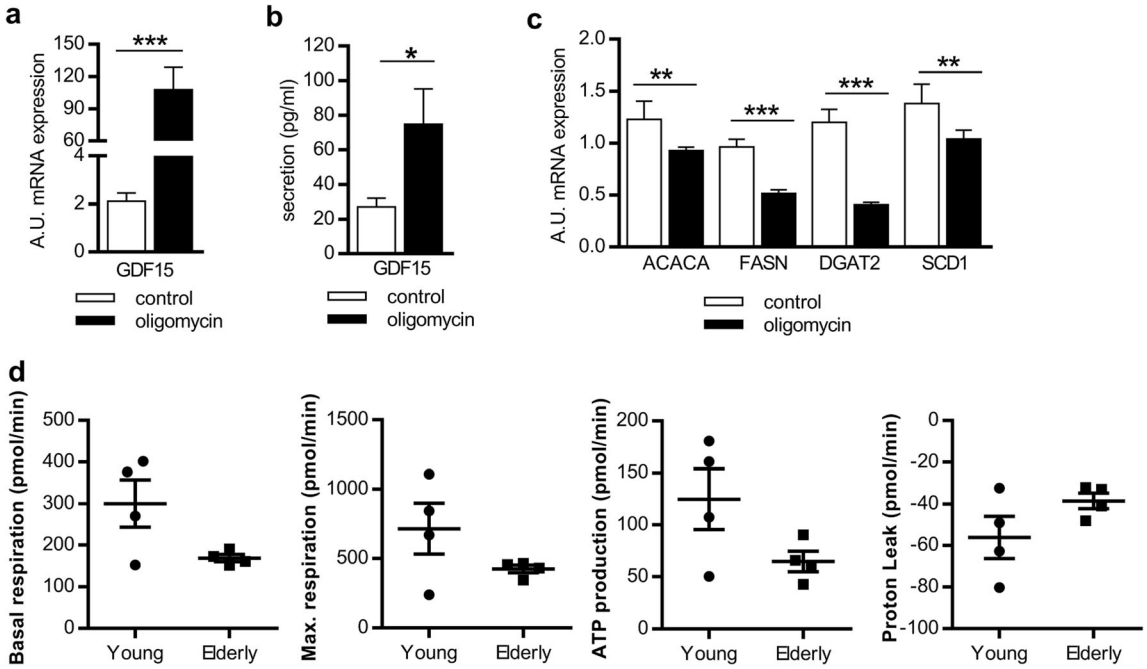

Fig. 3 Effect of oligomycin and aging on mitochondrial function in in vitro differentiated adipocytes. mRNA expression (**a**) and secretion of GDF15 (**b**) in control and oligomycin-treated adipocytes ($n = 8$). **c** mRNA expression of lipogenic enzymes in control and oligomycin treated adipocytes ($n = 8$). Data are means ± SEM, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. **d** Mitochondrial respiratory parameters in adipocytes differentiated in vitro of young and older women ($n = 4$ in each group). **d** Correlation of selected markers

Discussion

In aging, accumulation of triglycerides in SAT is diminished, but the molecular basis of this phenomenon remains elusive. In fact, to our knowledge, no analysis of the activity of lipogenic genes in SAT with respect to aging has ever been done in humans. To investigate the effect of aging on lipogenesis, we analyzed gene expression of lipogenic enzymes in SAT from two age-differing groups of women. Since SAT consists of a variety of cells including stem, endothelial, and immune cells, we also employed a model of in vitro differentiated adipocytes originating from the same donors to analyze aging-related changes in expression pattern specifically in adipocytes.

Our study brought the evidence of lower mRNA expression of two major lipogenic genes, *FASN* and *DGAT2*, in both, whole tissue and adipocytes from older women (Figs. 1a and 2a). This could represent a prerequisite for lower capacity of aged SAT to accumulate fat (mainly through reesterification of dietary fatty acids) and/or to maintain insulin sensitivity (through DNL) because lower mRNA expression of lipogenic markers in adipocytes is mirrored by lower activity of lipogenic enzymes and lower accumulation of triglycerides as previously documented by tracer studies performed in our and other laboratories [5, 14].

Since lipogenesis occurs in the ER and can be regulated by ER stress [9, 14, 34], a condition that was also proposed as one of the hallmarks of aging [16], we quantified mRNA expression of ER stress markers and correlated it with lipogenic gene expression. In contrast to previously published findings in aged murine AT [7], aged human SAT exhibited lower expression of major ER chaperone *HSPA5*, together with lower expression of its co-chaperone *DNAJC3* and ATP/ADP exchange factor *HYOU1* (Figs. 1c and 2c) [1, 29]. It is possible that higher expression of *HSPA5* and other markers in AT of aged mice reflected not only the aging process but also aging-related alterations of body composition, because the groups in the mice study were not matched for fat mass parameters that could substantially differ in young (4–6 months) vs. old animals (18–20 months). On the other hand, the results of our study are in accordance with several other studies performed on various (but not adipose) tissues showing aging-related reduction of the expression and activity of many ER chaperones and enzymes. It was suggested that their functional decline results in chronic ER stress [2, 16, 21]. Thus, it is possible that with aging, levels of ER chaperones become mildly reduced, which leads to chronic but low-intensity ER stress that cannot fully activate *IRE1* branch of the UPR necessary to restore ER homeostasis. This hypothesis is supported by our observations that mRNA expression of *HSPA5* and its co-chaperones was lower despite higher levels of *XBP1s*, a powerful transcription factor controlling the expression of a cluster of genes related to folding [13]. On the other hand, we found higher expression of both *ATF4* and its target gene *CHOP* [31] in adipocytes from older women, and thus, low-intensity ER

stress in aging adipocytes appears to be sufficient to trigger *PERK-ATF4* axis of the UPR.

Although the expression of major chaperone *HSPA5* and its co-chaperones in SAT correlated with expression of lipogenic markers, this relationship was not found in adipocytes, which implies an involvement of other AT resident cells [3] in this relationship. Indeed, both immune and endothelial cells are sensitive to ER stress and upon activation of the UPR they can produce a number of pro-inflammatory cytokines, which are involved in the worsened physiological functions of adipocytes [19]. Besides, we have previously observed that lipogenic capacity of mature adipocytes is not influenced by experimentally induced chronic low ER stress that could resemble the type of ER stress occurring in aging [14].

To address the impact of accumulation of senescent cells on AT lipogenesis, we analyzed several markers of senescent cells, namely inhibitors of cyclin-dependent kinases and markers of oxidative stress and mitochondrial dysfunction that are considered also as markers of senescence and whose expression can be monitored on mRNA level [6, 10, 18, 32]. Concomitantly increased levels of *p16*, *p21*, and *NOX4* mRNA are indeed suggestive of higher numbers of senescent cells in SAT from older women. The lack of correlation between the expression of these markers and lipogenic genes, however, does not corroborate the existence of cause–effect relationship between the accumulation of dysfunctional senescent cells and decreased lipogenesis in aged SAT. Thus, cells contributing to increased expression of senescent markers in aged SAT were probably not adipocytes. Nevertheless, the expression of *GDF15*, one of the genes implicated in aging, was strongly correlated with the expression of lipogenic markers in both SAT and adipocytes. *GFD15* is a cytokine that is induced by various cellular stresses, including mitochondrial dysfunction that may contribute to senescence and aging [6]. Indeed, *GDF15* was strongly induced by ATP synthase inhibition as shown by us (Fig. 3a, b) and others [20]. Inhibition of oxidative phosphorylation can at the same time limit lipogenesis as shown previously [25] and confirmed also in our experiment with oligomycin (Fig. 3c). Thus, the strong negative relationship between *GDF15* expression and lipogenesis could suggest that the aging-associated decline of lipogenesis in SAT is related to mitochondrial dysfunction in adipocytes. We further supported this hypothesis by finding that adipocytes from older women have a tendency to have lower ATP production in normal growth conditions. Moreover downregulation of mitochondrial enzymes in adipose tissue in vivo was shown in aging mice [17].

A major limitation of this study is the fact that it is based only on the analysis of mRNA levels of marker genes and as such it cannot provide final conclusions on SAT activity of certain UPR proteins that are regulated mostly post-transcriptionally. Nevertheless, mRNA levels of lipogenic and senescence markers are quite strong determinants of actual protein levels as mentioned above. Mitochondrial dysfunction was assessed on adipocytes in vitro by direct measuring ATP production, proton leak, and basal and maximal respiration using Seahorse XFe24 Analyzer. The obvious limitation of this experiment was a small sample size ($n = 4$ in each group) that did not allow to reach statistically significant difference between the two groups despite the clear trend. Another limitation of the presented study is the discordance of BMI and weight between the groups, which appears to be driven by difference in fat free mass. This discordance is rather impossible to overcome when comparing young and older women, because both factors (lean and fat mass) are changing with age. Thus, we believe that the matching for fat mass instead of weight and BMI is more suitable to analyze aging-dependent changes in adipose tissue.

In conclusions, decreased capacity of SAT in older women to accumulate triglycerides appears to be linked to diminished expression of lipogenic enzymes and appears to be driven at least partially by mitochondrial dysfunction.

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Authors' contribution Ve.Š. performed experiments and data analysis and wrote the manuscript; E. K. performed adipose tissue biopsies; M.K, M.E., and J.K. performed experiments; M. Š and D.L. contributed to the discussion and writing of the manuscript; V.Š. performed adipose tissue biopsies and contributed to the discussion and writing of the manuscript; and L.R. designed the study, performed experiments and data analysis, and wrote the manuscript. L.R. is a guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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