Biochemical and Biophysical Research Communications 457 (2015) 426–432

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Telomere length differences between subcutaneous and visceral adipose tissue in humans

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ARTICLE INFO

Article history:
Received 15 December 2014
Available online 13 January 2015

Keywords:
Telomere length
Obesity
Weight loss
Adipose tissue
T/S ratio

ABSTRACT

Adipocyte hypertrophy and hyperplasia have been shown to be associated with shorter telomere length, which may reflect aging, altered cell proliferation and adipose tissue (AT) dysfunction. In individuals with obesity, differences in fat distribution and AT cellular composition may contribute to obesity related metabolic diseases. Here, we tested the hypotheses that telomere lengths (TL) are different between: (1) abdominal subcutaneous and omental fat depots, (2) superficial and deep abdominal subcutaneous AT (SAT), and (3) adipocytes and cells of the stromal vascular fraction (SVF). We further asked whether AT TL is related to age, anthropometric and metabolic traits.

TL was analyzed by quantitative PCR in total human genomic DNA isolated from paired subcutaneous and visceral AT of 47 lean and 50 obese individuals. In subgroups, we analyzed TL in isolated small and large adipocytes and SVF cells.

We find significantly shorter TL in subcutaneous compared to visceral AT ($P < 0.001$) which is consistent in men and subgroups of lean and obese, and individuals with or without type 2 diabetes (T2D). Shorter TL in SAT is entirely due to shorter TL in the SVF compared to visceral AT ($P < 0.01$). SAT TL is most strongly correlated with age ($r = -0.205$, $P < 0.05$) and independently of age with HbA1c ($r = -0.5$, $P < 0.05$). We found significant TL differences between superficial SAT of lean and obese as well as between individuals with our without T2D, but not between the two layers of SAT. Our data indicate that fat depot differences in TL mainly reflect shorter TL of SVF cells. In addition, we found an age and BMI-independent relationship between shorter TL and HbA1c suggesting that chronic hyperglycemia may impair the regenerative capacity of AT more strongly than obesity alone.

1. Introduction

Metabolic disorders such as obesity and diabetes may accelerate the aging process [1]. Cellular and tissue telomere length (TL) represents a strong biological marker for the aging process, cell proliferation, tissue regeneration and a dysbalance between these processes [2]. Short telomeres have become a widely accepted molecular/cellular measure of aging [3]. Although telomere length seems to be tissue specific, the age-associated TL shortening occurs in parallel and proportionally in skeletal muscle, skin, leukocytes, and subcutaneous adipose tissue (SAT) [4]. At the tissue level, aging is characterized by a substantial decrease in the regenerative potential of several cell types [4,5]. Both genetic and environmental factors determine the rate and balance between cell death and regeneration [5].

Recently, it has been demonstrated that at least in extremely obese individuals, adipocyte TL is a marker of adiposity, and whole

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http://dx.doi.org/10.1016/j.bbrc.2014.12.122
0006-291X/

adipose tissue (AT) TL shortening may reflect AT dysfunction [2]. The number of adipocytes is determined in childhood and adolescence and remains largely constant in lean and obese individuals during adulthood [6]. Spalding and co-workers showed that each year approximately 10% of the adipocyte population is renewed [6]. However, these data are based on measurements of subcutaneous fat [6]. It is not yet known whether different fat depots have a specific fat cell turnover rate, and whether the renewal of AT is related to anthropometric and metabolic parameters.

It has been shown that individuals with obesity, type 2 diabetes (T2D) and additional cardiovascular risk factors have shortened telomeres in the peripheral blood and in SAT [1,7]. The TL in circulating blood cells is negatively associated with age, BMI, hip and waist circumference, systolic blood pressure, triglycerides, fasting glucose levels and body fat percentage [1]. Moreover, TL in adipocytes correlates negatively and independently of age with waist circumference and adipocyte size [2]. The finding that TL in SAT and/or visceral adipose tissue (VAT) is negatively associated with BMI, systolic blood pressure, hyperglycemia and adipocyte size is highly consistent across independent cohorts [2,8–10]. Since obesity seems to be related to shorter telomeres and obesity related metabolic disturbances are related to adipose tissue mass, depot distribution and gender, we hypothesize that TL are different between SAT and VAT depots in humans. We further tested the hypotheses that TLs are different between superficial and deep SAT, and between adipocytes and cells of the stromal vascular fraction (SVF). In addition, we sought to investigate whether telomere length is associated with parameters of obesity and fat distribution, adipocytes size or with metabolic traits including insulin sensitivity and glucose tolerance.

2. Material and methods

2.1. Subjects

For this cross-sectional study, paired visceral (VAT) and subcutaneous (SAT) adipose tissue samples were obtained from 97 extensively characterized Caucasian lean ($n = 47$) or obese ($n = 50$) men ($n = 46$) and women ($n = 51$) from the University Hospital Leipzig (Table 1). Patients either underwent elective bariatric surgery ($n = 43$), cancer related surgery ($n = 33$) or cholecystectomy ($n = 11$). The mean age of the study cohort was 55 ± 17 years (range: 16.7–93.2 years) with a mean BMI of 37.2 ± 15.3 kg/m^2 (range: 18.6–78.8 kg/m^2). 36 participants had a diagnosis of T2D. For each participant, demographic and anthropometric data, concomitant medication before surgery and routine laboratory results were recorded. The study was approved by the local ethics committee (Reg. No. 031-2006 and 017-12-23012012) and the participants gave their written informed consent.

In a subgroup of 22 subjects, we analyzed TL in SVF and isolated adipocytes. The mean age of this subcohort was 49 ± 13.8 years (range: 16.7–75.5years) with a mean BMI of 43.9 ± 10.6 kg/m^2 (range: 20.7–60.1 kg/m^2). 10 participants had a diagnosis of T2D. In a third subgroup (n = 13), we measured TL in small (<100 μm) and large adipocytes (>100 μm).

Table 1
Clinical characterization of lean and obese study participants. Data represent means ± SD. Differences between lean and obese individuals: *P < 0.05, **P < 0.01, ***P < 0.001.

	BMI <25 kg/m^2 n = 47	BMI >40 kg/m^2 n = 50
Age (years)	63.7 ± 16	46.1 ± 13.6***
Gender (women/men)	26/21	25/25
BMI (kg/m^2)	23.0 ± 1.8	50.6 ± 8.9***
Type 2 Diabetes (no/yes)	41/3	17/33***
HbA_{1c} (%)	4.92 ± 0.96	5.93 ± 1.80**
Fasting plasma glucose (mmol/l)	5.41 ± 1.6	6.23 ± 2.93
Fasting plasma insulin (pmol/l)	23 ± 29	133 ± 87
HOMA-IR	0.86 ± 1	5.4 ± 4.3
Triglycerides (mmol/l)	1.11 ± 0.12	1.81 ± 0.75**
Total cholesterol (mmol/l)	5.21 ± 0.19	4.72 ± 0.92*
HDL cholesterol (mmol/l)	1.79 ± 0.15	1.18 ± 0.3**
LDL cholesterol (mmol/l)	2.93 ± 0.28	2.77 ± 0.85

2.2. Measurement of body fat content, glucose metabolism and insulin sensitivity

BMI was calculated as weight divided by squared height. Hip circumference was measured over the buttocks; waist circumference was measured at the midpoint between the lower ribs and iliac crest. Percentage body fat was measured by bioelectrical impedance analysis (BIA). Abdominal visceral and subcutaneous fat areas were calculated using computed tomography (CT) or MRI scans at the level of L4–L5. Insulin sensitivity was assessed using the HOMA-IR index. All baseline blood samples were collected between 8 and 10 am after an overnight fast. Plasma insulin was measured with an enzyme immunometric assay for the IMMULITE automated analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA).

2.3. Adipocyte- and SFV isolation

AT samples were digested with 1 mg/mL (Type 1 collagenase, CellSystems Troisdorf, Germany) for 45 min in 37 °C shacking water bath (90 bpm). After digestion, samples are centrifuged at 362 g for 5 min at room temperature. Supernatant containing mature adipocytes were filtered through a 100 μm nylon mesh (NeoLab, Heidelberg, Germany) to separate small and large adipocytes. Cell pellets (SVF) as well as adipocytes were snap frozen in liquid nitrogen immediately.

2.4. TL studies in different SAT layers

The separation of paired superficial SAT (sSAT) and deep SAT (dSAT) was performed in a subgroup of 25 subjects. We defined sSAT in parallel histological sections as the region of AT between the Scarpa's fascia and lower dermis, whereas dSAT was defined as the AT below Scarpa's fascia. These layers were obtained from male ($n = 9$) and female ($n = 16$) patients underwent abdominal plastic surgery (e.g. body lift). The mean age of the study cohort was 50.6 ± 15.4 years (range: 23–82years) with a mean BMI of 33.2 ± 6.4 kg/m^2 (range: 22.1–52.5 kg/m^2). 17 participants had a diagnosis of T2D. The study was approved by the local ethics committee and the participants gave their written informed consent.

2.5. Measurement of telomere length

SAT, VAT, SVF, sSAT, dSAT and isolated adipocytes were immediately frozen in liquid nitrogen after explantation or collagenase digestion. Genomic DNA was extracted from frozen samples using QIAamp DNA blood & tissue kit (Qiagen, Hilden, Germany). TL was quantified using quantitative RT-PCR assay as described by Cawthon [11]. For each sample, containing 10 ng/μl genomic DNA, we assessed telomeric DNA and a single-copy control gene (36B4). The following primers were used: *36B4* 5′-CAGCAAGTGGGAAGGTGT AATCC-3′ (forward) and 5′-CCCATTCTATCATCAACGGGTACAA-3′ (reverse); *TEL* 5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGG GT-3′ (forward) and 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTA

TCCCTA-3′ (reverse). A serial diluted standard curve, based on pooled children genomic DNA (mean age 3.3 ± 1.5 years), served as reference. For determining relative telomere length we calculated for all samples the factor by which they differ from reference children's DNA in its ratio of telomere repeat copy number to single-copy gene copy number

$$\left(\mathrm{T/S-ratio}=\frac{\text{housekeep Efficiency Cp}-\frac{\text{sample}}{\text{telomere Efficiency Cp}}-\text{sample}}{\text{housekeep Efficiency Cp}-\frac{\text{sample}}{\text{telomere Efficiency Cp}}-\text{control}}\right).$$

The average T/S ratio is an indicator of telomere length. Lower T/S ratio reflects shorter telomere length.

2.6. Statistical analyses

Statistical analysis including multivariate linear regression analyses were performed with SPSS version 20 (SPSS; Inc., Chicago, IL, USA). All experimental data are presented as means ± SD unless stated otherwise. Non-normally distributed parameters were logarithmically transformed. Differences in TL between VAT and SAT were assessed using paired Student's t test. Results of univariate correlation analysis were described with Pearson's correlation coefficient. All *P* values presented are two-tailed, and *P*-values less than 0.05 are considered statistical significant.

3. Results

3.1. Shorter telomere length in SAT compared to VAT

We investigated TL in SAT and VAT in 97 AT donors (51 women, 46 men), which have been further classified into individuals with a BMI <25 kg/m^2 or >40 kg/m^2 (Table 1). The AT donors were selected to represent a wide range of age, BMI and parameters of insulin sensitivity and glucose metabolism. Analysis of the entire cohort revealed significantly shorter TL in SAT compared to VAT (Fig. 1A). Shorter TL in SAT compared to VAT was consistent across subgroups of men (but not women, Fig. 1B), lean versus obese (Fig. 1C) and individuals with or without T2D (Fig. 1D). There were no significant TL differences in both SAT and VAT for the comparisons of men versus women (Fig. 1B), lean versus obese (Fig. 1C) and AT donors with or without T2D (Fig. 1D).

3.2. Shorter telomere length in SAT is due to shorter TL in subcutaneous SVF cells

Adipose tissue is composed of several cell types including adipocytes and cells of the SVF. To further elucidate the contributions of these two cell fractions on whole adipose tissue TL differences between SAT and VAT, we analyzed TL in isolated adipocytes and cells of the SVF in a subgroup of AT donors (n = 22). There was no TL difference between isolated adipocytes from SAT and VAT (Fig. 2A), whereas TL was significantly shorter in SVF cells from the subcutaneous compared to the visceral fat depot (Fig. 2B). Isolated adipocytes were then further divided into small (diameter<100 μm) and large adipocyte (diameter>100 μm) subfractions. TL was not significantly different between small and large adipocytes, independently of the fat depot (Fig. 2C).

3.3. Telomere length in SAT negatively correlates with age and HbA1c

To further investigate the association between whole adipose tissue TL and parameters of obesity, fat distribution and metabolism, we performed univariate correlation analyses using data from the entire study population (n = 97). In SAT, we find significant negative correlations between SAT TL and age (Fig. 3A) and HbA_{1c} (Fig. 3B). The only significant correlate of VAT TL is mean visceral adipocytes size (Fig. 3C), which is however not significant after adjusting for age and BMI (r = −0.14, P = 0.26). Multivariate

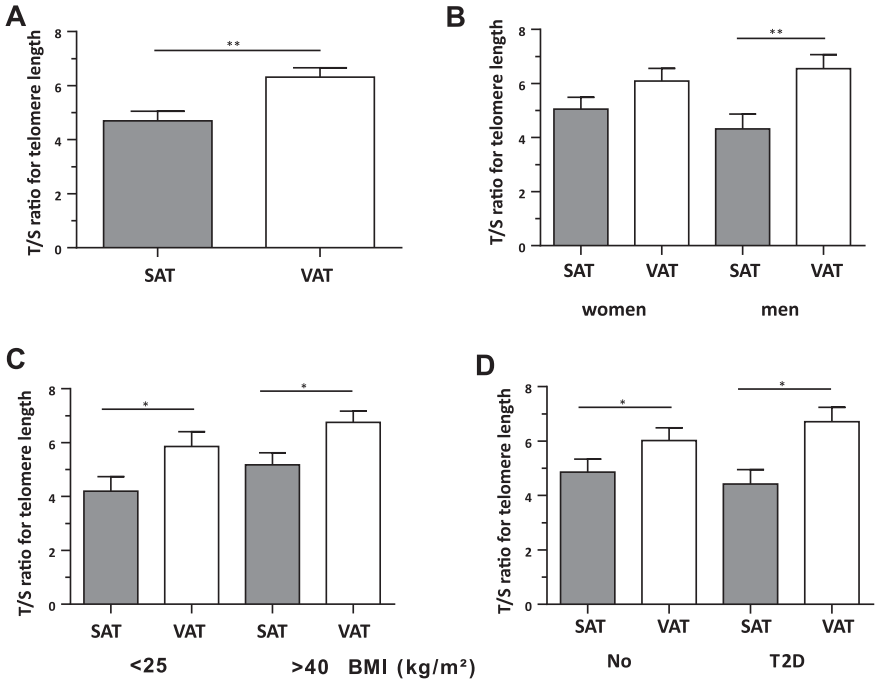

Fig. 1. Fat depot specific differences in telomere length. (A) Telomere length difference between human subcutaneous (SAT) and omental visceral (VAT) adipose tissue from 97 donors of paired samples. Telomere length differences in SAT and VAT of (B) men (n = 46) and women (n = 51); (C) adipose tissue donors with a BMI <25 kg/m^2 (n = 47) versus >40 kg/m^2 (n = 50); (D) as a function of absence (no) or diagnosis of type 2 diabetes (T2D). Data were log transformed to achieve normal distribution. *, p < 0.05, **p < 0.01.

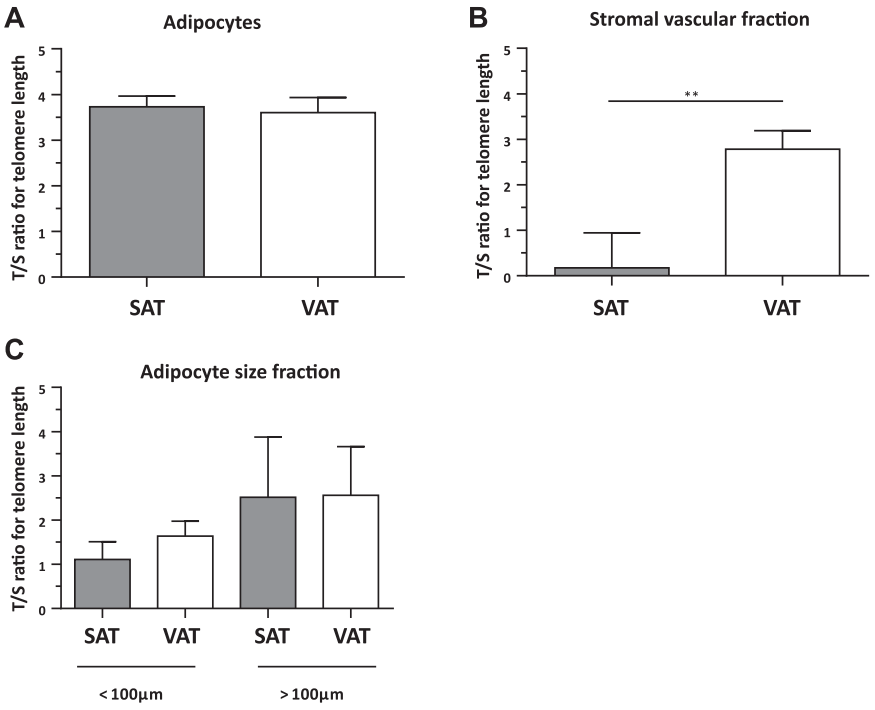

Fig. 2. Telomere lengths analyses in adipocytes and cells of the stromal vascular fraction. Telomere length in isolated adipocytes (A) and stromal vascular fraction (B) from human subcutaneous (SAT) and omental visceral (VAT) adipose tissue (n = 97). (C) Isolated adipocytes from SAT and VAT were further subdivided into small (diameter < 100 µm, n = 13) and large (diameter > 100 µm, n = 13) adipocytes. **P < 0.01.

linear regression models revealed that the negative correlation between SAT TL and HbA1c remained significant upon adjusting for age and BMI (Table 2). Noteworthy, there were no significant correlations between VAT TL and any of the parameters (see Table 1) tested.

3.4. Telomere length of superficial SAT is significantly shorter in patients with T2D

We tested the hypothesis that TL differs between different layers of abdominal subcutaneous AT. In the entire group, we do not find

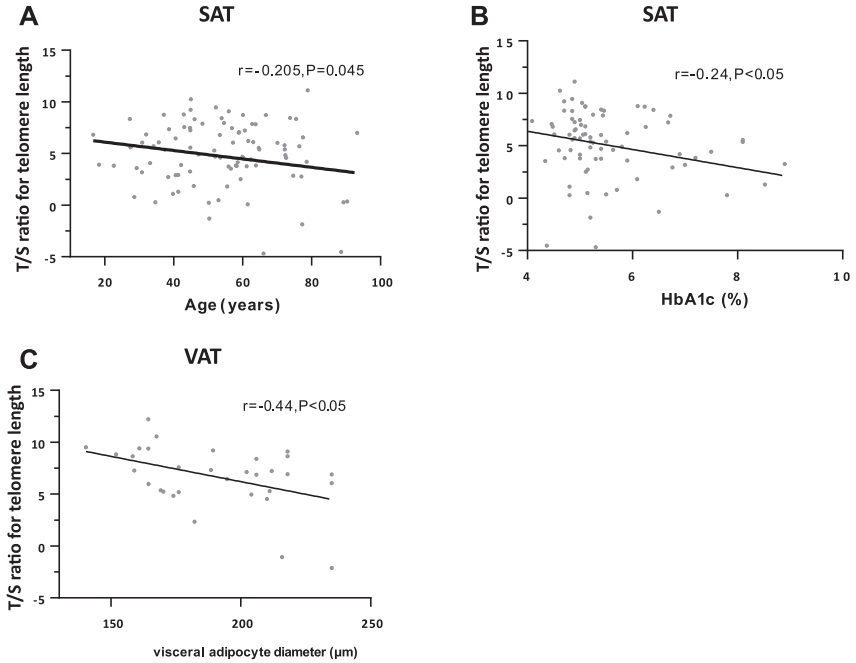

Fig. 3. Significant correlates of telomere lengths in adipose tissue and age, HbA1c and visceral adipocyte size. Linear relationships between telomere length in subcutaneous adipose tissue (SAT) and age (A), HbA_{1c} (B), and mean visceral (visc.) adipocyte size T/S ratio data (C) were log transformed to achieve normal distribution.

Table 2
Multivariate linear regression analysis of telomere length in subcutaneous (SAT) and visceral (VAT) adipose tissue. r = correlation coefficient (significant correlations are highlighted in bold).

Telomere length	SAT (*r*; *P*-value)	VAT (*r*; *P*-value)
Model 1:		
Age (years)	**−0.041; 0.045**	−0.019; 0.347
Model 2:		
Age (years)	−0.028; 0.187	0.002; 0.924
HbA_{1c} (%)	**−0.426; 0.05**	0.193; 0.389
Model 3:		
Age (years)	−0.018; 0.491	0.011; 0.677
HbA_{1c} (%)	**−0.501; 0.047**	0.129; 0.605
BMI (kg/m^2)	0.021; 0.503	0.018; 0.550

significant TL differences between sSAT and dSAT (Fig. 4A) which is consistent in men and women (Fig. 4B). There is a trend for shorter TL in both sSAT and dSAT in individuals with a BMI >30 kg/m^2 compared to lean controls (Fig. 4C).

SAT of patients with T2D is characterized by a significantly shorter TL in sSAT (but not in dSAT) compared to individuals with normal glucose metabolism (Fig. 4D).

4. Discussion

In obese individuals, adipocyte TL has been shown to reflect the degree of adipose tissue accumulation and AT function [2]. In addition, shortened telomeres in AT appear to be significantly associated with hypertrophy of adipocytes, low circulating adiponectin levels, and impaired glucose and lipid metabolism [9]. These associations were reported for both individuals with obesity, but also for lean individuals with metabolic diseases including T2D [9]. Based on these previous findings, we tested here the main hypothesis that TL is related to obesity, AT function and metabolic abnormalities in fat depot-specific manner.

We find that TL is significantly shorter in SAT compared to VAT due to shorter TL in cells of the SVF in SAT. Fat depot TL difference is independent of gender, diabetes status and BMI in our Caucasian cross-sectional study population. In addition, TL in SAT, but not in VAT is significantly negatively associated with age and independently of age and BMI with HbA1c. Further subclassification of SAT into superficial and deep SAT revealed shorter TL in sSAT from T2D patients compared to controls.

As telomeres shorten when cells proliferate, our data suggest that cell turnover rate of SVF cells in VAT is lower compared to SAT. Our data may therefore support previous findings that human abdominal subcutaneous preadipocytes had the highest and omental cells the lowest capacity to differentiate [12,13]. In this context, van Harmelen and colleagues found that the correlation between age and preadipocyte proliferation was restricted to subcutaneous AT and could not be detected in omental AT [13]. Together with these previous data, our findings confirm that aging has distinct effects on preadipocytes from different fat depots and could explain loss of subcutaneous and relative preservation of omental fat with aging. The TL differences between SAT and VAT in our study was consistently found in men, and across subgroups of lean versus obese, and individuals with or without T2D suggesting that gender, fat mass itself and chronic hyperglycemia do not significantly alter preadipocyte proliferation rate. TL differences between SAT and VAT have not been detected in women, which could be due gender differences in TL shortening with aging. In accordance with this postulate, it has been repeatedly shown that there are no gender differences in TL at birth, but in the aging process telomeres shorten faster in men than in women [14,15]. However, many of these previous data rely on TL measurements of leukocytes [14]. Although age-associated telomere shortening seems to occur proportionally in muscle, skin, leukocytes, and SAT

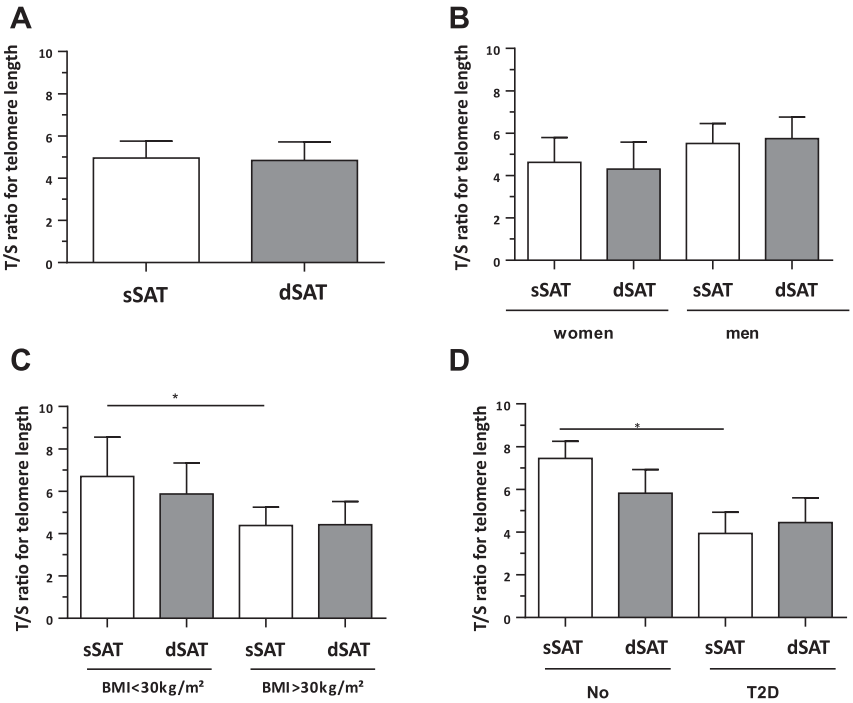

Fig. 4. Telomere length analyses in different abdominal subcutaneous adipose tissue segments. (A) Telomere length comparison between human superficial subcutaneous (sSAT) and deep subcutaneous (dSAT) adipose tissue from 25 donors who underwent plastic surgery of the abdominal region. Telomere length differences in sSAT and dSAT of (B) adipose tissue donors with a BMI <30 kg/m^2 (n = 7) versus >30 kg/m^2 (n = 18); (C) men (n = 9) and women (n = 16); (D) as a function of absence (no, n = 8)) or diagnosis of type 2 diabetes (T2D, n = 17). Data were log transformed to achieve normal distribution. *, P < 0.05.

[4], there are no longitudinal data available for the dynamic of TL shortening in adipose tissue.

In isolated adipocytes, we do not find significant fat depot-related differences in TL supporting the notion that the number of adipocytes remains largely constant during adulthood [6]. Higher preadipocyte proliferation rate in SAT compared to VAT may not be reflected by different TL in mature adipocytes due to the relatively low renewal rate of adipocytes [6]. One limitation of our study is that we can only evaluate TL at a given time point and can therefore not exclude that differences in the dynamic of adipocyte turn over exist between different fat depots. Interestingly, we do not find a significant TL difference between small and large adipocytes from both SAT and VAT. This is in contrast with a recent report demonstrating that shortened TL is associated with hypertrophied adipocytes [9]. The tendency for shorter TL in small versus large adipocyte observed in our study could support the hypothesis that smaller adipocytes may represent a younger generation of preadipocytes, which underwent more cell cycles (and have therefore a higher probability of shortened TL) than the mature larger adipocytes. In contrast to that, we found a significant correlation between shorter TL in VAT and larger visceral adipocytes. However, this correlation was not statistically significant upon adjusting for age and BMI.

The internal integrity of our TL measurements is at least suggested by the significant negative correlation between TL in SAT and age. Tissue aging is characterized by shortened TL due to a substantial decrease in the regenerative potential of several cell types and both genetic and environmental factors determine the rate of tissue regeneration and the balance between accumulation and removal of cellular damage [5]. Independently of the correlation between TL in SAT and age, we found a significantly negative correlation between TL and HbA1c, suggesting that chronic hyperglycemia may affect the cell turnover in SAT. The association between shorter TL and impaired glucose metabolism has been reported in previous studies [2,7–10]. Further studies are required to define the mechanism how chronic hyperglycemia may affect the balance between tissue damage and regeneration.

Abdominal SAT is divided by the Scarpa's fascia into a dSAT and sSAT layer [16]. These two layers of SAT are characterized by distinct structural and functional properties. Compared to sSAT, dSAT has a higher expression of proinflammatory, lipogenic, and lipolytic genes and is more closely related to visceral fat mass and whole body insulin resistance [16,17]. We therefore sought to determine potential TL differences between sSAT and dSAT. Interestingly, we did not find significant differences in TL between these two SAT layers neither in the entire cohort nor in subgroups of men and women or obese versus lean individuals. It has been shown that dSAT expands disproportionally more than sSAT with increasing obesity [16], but we cannot provide data that this difference is reflected by differences in TL between the two TL layers.

In patients with T2D, TL was significantly shorter in sSAT (but not in dSAT) compared to non-diabetic controls suggesting that hyperglycemia may affect AT turnover differentially in these two SAT layers. Our observation may point to a limited expandability of SAT, which may contribute to the development of obesity-related insulin resistance and T2D [18,19]. Interestingly, shorter TL has been recently associated with limited expandability of SAT and the expression of polymerase I and transcript release factor, which may inhibit adipocyte differentiation [20].

Taken together, our data indicate that fat depot differences in TL mainly reflect shorter TL of SVF cells supporting the notion that subcutaneous preadipocytes have higher capacity to differentiate than omental preadipocytes. In addition, we found an age and BMI-independent relationship between shorter TL and HbA1c suggesting that chronic hyperglycemia may impair the regenerative capacity of AT more strongly than obesity alone.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

We thank all participants of the study. We would also like to thank Lutz Feige for technical assistance. This work was supported by grants of the Deutsche Forschungsgemeinschaft, SFB1052 “Obesity mechanisms” (B1 to MB, B4 to NK), Federal Ministry of Education and Research (BMBF), Germany, FKZ: 01EO1001 (NK) and German Diabetes Association (NL) (grant number 981). This work was further supported by the Kompetenznetz Adipositas (Competence network for Obesity) funded by the Federal Ministry of Education and Research (German Obesity Biomaterial Bank; FKZ 01GI1128) and by the Helmholtz Alliance ICEMED – Imaging and Curing Environmental Metabolic Diseases, through the Initiative and Networking Fund of the Helmholtz Association (HA-314). No conflicts of interest, financial or otherwise, are declared by the authors.

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