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Telomere length differences between subcutaneous and visceral adipose tissue in humans

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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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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

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