International Journal of Obesity (2019) 43:2189–2199
https://doi.org/10.1038/s41366-019-0351-y

ARTICLE

Bariatric surgery

Effects of bariatric surgery on telomere length and T-cell aging

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Received: 2 July 2018 / Revised: 29 January 2019 / Accepted: 7 February 2019 / Published online: 12 April 2019

Abstract

Background Obesity adversely affects health and is associated with subclinical systemic inflammation and features of accelerated aging, including the T-cell immune system. The presence of metabolic syndrome (MetS) may accelerate, while bariatric surgery might reverse these phenomena. To examine the effects of MetS and bariatric surgery on T-cell aging, we measured relative telomere length (RTL) and T-cell differentiation status in obese patients before and after bariatric surgery.

Methods WHO II/III classified obese patients scheduled for bariatric surgery were included: 41 without MetS and 67 with MetS. RTL and T-cell differentiation status were measured in circulating $CD4^+$ and $CD8^+$ T cells via flow cytometry. T-cell characteristics were compared between patients with and without MetS prior to and at 3, 6, and 12 months after surgery considering effects of age, cytomegalovirus-serostatus, and weight loss.

Results Thymic output, represented by numbers of CD31-expressing naive T cells, showed an age-related decline in patients with MetS. MetS significantly enhanced $CD8^+$ T-cell differentiation. Patients with MetS had significant lower $CD4^+$ RTL than patients without MetS. Within the first 6 months after bariatric surgery, RTL increased in $CD4^+$ T cells after which it decreased at month 12. A decline in both thymic output and more differentiated T cells was seen following bariatric surgery, more pronounced in the MetS group and showing an association with percentage of body weight loss.

Conclusions In obese patients, MetS results in attrition of RTL and accelerated T-cell differentiation. Bariatric surgery temporarily reverses these effects. These data suggest that MetS is a risk factor for accelerated aging of T cells and that MetS should be a more prominent factor in the decision making for eligibility for bariatric surgery.

Introduction

Obesity (body mass index (BMI) > 30 kg/m^2) is a risk factor for a wide variety of diseases including hypertension, liver steatosis, and cancer [1]. The metabolic syndrome (MetS), characterized by biochemical dysregulation of triglycerides, high-density lipoprotein (HDL) cholesterol, glucose, blood pressure, and increase in abdominal waist circumference, increases this risk [2]. MetS in the context of obesity is associated with the development of a chronic subclinical systemic inflammatory state [3]. Major players in the development of this inflammatory milieu are adipocytokines, adipokines, and cytokines produced by white adipocytes that closely regulate lipid metabolism and the inflammatory response [4], and contribute to the development of insulin resistance [3, 5, 6].

Naive, antigen-inexperienced T cells that have recently left the thymus, express CD31 and are called recent thymic emigrants (RTEs). In response to infectious agents, either T-helper ($CD4^+$) or T-cytotoxic ($CD8^+$) cells differentiate from naive into memory, antigen-experienced T cells to

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Supplementary information The online version of this article (https://doi.org/10.1038/s41366-019-0351-y) contains supplementary material, which is available to authorized users.

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enable a potent second response to the same stimuli. Within the memory T-cell pool, central (CM), effector memory (EM), and terminally differentiated CD45RA$^+$ EM (EMRA) T cells can be distinguished with different phenotypical and functional characteristics. Changes in this response and in T-cell characteristics have been linked to the reduced lifespan seen in morbidly obese patients [7]. The link between chronic (sub)clinical inflammation and advancing age, "inflammaging", leads to accelerated aging and frailty [8]. The release of pro-inflammatory cytokines associated with inflammaging activates the immune system, which eventually leads to sustained damage on cells and tissues [9]. With aging, the thymus involutes resulting in a decrease in circulating naive T cells and a subsequent increase in more differentiated T cells, such as EMRA T cells and T cells lacking expression of the costimulatory molecule CD28 (CD28null), causing an enhanced T-cell differentiation state [8, 9].

Both a reduced thymic output and an enhanced T-cell differentiation status are validated biomarkers for human T-cell aging, as well as attrition of telomeres [10–12]. Telomeres are small DNA repeats located at the end of chromosomes that protect from fusion but shorten with each cell division [13].

Cytomegalovirus (CMV) seropositivity should be considered when studying T-cell aging as it leaves a clear fingerprint on circulating T cells, resembling T-cell aging. CMV-seropositivity has been associated with a more differentiated memory T-cell compartment, expansion of the pool of CD28null T cells and attrition of telomeres in T cells [10, 14–17]. CMV prevalence ranges from 30 to 100% and depends on socioeconomic and ethnic background [18].

Total T-cell numbers, as well as cytotoxic CD8$^+$ and CD4$^+$ T cells are reported to be positively associated with BMI and the prevalence of MetS by some authors [19–21], but not by others [22, 23]. Also, several studies find that BMI is inversely correlated with telomere length in T cells [13, 24]. However, this has not been established in all studies [13, 25]. The effect of MetS on T-cell aging has not yet been investigated.

Bariatric surgery may be indicated as a treatment for morbidly obese patients. [26]. Besides rapid loss of body weight, bariatric surgery has been reported to reverse obesity-related diseases including diabetes mellitus and dyslipidemia [26]. Whether this procedure also induces reversal of aging parameters, in particular premature T-cell aging, is unclear.

Therefore, we aimed to determine the effects of MetS on aging of circulating T-cell subsets, as well as the potential reversal of T-cell aging by bariatric surgery.

Subjects and methods

Study design

This study was designed as a non-randomized prospective cohort study. The study was approved by the general Medical Ethical Committee (METC) with MEC identification number 2012–134 of the Erasmus University Medical Center, Rotterdam, The Netherlands. Approval of the inclusion center occurred via the Board of Directors of the Maasstad Hospital, Rotterdam, The Netherlands, with local identification number 2012-51. The study is performed in accordance with the local METC guidelines. The trial is registered as part of the PROTECT trial in the Dutch trial registry database using trial code 3663 (www.trialregister.nl). This study was performed in accordance with the CONSORT 2010 statement, according to the Declaration of Helsinki [27].

Study population

Patients with obesity and morbid obesity scheduled to undergo bariatric surgery who visited the outpatient clinic at the Maasstad Hospital between March 2014 and August 2015 were invited to participate in the study. All participating patients gave written informed consent before inclusion. A patient flowchart showing all inclusions and exclusions is depicted in Figure S1. To be eligible for bariatric surgery, patients had to have a BMI (kg/m^2) corresponding with obese class II or III as defined by the World Health Organization (WHO) with or without the presence of the MetS [28, 29]. MetS was defined in accordance to the National Cholesterol Education Program ATPIII Guidelines, as fulfilling three out of five criteria [30]. Exclusion criteria were obesity class I, other comorbidities than MetS, patients without basic understanding of the Dutch or English language, or patients undergoing another form of bariatric surgery than a laparoscopic gastric bypass procedure (LGBP).

Bariatric surgery

All patients were scheduled to undergo the laparoscopic Roux-and-Y gastric bypass procedure (LGBP). During a LGBP, the jejenum is divided at 50 cm from the ligament of Treitz into a biliopancreatic limb and a 150-cm alimentary Roux limb. The proximal segment of the stomach is made into a small pouch with stapling devices. A side-to-side anastomosis is created between the pouch and the Roux limb. The biliopancreatic limb is connected to the Roux limb, 150 cm distally.

Blood collection

After providing written informed consent, a venous blood sample was obtained prior to surgery. The duration until scheduled surgery was between several days and five months after first blood sample. Prior to bariatric surgery, venous samples of 107 patients were collected for analysis. A selection of patients was asked to donate another venous blood sample at time points 3 ($n = 47$), 6 ($n = 10$), and 12 ($n = 11$) months after surgery (Figure S1). The largest subgroup at time point 3 months was also analyzed separately (Table S1). The selection was made since not all patients showed up in the outpatient clinic during their scheduled follow-up visits, or patients decided to be followed-up elsewhere (for example, by their general practitioner). Blood samples were collected in 10.0 mL BD Lithium-Heparin tubes (Franklin Lakes, NJ, USA), with a maximum of two tubes per time point.

CMV serology

CMV serology was assessed of all participants included in the study at the diagnostic Department of Virology of Erasmus University Medical Center, by determining the presence of plasma IgG antibodies to CMV with an enzyme immune assay (Biomerieux, VIDAS, Lyon, France). The results were expressed as arbitrary units/mL (AU/mL), and an outcome of ≥ 6 AU/mL was considered positive.

T-cell phenotyping and PBMC isolation

A whole blood staining was performed and analyzed on the BD FACSCanto II (BD (Erembodegem, Belgium) using FACSDiva software version 6.1.2 (BD) in order to determine percentages and absolute numbers of T-cell subsets (Table 1). The analysis procedure, as well as further characterization of the T cells has been described previously [31]. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples by Ficoll gradient centrifugation as described in detail before [32]. PBMC were stored at −150 °C at 10×10^6 per vial until further experiments.

Table 1 T-cell subsets and their corresponding staining markers

T-cell subset	Marker
Recent thymic emigrants (RTEs)	$CD31^+$ naive
Naive T cells	$CD45RO^-/CCR7^+$
Central memory T cells (CM)	$CD45RO^+/CCR7^+$
Effector memory T cells (EM)	$CD45RO^+/CCR7^-$
Terminally differentiated effector memory T cells (EMRA)	$CD45RO^-/CCR7^-$
Total memory T cells (MEM)	Sum of CM, EM, and EMRA
Advanced differentiated T cells	CD28null

Relative telomere length

The relative telomere length (RTL) of peripheral blood T cells was determined by flow fluorescent in situ hybridization (flowFISH) technique, as described previously [10]. PBMCs were fixed and permeabilized and using the FITC-labeled PNA-kit (DakoCytomation, Glostrup, Denmark), the telomere length of $CD4^+$ and $CD8^+$ T cells was determined. As an internal standard, the sub cell line 1301 of CCRF-CEM (known for its long telomeres) was taken along in this procedure as a reference. The median fluorescence intensity (FL1) with probe minus the median FL1without probe of $CD4^+$ and $CD8^+$ T cells was related to that of the cell line, both multiplied by the index of (non-dividing) single cells (DNA index), and RTL could be calculated using the following formula:

$$\mathrm{RTL} = \frac{\left(\begin{array}{c}\text{median FL1 sample cells with probe} - \text{median FL1 sample cells}\\ \text{without probe}\end{array}\right) \times \text{DNA index of control } (=2) \text{ cells}}{\left(\begin{array}{c}\text{median FL1 control cells with probe} - \text{median FL1 control cells}\\ \text{without probe}\end{array}\right) \times \text{DNA index of sample } (=1) \text{ cells}} \times 100$$

Statistical analysis

For all individual parameters, median and interquartile ranges were computed. Comparison of two or more parameters was done with the parametric *T*-test or the non-parametric variant Kruskall–Wallis test. Related samples from the same patient were analyzed via the non-parametric-related samples Friedman's test. Prior to bariatric surgery, a multivariate analysis was performed via general linear models option, considering the Wilks' Lambda test to evaluate which variables contributed to T-cell characteristics measured in the circulation. Generalized estimating equations (GEEs) were used to study the development of the selected outcome variables over time in patients after bariatric surgery, considering the relation between the repeated measures in the same patient. Interactions were investigated for outcome variables age, extent of body weight loss, and MetS, with preset cutoffs age (≤50/>50 years), percentage of body weight loss (≤66%/>66%) [33], and MetS (yes/no). Repeated measures of body weight were added to the model in case of a statistically significant interaction ($P < 0.05$). Statistics were computed with use of SPSS version 23 (IBM Corp, released 2015, IBM SPSS Statistics for Mac, Version 23.0, Armonk, NY: IBM Corp), Microsoft® Office Excel 2016 (version 16.12), SAS software version 9.4 (SAS Institute, Inc., Cary, NC), and GraphPad Prism (GraphPad Software

Table 2 Baseline characteristics of the different patient cohorts

Parameter	BMI ≥ 35 kg/m^2 no MetS ($n = 41$)	BMI ≥ 35 kg/m^2 with MetS ($n = 66$)	*P*-value
Gender (male/female)	5/36	20/46	**0.03**
Body weight (kg)	126 (103–184)	129 (98–200)	0.11
BMI (kg/m^2)	44 (35–65)	43 (35–57)	0.35
Age (mean, years)	34 (18–62)	43 (22–60)	0.07
Age (≤50/>50 years)	27/13	41/26	0.46
Age (mean (≤50/>50 years)	31 (18–46)/54 (51–62)	37 (22–50)/56 (51–60)	**0.009**/0.13
CMV (negative/positive/unknown)	27/13/0	33/30/4	0.08
Age (mean CMV negative/positive)	39 (18–62)/37 (25–56)	42 (22–60)/45 (25–60)	0.30/0.09

Significances ($P < 0.05$) are depicted in bold

BMI body mass index, *MetS* metabolic syndrome, *CMV* cytomegalovirus

Inc., version 5.01). Figures were made in Graphpad. For all parameters, $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

A total of 107 patients were included in this study, consisting of 41 patients without MetS and 66 patients with MetS. Table 2 summarizes the baseline characteristics of the included participants. Patients without MetS were more often female than patients with MetS. In the group of patients ≤50 years, patients with MetS were on average significantly older (37 years) than patients without MetS (31 years, $P = 0.009$). No significant differences were observed with respect to distribution of CMV-seropositivity between the study groups. Baseline characteristics of the subgroup analyses at time point 3 months after surgery are depicted in Table S1.

Lower numbers of RTEs due to MetS and age

A significant age-related decline of RTE (CD31$^+$ naive T cells) was observed in the CD8$^+$, but not CD4$^+$, T-cell compartment (Table 3) ($P = 0.001$). Subgroup analyses based on the presence of MetS, CMV-serostatus, or gender, showed this significance persisted in the presence of MetS ($P = 0.02$). These data suggest an age-related decline in RTE in the CD8$^+$ T-cell compartment with persistence in the presence of MetS.

MetS enhances T-cell differentiation status

Patients with MetS had a significant lower number of CD4$^+$ T cells ($P = 0.02$) and a higher number of more differentiated CD28null CD4$^+$ T cells ($P = 0.04$) (Table 3). In the CD8$^+$ T-cell compartment, patients with MetS showed significantly higher numbers of total memory T cells ($P = 0.02$), CM (CD45R0$^+$/CCR7$^+$) ($P = 0.01$), and terminally differentiated effector CD45RA$^+$ memory T cells (EMRA, CD45RO$^-$/CCR7$^-$) ($P = 0.03$). Advanced age (>50 years) led to a significant lower number of CD8$^+$ naive (CD45RO$^-$/CCR7$^+$) T cells ($P = 0.01$) (Table 3). In patients ≤50 years, the presence of MetS was associated with a lower number of CD4$^+$ cells ($P = 0.046$). These data point toward an advanced T-cell differentiation status in patients with MetS.

Subgroup analyses showed that CMV-seropositivity resulted in more differentiated CD4$^+$ and CD8$^+$ T cells (EMRA and CD28null), whereas female gender resulted in more CD8$^+$ memory and CM T cells (Table 3). A multivariate analysis including MetS, age, CMV-seropositivity and gender showed that MetS, CMV-seropositivity and female gender were independent factors for the enhanced CD4$^+$ T-cell differentiation status, whereas for the total cohort of CD8$^+$ T-cell markers, independent factors were age, CMV-seropositivity, and gender (Table S2).

MetS and age affect RTL of T cells prior to bariatric surgery

RTL of CD4$^+$ T cells was significantly shorter in the MetS group compared with the no MetS group ($P = 0.02$) (Fig. 1a). Age did not influence RTL (Fig. 1b). In patients ≤50 years, CD4$^+$ RTL was significantly shorter in the presence of MetS ($P = 0.03$) (Fig. 1c). No significance was seen in the group >50 years with or without MetS, however, a large spread in interquartile ranges was seen in both groups. No significant differences were seen in CD8$^+$ RTL in either the different MetS (Fig. 1d), age (Fig. 1e), or combined MetS and age (Fig. 1f) groups. These results suggest enhanced telomere attrition in the CD4$^+$ compartment of patients with MetS, with most pronounced changes

Table 3 *P*-values of T-cell differentiation markers of the different patient cohorts prior to surgery

	MetS (+ vs. –)	Age (old vs. yng)	MetS × age (+/old vs. –/yng)	CMV (pos. vs. neg.)	Gender (M vs. F)
CD4 T cells	**0.02** (↓)	0.38	**0.046** (↓)	0.12	0.68
Naive T cells	0.78	0.25	0.61	0.15	0.28
CD31 naive T cells	0.86	0.11	0.28	0.18	0.08
Memory T cells	0.33	0.88	0.57	0.92	0.75
Central memory T cells	0.30	0.65	0.74	0.27	0.56
Effector memory T cells	0.98	0.59	0.68	**0.03** (↑)	0.09
EMRA T cells	0.21	0.73	0.26	**0.02** (↑)	0.07
CD28null T cells	**0.04** (↑)	0.74	0.13	**<0.001** (↑)	0.27
CD8 T cells	0.12	0.40	0.14	0.16	0.52
Naive T cells	0.79	**0.01** (↓)	0.07	0.51	0.47
CD31 naive T cells	0.98	**0.001** (↓)	**0.02** (↓)	0.98	0.39
Memory T cells	**0.02** (↑)	0.51	0.08	**0.003** (↑)	**0.03** (↑)
Central memory T cells	**0.01** (↑)	0.81	0.14	0.29	**0.004** (↑)
Effector memory T cells	0.36	0.72	0.83	0.25	0.92
EMRA T cells	**0.03** (↑)	0.41	0.11	**<0.001** (↑)	0.07
CD28null T cells	0.06	0.20	0.06	**<0.001** (↑)	0.18

Significant comparisons are depicted in bold. Arrows indicate direction of significance

MetS MetS vs. no MetS, *Age* total group >50 years vs. ≤50 years, *MetS* × *age* MetS group >50 years vs. no MetS ≤50 years, *CMV* total group CMV vs. no CMV, *Gender* total group male vs. female patients, *EMRA* terminally differentiated effector memory T cells, *MetS* metabolic syndrome, *CMV* cytomegalovirus

in the younger patients. CMV-related attrition of telomeres was only observed within the $CD4^+$, but not the $CD8^+$ T-cell compartment of morbidly obese patients without Mets ($P = 0.03$). In a multivariate analysis, MetS was suggested to be an independent factor for $CD4^+$ or $CD8^+$ RTL (Table S2).

T-cell aging is partially reversed following surgery and is associated with body weight loss

To study the effects of bariatric surgery on the changes seen in the T-cell immune system and RTL over time, GEEs were used. These GEEs considering the correlation between the repeated measures in the same patient. Using $T = 0$ before surgery as a reference time point, we analyzed all repeated measurements included during at least one time point after surgery, i.e., at 3, 6, or 12 months after bariatric surgery. Since most outcome parameters are influenced by age and gender, the analyses were performed after adjustments for both characteristics.

Up until 12 months, bariatric surgery resulted in a vast decline in absolute $CD4^+$, but not $CD8^+$, RTE ($P < 0.001$) especially in the first 6 months ($P < 0.001$) (Table 4a). Between 6 and 12 months, the numbers of $CD4^+$ RTE significantly increased ($P = 0.03$). When adjusted for body weight, the significance marginally disappeared ($P = 0.07$), indicative of a correlation between body weight loss and numbers of $CD4^+$ RTE. Subgroup analyses showed that these significances were due to the presence of MetS ($P = 0.008$) and seen in patients of younger age ($P = 0.002$), indicating that the correlation between body weight and RTE-decline was seen in patients ≤50 years with MetS.

In the more differentiated T-cell subsets, a significant decrease in absolute numbers of $CD4^+$ EM ($P = 0.006$) and EMRA ($P = 0.03$), and a trend toward lower CD28null T cells ($P = 0.06$) was seen, which was most significant in the first 6 months postoperatively. Adjusting for body weight revealed remaining significance, suggesting of a weak association between body weight loss and the numbers of $CD4^+$ EM and EMRA. In subgroup analyses, the numbers of CD28null T cells were also significantly associated with CMV-seropositivity status ($P = 0.047$). In the $CD8^+$ T-cell compartment, the numbers of EM were significantly decreased as well ($P = 0.04$). In contrast, the absolute numbers of the less-differentiated $CD8^+$ CM T cells were increased by bariatric surgery ($P = 0.05$), especially in the first 3 months postoperatively ($P = 0.001$). Adjusted for body weight, the significance in $CD8^+$ CM disappeared as well, whereas it remained in the EM T cells (Table 4a).

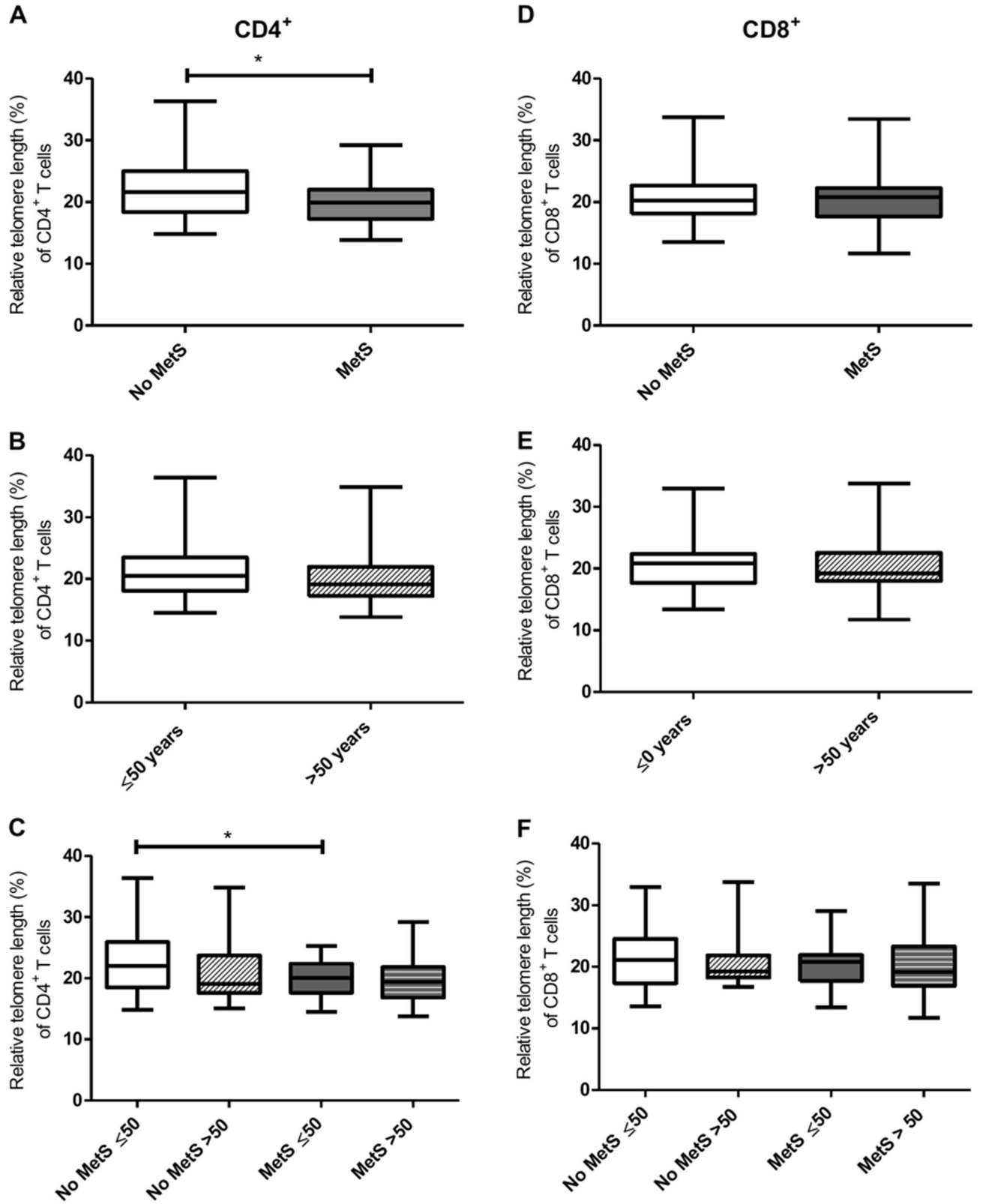


Fig. 1 RTL prior to bariatric surgery (total group). **a** RTL of $CD4^+$ T cells was significantly lower in the patients with MetS. **b** Age did not have an effect on RTL of $CD4^+$ T cells. **c** In patients ≤50 years, MetS resulted in significantly lower $CD4^+$ RTL than in the absence of MetS. In patients >50 years, a large spread in interquartile range was seen in both groups. **d** No changes were seen in the $CD8^+$ RTL due to MetS, **e** due to age or **f** due to both MetS and age. RTL relative telomere length, MetS metabolic syndrome. $^*P < 0.05$

Table 4a T-cell differentiation markers after surgery including interaction and subgroup analyses

Parameter	Adjusted for age and gender	Adjusted for body weight	Interactions			Subgroup analyses		
			MetS	Age	BWL	MetS (+/–)	Age (≤/>50 yrs)	TBWL (≤/>66%)
CD4 CD31 naive	**<0.001**	0.07	0.29	0.14	0.96	**0.008**/0.08	**0.002**/0.45	**0.005**/0.11
CD4 CM	0.60	N/A	0.10	0.93	0.34			
CD4 EM	**0.008**	**0.03**	0.71	0.60	0.08			
CD4 EMRA	**0.03**	**0.02**	0.89	0.19	**0.04**			
CD4 CD28null	**0.06**	0.36	0.62	0.36	0.08	0.12/0.41	0.11/0.06	0.26/0.05
CD8 CD31 naive	0.52	N/A	0.08	0.87	0.79			
CD8 CM	**0.05**	0.10	0.22	0.46	**0.01**			
CD8 EM	**0.02**	**0.005**	0.23	0.79	0.35			
CD8 EMRA	0.11	N/A	0.10	0.16	0.07			
CD8 CD28null	0.30	N/A	0.22	0.50	0.04			

Numbers correspond to *P*-values, significances ($P < 0.05$) are depicted in bold. Only in case of significant differences after adjustment for age and gender (column 2), subgroup analyses were performed

CM central memory, *EM* effector memory, *EMRA* terminally differentiated effector memory T cells, *N/A* not calculated, *MetS* metabolic syndrome, *Age* >50 years, *TBWL* total body weight loss >66% as from starting weight preoperatively

Table 4b Relative telomere length after surgery including interaction and subgroup analyses

Parameter	Adjusted for age and gender	Adjusted for body weight	Interactions			Subgroup analyses		
			MetS	Age	BWL	MetS (+/–)	Age (≤/>50 yrs)	TBWL (≤/>66%)
$CD4^+$ RTL	**0.04**	**0.03**	0.19	0.17	**0.01**	0.14/0.23	0.14/0.18	**0.03**/0.10
$CD8^+$ RTL	0.12	N/A	0.81	0.05	0.08	0.40/0.43	0.06/0.26	0.08/0.16

Numbers correspond to *P*-values, significances ($P < 0.05$) are depicted in bold. Generalized estimating equations were used, taking into account the correlation between the repeated measures in the same patient. Outcome variables were added as continuous dependent variable, time of measurement (expressed in months) as independent categorical variable. When outcome variables were not normally distributed, they were log-transformed before analyses. Time before bariatric surgery was used as reference. All models were adjusted for age at baseline and gender. When a statistically significant association was observed ($P < 0.05$ for time variable with Type 3 test), adjusted mean values were tested for statistically significant difference

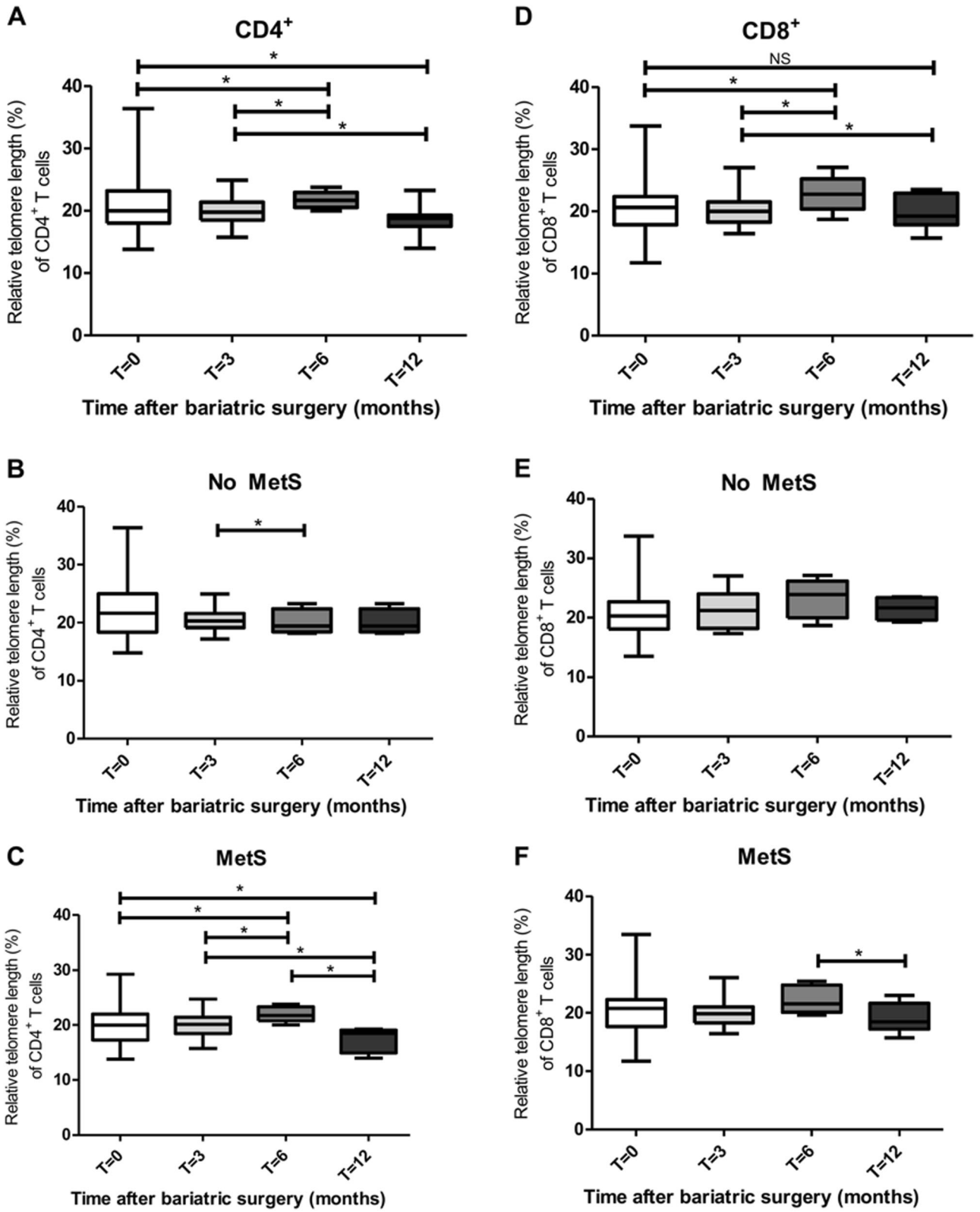
RTL relative telomere length, *MetS* metabolic syndrome, *MetS+* presence of MetS, *MetS–* absence of MetS, *Age* ≤ or >50 years, *TBWL* total body weight loss >66% as from starting weight preoperatively

Variations in RTL after bariatric surgery

A significant increase in $CD4^+$ RTL was seen after 3 ($P = 0.007$) and 6 ($P = 0.002$) months postoperatively. However, at month 12, a decrease in RTL was observed ($P < 0.001$) (Fig. 2a), thus resulting in an overall decrease in $CD4^+$ RTL throughout the first year after surgery ($P = 0.017$). Patients without MetS (Fig. 2b) or with MetS (Fig. 2c) did not show an overall interaction, however, in the MetS group in-between time points showed a steady increase of RTL within the first 6 months after which a decrease occurred. The GEE model showed a decline in $CD4^+$, as well ($P = 0.04$), and an interaction was found with more profound weight loss ($P = 0.01$) (Table 4b). Subgroup analyses revealed no additional changes due to the presence of MetS or for the different age categories. The same trend was observed in the $CD8^+$ RTL, with significant increase in length between 3 ($P = 0.01$) and 6 ($P = 0.01$) months followed by a decrease at months 12 ($P = 0.01$). Overall, these changes did not result in a significant change throughout the first 12 months (Fig. 2d). Also, the mixed model analysis revealed no overall significant changes in patients without MetS (Fig. 2e) or with MetS (Fig. 2f).

Discussion

In present study, we show that MetS is a suggested risk factor for accelerated attrition of telomeres in obese patients, and a more differentiated T-cell compartment. Bariatric surgery leads to a temporary, short-term increase of telomere length and decreased T-cell differentiation status. Our results suggest that bariatric surgery may temporarily reverse this accelerated T-cell aging, and that patients with



WHO II/III obesity and MetS, who are currently not included in the guidelines to undergo bariatric surgery, may benefit from surgery.

Changes in the T-cell-mediated adaptive immunity due to obesity have been observed previously. Obesity increases both the total numbers of $CD4^+$ and $CD8^+$ T cells [34]

◀ **Fig. 2** RTL after bariatric surgery (subgroup analysis). **a** $CD4^+$ RTL of the total obesity group significantly increased between time points 0–6 and 3–6 months postoperatively, after which RTL significantly declined again between 6 and 12 months. **b** Between 3 and 6 months, a significant increase in RTL of patients without MetS was seen, not leading to an overall significant change in the total time period. **c** MetS resulted in an overall decline in the first 12 months postoperatively; an increase was seen between 0–6 and 3–6 months, and a decrease was shown between 3–12 and 6–12 months. **d** No overall changes were seen in the $CD8^+$ RTL due to obesity, whereas subgroup analyses showed a significant increase between 0–6 and 3–6 months, and a decrease between 3 and 12 months. **e** No changes were seen in $CD8^+$ RTL in the group without MetS, whereas the group with MetS (**f**) only showed a significant decline between 6 and 12 months. RTL relative telomere length, MetS metabolic syndrome. $^*P < 0.05$. Time point 3, $n = 47$; time point 6, $n = 10$, time point 12, $n = 11$

while causing a decrease in $CD4^+$ regulatory T cells [21, 35, 36]. The effects of obesity on maturation of the T-cell system has only been investigated in children, showing an increase in more differentiated T cells [37]. In patients with renal failure, uremia induces a severe depletion of naive T cells and a shift to more differentiated T cells [10, 31, 38]. In our study, the observed detrimental effects of MetS are in line with earlier findings, namely a depletion in the total numbers of T cells as well as an increase in more differentiated EMRA and CD28null T cells, which are associated with aging. Presumably, the number of differentiated T cells increases with age while the number of naive T cells decreases [39]. This hypothesis could partially be confirmed by the marked decrease of naive T cells and RTE due to age. This was much more pronounced in the $CD8^+$ T-cell compartment, which is in line with the latest literature showing that the immunological changes due to obesity affect mostly $CD8^+$ T cells [23]. Since T-cell immunity and telomere length are linked to accelerated aging and age-associated diseases, these pronounced effects of MetS on T-cell immunity and telomere length suggest that patients with MetS have a higher immunological age, making them more prone to acquire infections and malignancies [10, 38]. Exactly these patients might especially benefit from bariatric surgery to reduce the accelerated aging-associated morbidity, in addition to weight-associated morbidity.

T-cell aging is associated with attrition of telomeres, which can be easily measured in circulating T cells using flow cytometry [13, 40]. The relationship between obesity and telomere length in circulating leukocytes and T cells is ambiguous [21, 25, 41, 42]. The additional effect of MetS on telomere length has not yet been established before [41]. Here, we underscore the deleterious role of MetS on T-cell aging by showing enhanced telomere attrition. Our study used a flow cytometry-based assay (flowFISH) to measure RTL, which has been shown to be more sensitive to detect differences between populations in contrast with the quantitative PCR assay used by others [41].

The hypothesis that bariatric surgery halts or reverses accelerated attrition of telomeres has been investigated previously with inconclusive results [41, 42]. Formichi et al. [42] showed in a comparable study design a significant decline of telomere length at 3, 6, and 12 months postoperatively. We too found a shortening of RTL at 12 months, but an increase of RTL in the first 6 months postoperatively, which suggests a beneficial effect of surgery on the T-cell immune system in the first postoperative period. Further studies should focus on the changes seen in the later months in order to understand the long-term effects of surgery. Interestingly, we found a relation between an increase in telomere length and percentage of total body weight loss, which marks the decrease in body weight after surgery as an important factor to measure the success rate of the surgical treatment [26]. The decline in RTL could be induced by the possible catabolic state induced by bariatric surgery [42], however, does not explain the change of RTL direction between 6 and 12 months and this should be studied in more detail. Also, the RTL could be inversely related to the number of CD28null T cells. As the RTL was higher at time points 3 and 6 months postoperatively, the numbers of CD28null cells were lower at these time points. In contrast, the number of CD28null cells was higher at time point 12 months, concomitant with a decline in RTL (Table 3). CD28null T cells are described to have shorter telomeres as compared with other T cells, possible due to their higher cell division rate [10]. Therefore, the number of CD28null T cells is suggested to be inversely linked to the RTL. Further studies should highlight this possible relation to confirm their association.

Bariatric surgery also induced changes in the T-cell differentiation state, an observation that has not been made previously. The increase in less-differentiated CM T cells in contrast to the decrease in more differentiated EM and CD28null T cells strongly direct toward reversal of the accelerated T-cell aging initiated by bariatric surgery. Again, high body weight loss might play a crucial role in mitigating these effects, while MetS had a modest role. Bariatric surgery is more effective than lifestyle or medical interventions in the reduction in body weight, as well as in reducing the metabolic complications of obesity [43]. The reduction in body weight is determined by the total body weight loss, and loss of >66% after 1 year is considered successful [44]. The direct correlation between T-cell differentiation and more pronounced total body weight loss again highlights the importance of the vast reduction in body weight that is induced by bariatric surgery. The only result not matching with these effects is the significant decline of $CD4^+$ RTE after bariatric surgery. Hypothetically, this decline in RTE might be due to a reduction in serum concentrations of interleukin 7 (IL-7). This cytokine is important for (naive) T-cell homeostasis, and a reduction

in IL-7 has been seen in obese patients [45–47]. We have previously shown a lower concentration of serum IL-7 in patients with end-stage renal failure compared with healthy controls and linked it to the decreased capacity in these patients to maintain the naive T-cell compartment [48]. Whether these effects also hold true for obesity, bariatric surgery, and MetS still needs to be elucidated.

Telomere attrition and T-cell differentiation status are influenced by various, mostly non-adjustable factors such as CMV-seropositivity and gender [13]. We confirm these data by showing the enhanced T-cell differentiation status in CMV-seropositive WHO II/III obese patients, as well as male gender, which is in line with previous findings [39]. Whether CMV-seropositive and/or male patients might benefit more from bariatric surgery remains unclear and further studies should focus on the role of CMV and gender on premature aging of morbidly obese patients.

There are several limitations attached to this study. These include the small sample size, especially the selected group of patients investigated after surgery, which could conceal effects on different subpopulations within our cohort. Also, the study was conducted at a single center. Despite the small sample sizes, significant differences could be detected. As we measured RTL in the total circulating $CD4^+$ and $CD8^+$ T-cell compartments, the effects seen in RTL might be the result of shifts in specific T-cell subsets as seen for the significant increase in CD28null T cells due to MetS. Further studies should identify changes in RTL within these specific subsets to correlate these findings. Finally, not all effects of obesity are reflected in the circulation and therefore investigating the effects on the corner stone of obesity, the fat tissue itself, will allow the association between the effect of excess of fat tissue and changes seen in the immune system.

In conclusion, we show that MetS in obese patients causes accelerated telomere attrition and enhanced T-cell differentiation in circulating $CD4^+$ and $CD8^+$ T cells. This strongly suggests accelerated aging of the T-cell compartment. Shortening of telomeres and enhanced T-cell differentiation state are temporarily reversed during the first 6 months after bariatric surgery and are associated with percentage of body weight loss. These data suggest that obese patients with MetS are at risk for accelerated aging of the T-cell immune system and might benefit from bariatric surgery at an earlier stage.

Acknowledgements This research was funded by a grant from the National Institute for Public Health and the Environment and the Ministry of Health, Welfare and Sport of the Netherlands (S/340005), and a grant from FitForMe B.V. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank the staff at the Maasstad Hospital Rotterdam, especially the assistants at the outpatient clinic, for their help with patient inclusion and follow-up.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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