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Short communication: Telomere lengths in different tissues of dairy cows during early and late lactation

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ABSTRACT

Telomeres create a protective cap on the ends of chromosomes that shorten with cell division and are influenced by stressful conditions. With the onset of lactation, high-yielding dairy cows are exposed to metabolic stress. In the present study, we aimed to analyze telomere length (TL) in key metabolic organs, such as liver, subcutaneous (sc) adipose tissue (AT), and mammary gland, as well as in peripheral blood cells during early and late lactation in German Holstein cows (n = 21). Animals were fed according to their requirement, and biopsies from scAT, liver, and mammary gland as well as blood cells were collected in early and late lactation. The relative quantity of telomere products (qT), which is proportional to the average TL, was determined in genomic DNA by multiplex quantitative PCR. In this study, relative qT varied widely in the investigated tissues and blood. In late lactation, slowly proliferating tissues, such as liver and scAT, had the highest qT, whereas peripheral blood cells and in the mammary gland had the lowest qT. Comparing early with late lactation, relative qT attrition was limited to blood and mammary gland. Relationships between relative qT in blood, mammary gland, scAT, and liver suggest that blood qT might serve as a surrogate marker for tissue-specific qT. Cows with high initial qT in tissues and blood in early lactation had greater qT attrition during the course of lactation than cows with lower qT. The determination of qT could be included when phenotyping dairy cattle to test for associations with performance and fitness traits.
Key words: dairy cow, lactation, telomere length

Short Communication

Telomeres are repetitive DNA sequences—(TTAGGG)n—capping the end of the chromosomes to protect them against degradation and fusion (Blackburn, 1991). Due to the inability of DNA polymerase to replicate the lagging strand of chromosomes, telomeres shorten with every cell division (Harley et al., 1990; Blackburn, 1991; von Zglinicki and Martin-Ruiz, 2005). The enzyme telomerase can maintain telomere length (**TL**) by adding tandem repeats de novo to the ends of the chromosomes; however, its activity in somatic cells is too low to enable full maintenance of TL (von Zglinicki et al., 2000). When TL declines to a critical point, the telomeres become dysfunctional, leading to cellular replicative senescence followed by cell death (Armanios and Blackburn, 2012). The TL is affected by genetics (Njajou et al., 2007), stress-related conditions, inflammation, oxidative stress, and environmental factors (Entringer et al., 2011). In humans, TL serves as a biomarker for cellular and biological aging (von Zglinicki and Martin-Ruiz, 2005), chronic stress (Epel et al., 2004), and longevity (Bakaysa et al., 2007).

During early lactation, most high-yielding dairy cows are in a phase of negative energy balance (**EB**), which is primarily compensated by mobilization of body reserves mainly from adipose tissue (**AT**), whereas in late lactation, fat depots are refilled by lipogenic processes (McNamara, 1989). During stressful periods such as parturition and lactation, dairy cows are more susceptible to metabolic and infectious diseases (Broom and Fraser, 2007) and often show reduced fertility (Sapolsky et al., 2000). Metabolic stress and reduced fertility are associated with reduced productive lifespan in dairy cows (Wathes, 2012; Pritchard et al., 2013). So far, data about TL in dairy cows are limited to blood measurements, relating peripheral blood TL and survival of Holstein cows (Brown et al., 2012). The study from Brown et al. (2012) indicated a potential role of TL to assess stress and health conditions in dairy cows.

However, no reports exist comparing TL in different tissues and TL changes during early and late lactation in dairy cows. Therefore, in the present study, we aimed to investigate (1) TL in physiologically relevant tissues of lactating dairy cows; namely AT, liver, mammary gland, and peripheral blood cells, and (2) changes of TL

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between early and late lactation. Moreover, we tested the use of TL in peripheral blood cells as a potential surrogate marker for TL in tissues avoiding biopsies in dairy cows.

The animal trial was conducted at the experimental station Frankenforst of the Faculty of Agriculture, University of Bonn, Königswinter, Germany. German Holstein cows (n = 21; age: 2 to 6 yr) were fed diets according to the recommendations of the Society of Nutrition Physiology in Germany (GfE, 2001) with a partial mixed ration (6.3 to 6.8 MJ of NE_L/kg of DM) offered for ad libitum intake and concentrate (7.7 MJ of NE_L/kg of DM) depending on the individual's milk yield. Animals were housed in a freestall barn with an adjacent milking parlor and were milked twice per day. The net EB was calculated by subtracting the daily requirement for maintenance (GfE, 2001) and the daily requirement for milk production (Tyrrell and Reid, 1965) from the daily energy intake with fixed values for fat and protein content (4 and 3.4%, respectively). Body weight (kg), feed intake (kg), and milk yield (kg) were recorded daily, and BCS (according to the 5-scale system by Edmonson et al., 1989) were monitored monthly and on the day of the biopsy.

Biopsies from subcutaneous (**sc**) AT of the tailhead region, from liver, and from the mammary gland were taken in early (21 to 28 DIM) and late (245 to 252 DIM) lactation. All biopsy samples were rinsed in saline, immediately snap frozen in liquid nitrogen, and stored at −80°C until further analyses. Blood samples were collected from the jugular vein before the biopsies and, after centrifugation (3,000 × *g*, 15 min, 4°C), serum and heparin-plasma were stored at −80°C, respectively. The DNA was isolated from whole heparin-blood. Concentrations (mmol/L) of BHB and nonesterified fatty acids were determined in serum by an automatic clinical chemistry analyzer (Eurolyser CCA180, Eurolab, Hallein, Austria).

The relative quantities of telomere products (**qT**), which strongly correlate with actual TL, were assessed after DNA isolation by a multiplex quantitative (**q**) PCR (Cawthon, 2009). Total genomic DNA from scAT biopsies was extracted using the PowerPlant Pro DNA Isolation Kit (MoBio, Carlsbad, CA), and DNA from whole blood, liver, and mammary gland tissue biopsies was isolated with the Wizard Genomic DNA Purification Kit (Promega, Mannheim, Germany). The concentration and purity of total DNA were assessed on a Nanodrop 1000 device (peQLab Biotechnology, Erlangen, Germany) at 260 and 280 nm. Gel electrophoresis was used to evaluate the integrity of the DNA. Total DNA (10 ng/μL) was mixed with 2 sets of primers: one amplified telomeres, whereas the other set was specific to the bovine β-globin gene, a housekeeping gene that operates as the nuclear control gene for determining the relative qT. Primer sequences and PCR conditions were performed as described previously, including minor modifications of the thermal conditions (Brown et al., 2012). In brief, the specificity of both primers was tested by gel electrophoresis. For multiplex qPCR, 10 μL of Dynamo SYBR Green (Thermo Scientific, Rockford, IL), 0.12 μL of ROX as passive reference dye (Thermo Scientific), both primers (1 μL each), and nuclease-free water (final volume of 20 μL) were mixed. All samples were run in triplicate, a DNA standard curve was used to estimate PCR efficiency for each qPCR run, and a pooled DNA sample served as inter-run calibrator. The analysis of the relative quantity of the telomeres to β-globin was calculated as follows: qT = PCR efficiency $(E)^n$, with $n = Ct_{\beta Globin} - Ct_{Telomere}$, and where Ct = cycle threshold.

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL). Data for all variables were tested for normal distribution using the Kolmogorov-Smirnov test and for homogeneity of variances by the Levene test. Data from early and late lactation were compared by using the pairwise Student's *t*-test and differences between tissues were analyzed using one-way ANOVA with Bonferroni post hoc test. All values are expressed as means ± standard errors of the mean. Correlations were assessed by Pearson analysis. Results with a *P*-value ≤0.05 were considered significant and $0.05 < P \leq 0.1$ was set as a trend.

Net EB, milk yield, BW, BCS, and concentrations of nonesterified fatty acids and BHB in early and late lactation are given in Supplemental Table S1 (http://dx.doi.org/10.3168/jds.2015-10095). The relative qT in scAT, liver, mammary gland, and peripheral blood from early and late lactation are presented in Figure 1. Relative qT decreased by 28% ($P = 0.012$) in peripheral blood from early to late lactation. Similar changes of TL in blood cells within a period of 6 mo were observed in overweight humans (Svenson et al., 2011). Given that blood cells are fast-replicating cells, determination of telomerase activity might be of particular interest, because tissues that renew throughout life may require consistent regulation by telomerase (Wang et al., 2005).

High milk yield during early lactation arises from increased activity of individual cells rather than from cell proliferation (Capuco et al., 2001). In the present study, relative qT in the mammary gland decreased by 16% ($P = 0.02$) from early to late lactation and was positively correlated with milk yield over the total lactation (r = 0.421; $P = 0.01$) when averaging both time points. However, the relative qT in the mammary gland was not related to milk yield when considering separate time points (early lactation: r = 0.358; $P = 0.144$; late lactation: r = 0.318; $P = 0.198$) nor to differences in