INTRODUCTION

In the United States, the prevalence of obesity is increasing (Hales, Carroll, Fryar, & Ogden, 2017) alongside the number of elderly individuals (He, Goodking, & Kowal, 2016). Both obesity and aging are independent risk factors for many chronic diseases, including type 2 diabetes (Barnes, 2011; Lindstrom & Tuomilehto, 2003), cardiovascular disease (Dhingra & Vasan, 2012; Sowers, 2003), and some forms of cancer (De Pergola & Silvestris, 2013; U.S. Cancer Statistics...
Working Group, 2013). Therefore, it is probable that similar physiological processes are central to the increased risk of disease found with aging and obesity. However, many questions about how aging and obesity lead to disease remain poorly resolved. In addition to physical inactivity and other lifestyle factors, a high-fat diet (HFD) is associated with obesity (Golay & Bobbioni, 1997). Obesity and aging are characterized by disruption of adipose tissue homeostasis (Goossens, 2008; Kirkland, Tchkonia, Pirtskhalava, Han, & Karagiannides, 2002); accordingly, understanding how obesity and advanced age influence adipose tissue may delineate mechanisms responsible for the increased risk of disease associated with obesity and advancing age.

Adipose tissue is a highly active organ involved in various physiological processes including energy storage, metabolism, and the secretion of hormones and signaling molecules (Frayn, 2002; Goossens, 2008). It has been established that dysfunctional adipose tissue is involved in the inflammatory response, (Trayhurn & Wood, 2004) the development of insulin resistance (Goossens, 2008), and both aging and obesity can disrupt normal adipose tissue function (Goossens, 2008; Kirkland et al., 2002). Disruption of adipose tissue homeostasis may be caused, in part, by an increase in oxidative stress (Zhang et al., 2011). According to the free-radical theory of aging, oxidative stress increases with age (Harman, 1992) leading to damage to DNA, lipids, and proteins – changes which affect physiological function by promoting inflammation and altering cell cycle control (Liguori et al., 2018). Likewise, a HFD has been shown to increase oxidative stress (Du et al., 2012) and inflammation (Liguori et al., 2018). Adipose tissue dysfunction and the concomitant diseases may therefore be caused by structural and functional damage at the cellular and molecular level.

Telomeres, the end caps of eukaryotic chromosomes, are made of hexanucleotide TTAGGG tandem repeats that are associated with a protein complex known as shelterin. Shelterin proteins are involved in structural formation and protection of telomeres, and in conjunction with the ribonucleoprotein reverse transcriptase known as telomerase, modulate telomere length (de Lange, 2005). Shelterin proteins regulate the structure of telomeric DNA, forming what is known as a t-loop to cap telomeres (de Lange, 2009). Telomere capping prevents recognition of chromosome ends as damaged DNA, thereby preventing the initiation of DNA damage response pathways that lead to cellular senescence or apoptosis (Takai, Smogorzewska, & de Lange, 2003). Dysfunctional telomeres have been linked to advancing age (Morgan et al., 2013), and a variety of diseases (Kong, Lee, & Wang, 2013) including cardiovascular disease (Liu, Bloom, & Donato, 2019; Morgan et al., 2014) and cancer (Shammas, 2011). Thus, it is important to understand telomere dynamics (e.g., telomere uncapping or altered shelterin complex) and the effects of various lifestyle factors, which may occur independent of telomere length (Morgan et al., 2013; Walker et al., 2016). The shelterin complex is comprised of protection of telomeres 1 (POT1) in humans, and its two paralogs, POT1a and POT1b in mice, which function to prevent the damage response along with telomere-repeat binding factor 1 (TRF1) and 2 (TRF2) (Hockemeyer, Daniels, Takai, & de Lange, 2006; de Lange, 2009; Sfeir et al., 2009). Despite these telomere maintenance and damage prevention mechanisms, it is known that oxidative stress can damage telomeres (Wang et al., 2010; von Zglinicki, 2002), and that inflammation is closely associated with this process (Zhang et al., 2016).

Both advancing age and obesity have been shown to increase oxidative stress (Liguori et al., 2018; Marseglia et al., 2014) and inflammation (Ellulu, Patimah, Khaza’ai, Rahmat, & Abed, 2017; Franceschi & Campisi, 2014), which may cause telomere dysfunction (Wang et al., 2010; Zhang et al., 2016) and cellular senescence (Liu et al., 2019). Furthermore, DNA damage signaling disrupts adipose tissue homeostasis and systemic metabolism (Vergoni et al., 2016), and telomeres are likely targets of DNA damage that can cause cellular senescence (Liu et al., 2019). Therefore, it is logical to examine the effects HFD induced obesity and age on regulators of telomere homeostasis. Additionally, it is important to examine markers of inflammation and cellular senescence to determine if these processes are accompanied by changes in genes that regulate telomere dynamics in adipose tissue. Investigating the relationship between HFD induced obesity, aging and telomere dynamics could help to determine if a HFD accelerates biological aging of adipose tissue. The impact of HFD induced obesity and aging on shelterin proteins that regulate and protect telomeric DNA have yet to be examined in adipose tissue. We hypothesized that both a HFD and aging would induce changes in shelterin protein gene expression indicative of telomere dysfunction, in conjunction with increased expression of pro-inflammatory cytokines and markers of cellular senescence.

2 | METHODS

2.1 | Ethical approval

All animal studies guidelines set forth by the National Research Council’s Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, 2011) were followed, and experimental protocols were approved by the Skidmore College Institutional Animal Care and use Committee (Protocol #123, approved 02/17/2014). The procedures were conducted in accordance with recent guidance provided by the journal (Grundy, 2015).
2.2 | Animals and diet

15 male C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME). Young (YG) mice were randomly assigned to be fed either a high-fat diet (YG + HFD; n = 5; 60% kcal from fat) or a low-fat diet (YG + LFD; n = 4; 10% kcal from fat) for 3 months (Test Diets). A subset of mice were fed a low-fat diet (n = 6; 10% kcal from fat) and aged until 16 months (Test Diets). Animals were housed at the Skidmore College animal facility on a 12:12 light:dark cycle and had access to food and water ad libitum.

2.3 | Tissue harvest

Mice were anesthetized with a 1:1:1 mixture of promace, ketamine hydrochloride, and xylazine by intraperitoneal injection (1.5 ml/Kg). After the removal of EWAT, unconscious mice were euthanized by cervical dislocation. Dissected EWAT was placed in liquid nitrogen, and then stored at −80°C until analysis.

2.4 | RNA extraction and gene expression quantification

RNA was extracted from EWAT using an RNA extraction kit specific to lipid rich tissues (QIAGEN). Total nucleic acid content was quantified using a spectrophotometer (NanoDrop, Thermo Scientific), and values were used to obtain 1 ug of RNA. RNA was reverse transcribed into cDNA using an Ambion RETROscript Kit (Austin, TX). Quantitative polymerase chain reaction (qPCR) was performed using TaqMan Gene Expression Assays and a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, Ca). The delta-delta cycle threshold (ΔΔCT) method was used to determine relative quantification.

2.5 | Statistical analysis

A one-way analysis of variance (ANOVA) was used to detect statistical differences between the YG + LFD, YG + HFD, and aged groups respectively. A Tukey HSD post-hoc analysis was used to locate the significance. Values that were two standard deviations away from the mean were considered as outliers and were not included in analysis. To assess the correlation between body weight, EWAT, and mRNA expression, a bivariate correlation analysis was performed. Data are presented as mean ± SD, and the level of statistical significance was set at p < .05.

3 | RESULTS

3.1 | Body weight and adipose tissue mass

To determine if a high-fat diet or aging induce obesity, body weight, and EWAT weight were assessed at the end of our study. As expected, YG mice fed a high-fat diet (YG + HFD) were significantly heavier than aged (p = .011) and young mice fed a low-fat diet (YG + LFD, p = .000) while aged mice weighed more than YG + LFD (Figure 1a; p = .000). YG + HFD mice had significantly greater absolute and relative (to body mass) EWAT weight compared to YG + LFD mice (Figure 1b and c; p = .000), and aged mice (p = .001), while aged mice had significantly higher absolute and relative EWAT weight compared to YG + LFD (Figure 1b and c; p = .015).

3.2 | Adipose tissue shelterin complex and telomerase-related gene expression

To investigate the effects of a high-fat diet and aging on telomere dynamics, we assessed gene expression of shelterin complex proteins that regulate telomere replication, protection, and length. There were no differences in adipose tissue mRNA expression of Terf1 between groups (Figure 2a; p = .233). Aged mice displayed a trend for more Terf2 mRNA expression than YG + LFD mice (Figure 2b; p = .129). There were no significant differences between groups for mRNA expression of POT1a (Figure 3a; p = .668). Aged mice had significantly greater mRNA expression of POT1b compared to YG + HFD (Figure 3b; p = .049) and a trend for greater expression compared to YG + LFD mice (p = .181). mTert, the gene that codes for the catalytic subunit of the enzyme telomerase, was significantly higher in aged compared to the YG + HFD mice (Figure 2c; p = .009). To determine if these changes in adipose tissue mRNA expression were associated with changes in body weight or EWAT weight, we performed a bivariate correlation analysis. mTert expression tended to be correlated with EWAT weight (r = −.502, p = .056).

3.3 | Adipose tissue inflammation and senescence

Next, we evaluated the effects of a HFD and age on markers of the inflammatory response. Specifically, we looked at gene expression of monocyte chemoattractant protein 1 (Mcp1) and tumor necrosis factor α (Tnfα). Adipose tissue mcpl mRNA expression significantly elevated in the YG + HFD group compared to the YG + LFD (Figure 4b; p = .002) and aged groups (p = .013). There was a trend for elevated Tnfα in YG + HFD and aged mice versus YG + LFD mice.
We also assessed gene expression of the tumor suppressor protein \( p53 \), which is involved in the DNA damage response and cellular senescence. There was no significant difference between groups for \( p53 \) mRNA expression (Figure 4c; \( p = .369 \)). To determine if these changes in adipose tissue mRNA expression were associated with changes in body weight or EWAT weight, we performed a bivariate correlation analysis. \( Tnf\alpha \) mRNA expression was

(Figure 4a; \( p = .113 \)).
significantly correlated to body weight ($r = .524, p = .045$) and Mcp1 mRNA expression was significantly correlated to body weight ($r = .683, p = .005$) and eWAT weight ($r = .861, p = .000$).

4 | DISCUSSION

The present study was the first to demonstrate the effects of HFD induced obesity and aging on the telomere shelterin complex and telomerase gene expression in mouse adipose tissue. We demonstrate that adipose tissue gene expression of the shelterin protein Terf2 tends to be elevated in adipose tissue of aged and HFD fed mice. Aged mice had significantly greater mRNA expression of POT1b compared to YG + HFD. Furthermore, HFD induced obesity decreased mTert expression, while age tended to increase its expression, changes which were correlated with body weight and tended to be correlated to EWAT weight. Mcp1, a chemokine that regulates immune cell infiltration and a marker of inflammation, was elevated in HFD mice, and its expression was correlated with body weight and EWAT weight. Therefore,
both HFD induced obesity and age significantly influence expression of genes involved in the regulation of telomere dynamics in adipose tissue, changes which may ultimately result in increased inflammation.

### 4.1 Shelterin regulation of telomeres

In the present study, it was demonstrated that aging tends to increase Terf2 gene expression. Changes in Terf2 expression may represent the deleterious effects of age and obesity on shelterin regulation of telomeres in adipose tissue, or demonstrate a compensatory mechanism, as TRF2 plays a role in double strand break repair (Bradshaw, Stavropoulos, & Meyn, 2005; Mao, Seluanov, Jiang, & Gorbunova, 2007). TRF2 is essential for maintenance of the telomere t-loop structure that prevents recognition of telomeric DNA by DNA repair machinery which would lead to chromosome fusions and senescence (van Steensel, Smogorzewska, & de Lange, 1998). Thus, in mice, genetic reduction of Terf2 results in an accelerated aging phenotype in mouse arteries (Morgan et al., 2019) and a rapid DNA damage response and cell death in skin (Martínez, Ferrara-Romeo, Flores, & Blasco, 2014). Contrary to this, Terf2 overexpression leads to an accelerated aging phenotype and cancer in mice, and is increased in some types of human cancer (Matsutani et al., 2001; Munoz, Blanco, Flores, & Blasco, 2005; Oh, Kim, Park, & Park, 2005). These findings indicate that regulation of Terf2 is critical for maintaining cell and tissue homeostasis. Accordingly, the findings of the present study may demonstrate that aging disrupts the regulation of Terf2 in adipose tissue. The consequences of these changes are unknown and warrant further investigation.

POT1, and its paralogs POT1a and POT1b in mice, serve to protect and regulate telomeric DNA (Hockemeyer et al., 2006). Previous research has demonstrated lifestyle interventions, such as exercise, induce tissue specific alterations in promoting gene expression of Pot1a and Pot1b (Ludlow, Gratidao, Ludlow, Spangenburg, & Roth, 2017; Ludlow et al., 2012). Furthermore, genetic reduction of Pot1b in mice results in reduced body size and cell death in highly mitotic tissues (He et al., 2009). Pot1b regulates the length of the single stranded and, in conjunction with Pot1a, contributes to preventing DNA damage response activation (Hockemeyer et al., 2006). In the present study, adipose tissue Pot1b gene expression increased in aged mice compared to YG + LFD and YG + HFD (Figure 3b). These findings suggest that advancing age, but not body weight or EWAT weight per se, lead to increased Pot1b expression and supports the notion that Pot1a and Pot1b are likely regulated in a tissue specific manner. However, the age associated increase in expression Pot1b could be compensatory in nature, or specific to the experimental model employed.

### 4.2 HFD induced obesity, aging and mTert

Obesity is associated with increased expansion of visceral adipose tissue depots (Sun, Kusminski, & Scherer, 2011). In humans, most somatic cells do not express appreciable levels of TERT, (Cong, Wright, & Shay, 2002) the catalytic subunit of the enzyme telomerase that elongates telomeres. However, Tert mRNA expression has been associated with tumorigenesis, and significant telomerase activity is found in a vast majority of human tumor cells (Cong et al., 2002), highlighting a role for mTert in highly mitotic tissues. Contrary to this, adult mice are thought to maintain telomerase expression in somatic tissues (Prowse & Greider, 1995). Interestingly, in the present study aged mice displayed significant upregulation of mTert compared to young mice fed a HFD and a trend for increased mTert compared to YG + LFD mice (Figure 2). While mTert gene expression is not necessarily indicative of telomerase activity, the finding that mTert regulation differs between aging and HFD induced obesity warrants further investigation into the regulation of telomerase in mice and might have implications for more effective use of mice as a model to explore the dynamics of telomerase more typical of humans.

### 4.3 HFD induced obesity, aging, and adipose tissue inflammation

HFD induced obesity and aging are associated with adipose tissue dysfunction, including an increase in expression of pro-inflammatory cytokines which contributes to the pathogenesis of insulin resistance (Goossens, 2008; Mau & Yung, 2018). In the present study, we evaluated gene expression in epididymal adipose tissue. Recent evidence suggests that diet induced obesity results in the greatest senescence burden in this adipose tissue depot when compared to other tissue types and adipose depots (Palmer et al., 2019). Furthermore, advancing age results in significant senescent cell accumulation in epididymal adipose (Yousefzadeh et al., 2020). Accordingly, it is logical to investigate the dynamics of shelterin complex gene expression, as the shelterin complex plays a key role in preventing cellular senescence and the associated inflammatory phenotype (Liu et al., 2019). Furthermore, mice with genetically reduced telomerase expression, which develop critically short telomeres after several generations, display elevated adipose tissue senescence burden accompanied by increased gene expression of Mcp1 and Tnfa (Minamino et al., 2009). The present study builds upon these findings by demonstrating that diet induced obesity and advancing age results in altered expression of the genes that regulate telomere dynamics and telomerase, changes accompanied by increased Mcp1 and Tnfa expression. In adipose tissue, Mcp1 expression increases with high-fat diet induced obesity.
(Kanda et al., 2006) and aging (Lumeng et al., 2011), and Mcp1 overexpression leads to insulin resistance in mice (Kanda et al., 2006). Similarly, adipose tissue expression of Tnfα is elevated in obesity (Hotamisligil, Shargill, & Spiegelman, 1993) and aging (Lumeng et al., 2011), and inhibition of Tnfα improves insulin sensitivity. These data, alongside those of the present study, indicate that changes in the regulation of telomere associated genes occur in response to stimuli that cause inflammation. Telomere dysfunction is associated cellular senescence and a proinflammatory state, and thus the findings of the present study warrant further investigation into the relationship between telomere dynamics and inflammation in adipose tissue.

4.4 | Experimental considerations

The present study demonstrates that HFD induced obesity and age alter expression of genes in involved in the regulation of telomeres, namely, Terf2, Pot1b, and mTert. However, limitations to this study include that our study only examined shelterin components and telomerase at the mRNA level, rather than at the protein level. Therefore, it is important to recognize that post transcriptional or translational modifications to these gene transcripts may ultimately affect the levels of proteins that are involved in the regulation of telomeric DNA. However, previous research has demonstrated that reduction in shelterin gene expression is sufficient to induce DNA damage and enhance cancer formation (Hartmann et al., 2016). Work in humans has also documented that changes in telomere uncapping and subsequent activation of senescence pathways can occur independent of changes in telomere length (Morgan et al., 2013; Walker et al., 2016). Though, we acknowledge that measurements of telomere length and protein expression would make our current study more comprehensive, we believe that the findings of our study are the first to demonstrate the effects of diet-induced obesity and aging on adipose tissue regulation of genes involved in telomere dynamics and thus provide novel insight as well as a detailed roadmap for future studies. One additional factor future studies might consider is the exploration of possible associations of altered telomere dynamics with glucose tolerance and insulin sensitivity, as three months HFD induces insulin resistance in C57BL/6j young mice (Liu et al., 2015) and we (Reynolds et al., 2019) and others (Oh et al., 2016) have documented that changes might begin at 16 months, but are more pronounced at 20 months of age.

5 | CONCLUSION

The main novel findings of the present study include that HFD induced obesity and aging alter adipose tissue regulation of telomeres. Furthermore, aged mice displayed upregulation of adipose tissue Terf2 and Pot1b. Finally, adipose tissue mTert mRNA expression was upregulated in aged mice. Concurrent to changes in genes that regulate telomere dynamics, HFD induced obesity and aging increase adipose tissue inflammation. These findings warrant further studies examining the regulation of shelterin and telomeres in adipose tissue, and the relationship between telomere dynamics and inflammation.

ACKNOWLEDGMENTS

This work was supported in part by the National Institutes of Health, National Institute of Aging (R15 AG053790-01) to THR and SJI and National Institutes of Health (T32 5T32HL139451-02) to SIB.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTIONS

S.I.B., S.J.I., and T.H.R. contributed to the conception and design of the work. S.I.B., A.T., and T.H.R. carried out the analysis and interpretation of data for the work. All authors contributed to drafting of the work and revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

ORCID

Samuel I. Bloom https://orcid.org/0000-0003-1440-8122

Thomas H. Reynolds https://orcid.org/0000-0002-0290-528X

REFERENCES

Barnes, A. S. (2011). The epidemic of obesity and diabetes: Trends and treatments. Texas Heart Institute Journal, 38(2), 142–144.

Bradshaw, P. S., Stavropoulos, D. J., & Meyn, M. S. (2005). Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. Nature Genetics, 37(2), 193–197. https://doi.org/10.1038/ng1506

Cong, Y. S., Wright, W. E., & Shay, J. W. (2002). Human telomerase and its regulation. Microbiology and Molecular Biology Reviews, 66(3), 407–425. https://doi.org/10.1128/MMBR.66.3.407-425.2002

de Lange, T. (2005). Shelterin: The protein complex that shapes and safeguards human telomeres. Genes & Development, 19(18), 2100–2110. https://doi.org/10.1101/gad.1346005

de Lange, T. (2009). How telomeres solve the end-protection problem. Science, 326(5955), 948–952. https://doi.org/10.1126/science.1170633
De Pergola, G., & Silvestris, F. (2013). Obesity as a major risk factor for cancer. *Journal of Obesity*, 2013, 291546. https://doi.org/10.1155/2013/291546

Dhingra, R., & Vasan, R. S. (2012). Age as a risk factor. *Medical Clinics of North America*, 96(1), 87–91. https://doi.org/10.1016/j.mcnca.2011.11.003

Du, Z., Yang, Y., Hu, Y., Sun, Y. U., Zhang, S., Peng, W., … Kong, W. (2012). A long-term high-fat diet increases oxidative stress, mitochondrial damage and apoptosis in the inner ear of D-galactose-induced aging rats. *Hearing Research*, 287(1–2), 15–24. https://doi.org/10.1016/j.heares.2012.04.012

Ellulu, M. S., Patimah, I., Khaza’ai, H., Rahmat, A., & Abed, Y. (2017). Obesity and inflammation: The linking mechanism and the complications. *Archives of Medical Science*, 13(4), 851–863. https://doi.org/10.5114/ams.2016.58928

Franceschi, C., & Campisi, J. (2014). Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *Journals of Gerontology Series A, Biological Sciences and Medical Sciences*, 69(Suppl 1), S4–S9. https://doi.org/10.1093/gerona/glu057

Frayn, K. N. (2002). Adipose tissue as a buffer for daily lipid flux. *Diabetologia*, 45(9), 1201–1210. https://doi.org/10.1007/s00123-002-0873-y

Golay, A., & Bobbioni, E. (1997). The role of dietary fat in obesity. *International Journal of Obesity and Related Metabolic Disorders*, 21(Suppl 3), S2–11.

Goossens, G. H. (2008). The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. *Physiology & Behavior*, 94(2), 206–218. https://doi.org/10.1016/j.physbeh.2007.10.010

Grundy, D. (2015). Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology. *Journal of Physiology*, 593(12), 2547–2549. https://doi.org/10.1111/jp270818

Hales, C. M., Carroll, M. D., Fryar, C. D., & Ogden, C. L. (2017). Prevalence of obesity among adults and youth: United States, 2015–2016. NCHS data brief, no 288. Hyattsville, MD: National Center for Health Statistics.

Harman, D. (1992). Free radical theory of aging. *Mutation Research*, 275(3–6), 257–266. https://doi.org/10.1016/0021-8374(92)90030-s

Hartmann, K., Illing, A., Leithäuser, F., Baisantry, A., Quintanilla-Martinez, L., & Rudolph, K. L. (2016). Gene dosage reductions of Trf1 and/or Tin2 induce telomere DNA damage and lymphoma formation in aging mice. *Leukemia*, 30(3), 749–753. https://doi.org/10.1038/leu.2015.173

He, H., Wang, Y., Guo, X., Ramchandani, S., Ma, J., Shen, M.-F., … Chang, S. (2009). Pot1b deletion and telomerase haploinsufficiency in mice initiate an ATR-dependent DNA damage response and elicit phenotypes resembling dyskeratosis congenita. *Molecular and Cellular Biology*, 29(1), 229–240. https://doi.org/10.1128/mcb.01400-08

He, W., Goodking, D., & Kowal, P. (2016). An aging world: 2015. Washington, DC: Government Publishing Office.

Hockemeyer, D., Daniels, J. P., Takai, H., & de Lange, T. (2006). Recent expansion of the telomeric complex in rodents: Two distinct POT1 proteins protect mouse telomeres. *Cell*, 126(1), 63–77. https://doi.org/10.1016/j.cell.2006.04.044

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science*, 259(5091), 87–91. https://doi.org/10.1126/science.7678183

Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K., Kitazawa, R., … Kasuga, M. (2006). MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *Journal of Clinical Investigation*, 116(6), 1494–1505. https://doi.org/10.1172/jci26498

Kirkland, J. L., Tchkonia, T., Pirtskhalava, T., Han, J., & Karagiannides, I. (2002). Adipogenesis and aging: Does aging make fat go MAD? *Experimental Gerontology*, 37(6), 757–767. https://doi.org/10.1016/s0531-5565(02)00014-1

Kong, C. M., Lee, X. W., & Wang, X. (2013). Telomere shortening in human diseases. *FEBS Journal*, 280(14), 3180–3193. https://doi.org/10.1111/febs.12326

Liguori, L., Russo, G., Cucito, F., Bulli, G., Aran, L., Della-Morte, D., … Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, 13, 757–772. https://doi.org/10.2147/cia.s158513

Lindstrom, J., & Tuomilehto, J. (2003). The diabetes risk score: A practical tool to predict type 2 diabetes risk. *Diabetes Care*, 26(3), 725–731. https://doi.org/10.2337/diacare.26.3.725

Liu, Y., Bloom, S. I., & Donato, A. J. (2019). The role of senescence, telomere dysfunction and shelterin in vascular aging. *Microcirculation*, 26(2), e12487. https://doi.org/10.1111/micc.12487

Liu, Z., Patil, I. Y., Jiang, T., Sancheti, H., Walsh, J. P., Stiles, B. L., … Cadenas, E. (2015). High-fat diet induces hepatic insulin resistance and impairment of synaptic plasticity. *PLoS ONE*, 10(5), e0128274. https://doi.org/10.1371/journal.pone.0128274

Ludlow, A. T., Gratidsao, L., Ludlow, L. W., Spangenberg, E. E., & Roth, S. M. (2017). Acute exercise activates p38 MAPK and increases the expression of telomere-protective genes in cardiac muscle. *Experimental Gerontology*, 102(4), 397–410. https://doi.org/10.1016/j.expger.2016.09.007

Ludlow, A. T., Witkowski, S., Marshall, M. R., Wang, J., Lima, L. C. J., Guth, L. M., … Roth, S. M. (2012). Chronic exercise modifies age-related telomere dynamics in a tissue-specific fashion. *Journals of Gerontology: Series A, Biological Sciences and Medical Sciences*, 67(9), 911–926. https://doi.org/10.1093/gerona/gls002

Lumeng, C. N., Liu, J., Geletka, L., Delaney, C., Delproposto, J., Desai, A., … Yung, R. L. (2011). Aging is associated with an increase in T cells and inflammatory macrophages in visceral adipose tissue. *The Journal of Immunology*, 187(12), 6208–6216. https://doi.org/10.4049/jimmunol.1102188

Mao, Z., Seluanov, A., Jiang, Y., & Gorbunova, V. (2007). TRF2 is required for repair of nontelomeric DNA double-strand breaks by homologous recombination. *Proceedings of the National Academy of Sciences*, 104(32), 13068–13073. https://doi.org/10.1073/pnas.0702410104

Marseglia, L., Manti, S., D’Angelo, G., Nicotera, A., Parisi, E., Di Rosa, G., … Arrigo, T. (2014). Oxidative stress in obesity: A critical component in human diseases. *International Journal of Molecular Sciences*, 15(1), 378–400. https://doi.org/10.3390/ijms15010378

Martinez, P., Ferrara-Romeo, I., Flores, J. M., & Blasco, M. A. (2014). Essential role for the TRF2 telomere protein in adult skin homeostasis. *Aging Cell*, 13(4), 656–668. https://doi.org/10.1111/acel.12221

Matsutani, N., Yokozaki, H., Tahara, E., Tahara, H., Kuniyasu, H., Haruma, K., … Tahara, E. (2001). Expression of telomeric repeat binding factor 1 and 2 and TRF1-interacting nuclear protein 2 in human gastric carcinomas. *International Journal of Oncology*, 19(3), 507–512. https://doi.org/10.3892/ijo.19.3.507

Mau, T., & Yung, R. (2018). Adipose tissue inflammation in aging. *Experimental Gerontology*, 105, 27–31. https://doi.org/10.1016/j.exger.2017.10.014
Minamino, T., Orimo, M., Shimizu, I., Kunieda, T., Yokoyama, M., Ito, T., ... Komuro, I. (2009). A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nature Medicine, 15*(9), 1082–1087. https://doi.org/10.1038/nm.2014

Morgan, R. G., Ives, S. J., Lesniewski, L. A., Cawthon, R. M., Andtbacka, R. H. I., Noyes, R. D., ... Donato, A. J. (2013). Age-related telomere uncapping is associated with cellular senescence and inflammation independent of telomere shortening in human arteries. *American Journal of Physiology-Heart and Circulatory Physiology, 305*(2), H251–H258. https://doi.org/10.1152/ajpheart.00197.2013

Morgan, R. G., Ives, S. J., Walker, A. E., Cawthon, R. M., Andtbacka, R. H. I., Noyes, D., ... Donato, A. J. (2014). Role of arterial telomere dysfunction in hypertension: Relative contributions of telomere shortening and telomere uncapping. *Journal of Hypertension, 32*(6), 1293–1299. https://doi.org/10.1097/HJH.0000000000000157

Morgan, R. G., Walker, A. E., Trott, D. W., Machin, D. R., Henson, G. D., Reihl, K. D., ... Donato, A. J. (2019). Induced Trf2 deletion leads to aging vascular phenotype in mice associated with arterial telomere uncapping, senescence signaling, and oxidative stress. *Journal of Molecular and Cellular Cardiology, 127*, 74–82. https://doi.org/10.1016/j.yjcc.2018.11.014

Munoz, P., Blanco, R., Flores, J. M., & Blasco, M. A. (2005). XPF nuclease-dependent telomere loss and increased DNA damage in mice overexpressing TRF2 result in premature aging and cancer. *Nature Genetics, 37*(10), 1063–1071. https://doi.org/10.1038/ng1633

Oh, B. K., Kim, Y. J., Park, C., & Park, Y. N. (2005). Up-regulation of telomere-binding proteins, TRF1, TRF2, and TIN2 is related to telomere shortening during human multistep hepatocarcinogenesis. *American Journal of Pathology, 166*(1), 73–80. https://doi.org/10.1016/j.ajpath.2004.10.033

Oh, Y. S., Geo, E. H., Lee, Y. S., Cho, S. C., Jung, H. S., Park, S. C., & Jun, H. S. (2016). Increase of calcium sensing receptor expression is related to compensatory insulin secretion during aging in mice. *PLoS ONE, 11*(7), e0159689. https://doi.org/10.1371/journal.pone.0159689

Palmer, A. K., Xu, M., Zhu, Y. I., Pirskkalava, T., Weivoda, M. M., Hachfeld, C. M., ... Kirkland, J. L. (2019). Targeting senescent cells alleviates obesity-induced metabolic dysfunction. *Aging Cell, 18*(3), e12950. https://doi.org/10.1111/acel.12950

Prowse, K. R., & Greider, C. W. (1995). Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proceedings of the National Academy of Sciences, 92*(11), 4818–4822. https://doi.org/10.1073/pnas.92.11.4818

Reynolds, T. H., Dalton, A., Calzini, L., Tulca, A., Hoyte, D., & Ives, S. J. (2019). The impact of age and sex on body composition and glucose sensitivity in C57BL/6J mice. *Physiological Reports, 7*(3), e13995. https://doi.org/10.14814/phy2.13995

Sfeir, A., Kosiartakul, S. T., Hockemeyer, D., MacRae, S. L., Karlsseder, J., Schildkraut, C. L., & de Lange, T. (2009). Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. *Cell, 138*(1), 90–103. https://doi.org/10.1016/j.cell.2009.06.021

Shammas, M. A. (2011). Telomeres, lifestyle, cancer, and aging. *Current Opinion in Clinical Nutrition and Metabolic Care, 14*(1), 28–34. https://doi.org/10.1097/MCO.0b013e32834121b1

Sowers, J. R. (2003). Obesity as a cardiovascular risk factor. *American Journal of Medicine, 115*(Suppl 8A), 37s–41s. https://doi.org/10.1016/j.amjmed.2003.08.012

Sun, K., Kusminski, C. M., & Scherer, P. E. (2011). Adipose tissue remodeling and obesity. *Journal of Clinical Investigation, 121*(6), 2094–2101. https://doi.org/10.1172/jci45887

Takai, H., Smogorzewska, A., & de Lange, T. (2003). DNA damage foci at dysfunctional telomeres. *Current Biology, 13*(17), 1549–1556. https://doi.org/10.1016/s0960-9822(03)00542-6

Trayhurn, P., & Wood, I. S. (2004). Adipokines: Inflammation and the pleiotropic role of white adipose tissue. *British Journal of Nutrition, 92*(3), 347–355. https://doi.org/10.1079/bjn20041213

U.S. Cancer Statistics Working Group. (2013). *United States Cancer Statistics: 1999–2009 incidence and mortality web-based report*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute. Retrieved from www.cdc.gov/uscs

van der Heijden, R. A., Bijzet, J., Meijers, W. C., Yakala, G. K., Kleemann, R., Nguyen, T. Q., ... Heeringa, P. (2015). Obesity-induced chronic inflammation in high fat diet challenged C57BL/6J mice is associated with acceleration of age-dependent renal amyloidosis. *Scientific Reports, 5*, 16474. https://doi.org/10.1038/srep16474

van Steensel, B., Smogorzewska, A., & de Lange, T. (1998). TRF2 protects human telomeres from end-to-end fusions. *Cell, 92*(3), 401–413. https://doi.org/10.1016/s0092-8674(00)80932-0

Vergoni, B., Cornejo, P.-J., Gilleron, J., Dzedaini, M., Ceppo, F., Jacquel, A., ... Cormont, M. (2016). DNA damage and the activation of the p53 pathway mediate alterations in metabolic and secretory functions of adipocytes. *Diabetes, 65*(10), 3062–3074. https://doi.org/10.2337/db16-0014

von Zglinicki, T. (2002). Oxidative stress shortens telomeres. *Trends in Biochemical Sciences, 27*(7), 339–344. https://doi.org/10.1016/s0968-0004(02)02110-2

Walker, A. E., Morgan, R. G., Ives, S. J., Cawthon, R. M., Andtbacka, R. H. I., Noyes, D., ... Donato, A. J. (2016). Age-related arterial telomere uncapping and senescence is greater in women compared with men. *Experimental Gerontology, 73*, 65–71. https://doi.org/10.1016/j.exger.2015.11.009

Wang, Z., Rhee, D. B., Lu, J., Bohr, C. T., Zhou, F., Vallabhaneni, H., ... Liu, Y. (2010). Characterization of oxidative guanine damage and repair in mammalian telomeres. *PLoS Genetics, 6*(5), e1000951. https://doi.org/10.1371/journal.pgen.1000951

Yousefradzadeh, M. J., Zhao, J., Bukata, C., Wade, E. A., McGowan, S. J., Angelini, L. A., ... Niedernhofer, L. J. (2020). Tissue specificity of senescent cell accumulation during physiologic and accelerated aging of mice. *Aging Cell, 19*(3), e13094. https://doi.org/10.1111/ace.13094

Zhang, J., Rane, G., Dai, X., Shanmugam, M. K., Arfuso, F., Samy, R. P., ... Sethi, G. (2016). Ageing and the telomere connection: An intimate relationship with inflammation. *Aging Research Reviews, 25*, 55–69. https://doi.org/10.1016/j.aggre.2015.11.006

Zhang, L. E., Ebenezer, P. J., Dasuri, K., Fernandez-Kim, S. O., Francis, B., Boettcher, C., ... Oh, B. K. (2011). Telomere shortening and telomere uncapping. *American Journal of Physiology-Endocrinology and Metabolism, 301*(4), E599–E607. https://doi.org/10.1152/ajpendo.00059.2011

**How to cite this article:** Bloom SI, Tuluca A, Ives SJ, Reynolds TH. High-fat diet induced obesity and age influence the telomere shelterin complex and telomerase gene expression in mouse adipose tissue. *Physiol Rep. 2020;8:e14461. https://doi.org/10.14814/phy2.14461*