ORIGINAL ARTICLE

Beneficial Primary Outcomes of Metabolic Surgery with Changes in Telomere Length and Mitochondrial DNA in Obese Asian Indians with Dysglycemia

Sundaramoorthy Chandru[1,2], Paramasivam Prabhu[3], Muthuswamy Balasubramanyam[1,4], Radhakrishnan Subhashini[1], Mangesh Tiwaskar[5], Thyparambil Aravindakshan Pramodkumar[1], Rajendra Pradeepa[1], Ranjit Mohan Anjana[1], Viswanathan Mohan[1*]

Abstract

Introduction: Although metabolic surgery has been shown to offer beneficial primary outcome results in obese individuals / obese Type 2 diabetes mellitus (T2DM) patients, there is paucity of information on the underlying mechanisms. In the recent years, estimations of non-invasive molecular parameters viz., telomere length and mtDNA copynumber (mtDNAcn) assume significance as robust biomarkers. However, there is lack ofevidence about this especially, in the Indian context. To assess the changes in the telomere length and mtDNAcn levels after metabolic surgery in obese Asian Indians withdysglycemia along with routine measurements of anthropometry, glycemic/lipidimic parameters and inflammatory markers.

Methods: This study is a prospective one-year follow-up study of 16 obese individuals with dysglycemia who underwent metabolic surgery at a tertiary diabetes centre in South India.Telomere length, mtDNAcn, serum adiponectin, glycated haemoglobin and high- sensitivity C-reactive protein (hs-CRP) levels were analysed before surgery and at 6 and12 months after surgery.

Results: There was a significant reduction in weight ($p<0.001$), BMI ($p<0.001$), waist circumference ($p<0.001$), fasting and postprandial glucose ($p<0.05$), HbA1c ($p<0.001$), triglycerides ($p<0.05$), hs CRP ($p<0.05$) and increase in serum adiponectin ($p<0.05$) at 6 and 12 months post-surgery compared to the preoperative status. There was a significant reduction in mtDNAcn ($p<0.001$) and a significant increase in telomere length ($p<0.001$) at 6 and 12 months post metabolic surgery.

Conclusion: We report an increase in telomere length and decrease in circulatory mtDNA copy number levels at 6 and 12 months post metabolic surgery in obese individuals with T2DM in India.

Introduction

Type 2 diabetes mellitus (T2DM) has a strong association with obesity and unhealthy lifestyle regardless of genetic predisposition.[1] Obesity is a complex multi- factorial disease that has genetic, behavioural, environmental and socio-economic origins leading to an increase in morbidity and mortality. Nitrogen compounds, free radicals and reactive oxygen species (ROS) are needed in low concentration for normal cell functioning, intracellular signalling and cell redox state. However there is an excess production of ROS and free radicals in certain disease conditions like T2DM and obesity. Higher concentration of ROS and free radicals can damage cellular proteins, lipids, carbohydrates, DNA and other cellular structures and impair cell function.[2]

Higher circulating levels of triglycerides in obesity and T2DM leads to excess infiltration and accumulation of triglycerides in the peripheral tissues and this excess in triglycerides is shown to induce mitochondrial (mt) dysfunction, increase oxidative stress and impair energy substrate metabolism as well as oxidative phosphorylation. Oxidative stress is known to cause cellular injury and release excess amount of mtDNA fragments into the circulation.[3] Higher circulating levels of plasma mtDNA fragments are associated with oxidative stress, mtDNA damage and in obese individuals with T2DM, it correlates with insulin resistance.[4]

Telomere length is determined by genetic factors but throughout human life it is also influenced by various non-genetic factors. Longest and shortest telomere length arepresent at birth and old age, respectively.[5] Aging is associated with a progressive shortening of telomere length and it varies with the type of the cell and mitotic tissue.[6] Telomere length was reported to shortened in both type 1 diabetes mellitus (T1DM) and T2DM as well as in obesity states.[7] Apart from diabetes and obesity, studies have also reported that telomere shortening is associated with impaired glucose tolerance, atherosclerosis, hypertension, dyslipidemia, diabetic kidney disease, insulin resistance and non-alcoholic fatty liver disease (NAFLD).[8]

Metabolic surgery is an effective strategy to reduce both obesity and

[1]Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialities Centre, Chennai, Tamil Nadu; [2]Ph.D. Scholar, University of Madras, Chennai, Tamil Nadu; [3]Department of Neurology, School of Medicine, University of New Mexico, USA; [4]SRM Medical College Hospital and Research Centre, SRM Institute of Science & Technology (SRMIST), Chennai, Tamil Nadu; [5]Shilpa Medical Research Centre, Mumbai, Maharashtra; *Corresponding Author
Received: 26.02.2021; Revised: 17.06.2021; Accepted: 06.07.2021

diabetes by altering or reversing various patho-physiological mechanisms. Studies have shown the effects of metabolic surgery in rescuing the cell from oxidative stress and delaying the progression of biological aging.[9] There is a lacuna of data among the obese Asian Indians with dysglycemia with regard to oxidative stress. The potential associations between telomere length and mitochondrial DNA copy number (mtDNAcn) with diabetes has been studied cross-sectionally.[10] Prospective studies in this field are scarce, with very few reporting that telomere length is increased after bariatric surgery compared to before.[11] The current study aimed to evaluate the benefits of metabolic surgery with a special emphasis on changes in telomere length and mtDNAcn, a year after surgery in obese Asian Indians with dysglycemia.

Methods Study Design

This is a prospective observational study of individuals with dysglycemia who underwent metabolic surgery between November 2013 and March 2019. A total of 36 individuals with pre diabetes or type 2 diabetes were recruited, who underwent metabolic surgery at a tertiary referral centre for diabetes in Chennai, South India. For purpose of this study sixteen (14 T2DM and 2 prediabetes, male 6 and female 10) individuals who agreed to take part in subset of studies and willing to give additional blood samples for analyzing various biochemical parameters were included in the present study. Written informed consent was obtained from all the study participants. All study procedures were conducted in accordance with the declaration of the Helsinki and approved by the EthicalCommittee of Madras Diabetes Research Foundation, Chennai India (MDRF/NCT/07- 02/2014).

All the individuals underwent pre-operative, multi-disciplinary evaluation by a bariatric surgeon, physician, anaesthetist, psychologist and dietician prior to surgery. Diabetes history, medications, risk factors and co morbidities associated with obesity were recorded.

Anthropometry and blood pressure measurements

Anthropometric measurements including weight, height, and waist circumference were measured using the standard techniques.[12] Height (in cm) was measured using a stadiometer (SECA Model 214, Seca GmbH Co, Hamburg, Germany). Weight (in kg) was measured with an electronic weighing scale (SECA Model 807, Seca GmbH Co, Hamburg, Germany) that was kept on a firm horizontal flat surface. Waist circumference was measured at the smallest horizontal girth between the costal margins and the iliac crest at the end of expiration. Blood pressure was recorded in the sitting position in the right arm to the nearest 1 mmHg using a mercury sphygmomanometer (Diamond Deluxe, Pune, Maharashtra, India). Two readings were taken 5 min apart and their mean was taken as the blood pressure.

Biochemical investigations

Biochemical analysis was done at our laboratory which is certified by the College of American Pathologist and the National Accreditation Board for Testing and Calibration Laboratories. A fasting venous blood sample was collected after an overnight fast of at least 10 hours for the estimation of fasting glucose and lipids and, after a standard South Indian breakfast, a 2 hour postprandial sample was obtained for postprandial plasma glucose estimation. Plasma glucose levels were analysed by the hexokinase method, serum cholesterol by cholesterol oxidase peroxidase amidopyrine method, serum triglyceride by the glycerolphosphate oxidase-peroxidase-amidopyrine method, high density lipoprotein(HDL) cholesterol by direct method- immunoinhibition method, hs-CRP by immunoturbidometry measured using Beckman Coulter AU680 (Fullerton, CA, USA) and Beckman kits. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[13] HbA1c was measured by high-performance liquid chromatography using the Variant II Turbo (Bio-Rad, Hercules, CA, USA). The intra and inter-assay coefficients of variation for the biochemical assays ranged between <3.1 and7.6%.

Adiponectin measurements

Adiponectin was measured by quantitative sandwich enzyme linked-immune sorbent assay technique (ELISA) (Adiponectin: Cusabio: Houston, USA). In brief, a monoclonal antibody specific to Adiponectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Adiponectin present is bound by the immobilized antibody. After removing any unbound substances, a biotin- conjugated antibody specific for Adiponectin is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of Adiponectin bound in the initial step. The colour development is stopped and the intensity of the color is measured at 450 nm.The values are expressed in (ng/ml). The intra- and inter-assay coefficients of variation were <8% and <10%, respectively.

Surgical technique

Of the 16 individuals, 9 underwent Restrictive procedure-Laparoscopic sleeve gastrectomy (SG) and 7 underwent Gastro intestinal diversionary procedures- Laparoscopic Rouxen-Y gastric bypass (RYGB).[14] The type of metabolic surgery was decided by the bariatric surgeon depending upon the duration of diabetes and other comorbid conditions of the patient. DNA was quantified using Nanodrop One (Thermo Scientific) and was stored at -20°c.

Human serum DNA isolation

Serum DNA was isolated using commercially available serum/plasma DNA isolation kit (#ab15893; abcam). Briefly, 500µL of serum sample was added into mixture 20 µL digestion and 500µL of DNA isolation buffer. The resultant cocktail was mixed properly and incubated at 65^0c for 10 minutes. After that, 500 µL of above mixture was added into column tube and centrifuged at 12000 rpm for 30 seconds and discarded flow through. Then F-spin column tube was washed with 70% and 90 % ethanol by centrifuged at 12000rpm for 20 seconds respectively. Finally, DNA was eluted by adding 20 µL DNA elution solution into F-spin column tube and centrifuged at 12000 rpm for 30 seconds. DNA wasquantified Nanodrop One (Thermo Scientific) and DNA was stored at -200c.

Telomere length measurement

Relative telomere length was determined by Real-time PCR approach as previously described by[10] with minor modifications.[15] This method measures

Table1: Clinical and biochemical characteristics of individuals with type 2diabetes (n=16)

Variables	Pre-operative	Post-operative	
		6 months	12 months
Age at metabolic Surgery	45 ±12	-NA-	-NA-
Duration of Diabetes (years)	8.3 ± 5.9	-NA-	-NA-
Male (%)	37.5	-NA-	-NA-
Weight (kgs)	107.9 ± 19.5	83.1±14.9 *	76.3±16.5 *
BMI (kg/m²)	41.7 ± 6.5	32.3 ± 4.5*	30.1 ± 4.7*
Waist circumference(cm)	126.1 ± 13.1	107.4 ± 11.5*	103.0 ± 10.4*
Excess body weight loss (%)	-	23.4 ± 7.9	29.7 ± 14.8 **
Systolic blood pressure (mmHg)	134 ± 16	115 ± 15 *	115 ± 15 *
Diastolic blood pressure (mmHg)	83 ± 11	72 ± 9 *	75 ± 9 **
Fasting blood sugar (mg/dl)	180.2 ± 60.4	107.6 ± 22.1*	103.3 ± 26.4*
Postprandial blood sugar (mg/dl)	249.2 ± 95.0	124.5 ± 37.9*	111.8 ± 51.5*
HbA1c (%)	8. 9 ± 2.0	6.3 ± 1.0*	6.2 ± 0.9*
Serum cholesterol (mg/dl)	161 ± 29	158 ± 34	157 ± 32 **
Serum triglyceride (mg/dl)	181± 85	117 ± 47 **	120 ± 64**
HDL-Cholesterol (mg/dl)	37 ± 7	43 ± 9**	46 ± 12 **
LDL-Cholesterol (mg/dl)	87 ± 27	91 ± 33	86 ± 24
hs-CRP (mg/l)	8.3±1	3.1 ± 0.9**	1.3±0.6*
Adiponectin (ng/ml)	24±12	44±28**	67±34*
White blood cells (10^3/µL)	7669±1633	7570 ±1857	6172±1127**
Hemoglobin (g/dL)	12.9 ± 1.7	12.9 ± 1.7	12.7 ± 2.0

**<0.05 -compared to pre-operative; *<0.001-compared to pre-operative; *HDL- HighDensity Lipoprotein; LDL-Low Density Lipoprotein; VLDL - Very Low Density Lipoprotein*

the factor by which the ratio of telomere repeat copy number to single - gene copy number differs between asample and that of a reference DNA sample. PCR amplification was achieved using telomere (T) and single copy gene, 36B4 (encodes acidic ribosomal phosphoprotein) primers (S) which serves as a quantitative control. The mean telomere repeat gene sequence (T) to a reference single copy gene (S) was represented as T/S ratio which was calculated to determine the relative telomere length.

Quantification mitochondrial DNA (mt DNA) copy number: Human mitochondrial DNA (mt DNA) monitoring primer Set (Takara Bio Inc, Kusatsu, Shiga, Japan) was purchased from Takara and the experiment was carried out as per manufacture's instruction. In brief, 20 ng of DNA and Nuclear DNA primers (SLCO2B1 and SERPINA1) and mitochondrial primers (ND1 -NADH dehydrogenase subunit 1- and ND5) were added to SYBR green mater mix (Clontech Laboratories, Mountain View, USA) and Quantitative PCR was performed on the Roche Light Cycler 96 (Roche GmbH, Mannheim, Germany). Relative quantification of mitochondrial DNA was determined using the 'ct' values for mtDNA and nDNA and data was expressed as mean mtDNAcn.

Statistical analysis

Quantitative variables were described with means and standard deviations (SD). Paired *t*-test as appropriate were used to compare groups for continuous variables and the Chi- square test or Fisher's exact test as appropriate was used to compare proportions. We used normalized log-transformed values for the correlation table. All analyses were done using the Windows-based SPSS statistical package (version 22.0, SPSS Inc, Chicago, IL) and p<0.05 was considered statistically significant.

Results

Table 1 illustrates the clinical and biochemical characteristics of the study individuals involved in the study. Metabolic surgery resulted in reduced body weight (p<0.001), BMI (p<0.001), waist circumference (p<0.05), systolic and diastolic blood pressure(p<0.001) in 6 and 12 months postoperative follow-up compared to the preoperative status. Improved glycemic variables included FPG (p<0.001), PPG (p<0.001) and HbA1c (p<0.001), total cholesterol (p<0.001) and triglyceride (p<0.05), VLDL (p<0.05); increased HDL cholesterol (p<0.001) in 6 and 12 months postoperative follow-up compared to preoperative. Interestingly, inflammatory marker hsCRP (p<0.05) was significantly reduced in 6 and 12 months postoperative follow-up compared to preoperative postoperative follow-up. Adiponectin

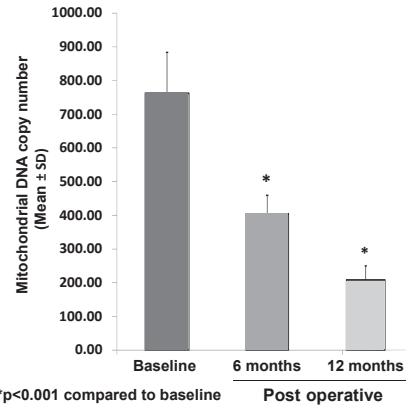

Fig. 1: Mitochondrial DNA (mt DNA) copy number in obese Asian Indians withdysglycemia prior to surgery, 6 and 12 months post-surgery

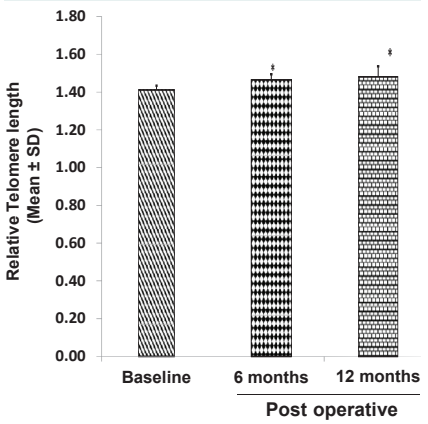

Fig. 2: Telomere length in obese Asian Indians with dysglycemia prior to surgery, 6 and 12 months post-surgery

was significantly increased post surgery (p<0.05).

Figure 1 shows that mtDNAcn (762± 122 vs. 404 ± 55 vs. 208 ± 42, p<0.001) was significantly decreased at 6 months and this improvement persisted 12 months postoperatively compared to baseline. **Figure 2**. Shows data on relative telomere length (1.41±0.02 vs. 1.46 ± 0.03 vs. 1.48 ± 0.06 p<0.001) was significantly increased at 6 months and persisted 12 months postoperatively compared to baseline. This increase in Telomere length was suggestive of a decrease in oxidative stress and inflammation levels.

Pearson correlation analysis was performed between telomere length as well as mtDNAcn with anthropometric and biochemical parameters. Telomere length was positively correlated with weight (r=0.571, p=0.010) and BMI

Table 2: Correlation of clinical and biochemical characteristics with pre-operative and 12-months change intelomere length and mitochondrial DNA in individuals with type 2 diabetes

Characteristics	Telomere length (n=16)				mtDNA-CN# (log transfer done) (n=16)			
	Correlation with baseline (r)	p value	Correlation with one year change (r)	p value	Correlation with baseline (r)	p value	Correlation with one year change (r)	p value
Age (years)	-0.356	0.088	-0.555	**0.013***	0.220	0.207	-0.364	0.083
Weight(kg)	0.571	**0.010***	-0.464	**0.035***	-0.334	0.096	-0.450	**0.040***
BMI (kg/m^2)	0.498	**0.025***	-0.330	0.106	-0.097	0.361	-0.405	0.060
Waist circumference (cm)	0.358	0.087	-0.413	0.056	-0.186	0.246	-0.688	**0.002***
Systolic blood pressure (mmHg)	0.165	0.271	-0.007	0.490	-0.334	0.103	0.008	0.488
Diastolic blood pressure (mmHg)	0.238	0.188	-0.047	0.431	-0.089	0.371	-0.590	**0.008***
HbA1c (%)	0.303	0.127	-0.455	**0.038***	0.375	0.076	0.010	0.486
Fasting plasma glucose (mg/dl)	0.247	0.178	-0.365	0.082	0.006	0.492	0.290	0.138
Postprandial plasma glucose (mg/dl)	0.250	0.175	-0.355	0.089	-0.242	0.184	-0.045	0.434
Hs C-Reactive Protein (mg/l)	0.187	0.253	-0.691	**0.002***	-0.238	0.196	-0.269	0.166
Adiponectin (ng/ml)	0.064	0.407	-0.371	0.078	-319	0.114	-0.220	0.207
Serum cholesterol (mg/dl)	0.050	0.430	-0.136	0.315	-0.331	0.114	0.028	0.460
Serum triglyceride (mg/dl)	-0.410	0.064	-0.293	0.145	0.091	0.373	-0.026	0.464
HDL-Cholesterol (mg/dl)	-0.221	0.214	0.219	0.217	0.028	0.461	0.042	0.440
LDL-Cholesterol (mg/dl)	0.374	0.085	-0.012	0.483	-0.428	0.056	0.041	0.442
White blood cells (10^3/μL)	-0.096	0.377	0.280	0.177	-0.319	0.144	0.284	0.174
Hemoglobin (g/dL)	0.226	0.228	.702	0.004	-0.322	0.141	0.182	0.276

*p for <0.05 and "r" Pearson's correlation

(r=0.498, p=0.025) at preoperative and negatively correlated with age (r=-0.555,p=0.013), weight (r=-0.464,p=0.035), HbA1c (r=-0.455,p=0.038) and hsCRP (r=-0.691,p=0.002) with one year change. mtDNAcn negatively correlated with weight (r=-0.450, p=0.040), waist circumference (r=-0.688,p=0.002), and diastolic blood pressure (r=-0.590,p=0.008) at 12 month postoperative follow-up **(Table 2)**.

Discussion

Although metabolic surgery has been shown to offer beneficial primary outcome results in obese individuals / obese T2DM patients in terms of weight loss as well as reductions in glycemic and lipidemic parameters, there is paucity of information on the underlying mechanisms. In this context, the present study assumes significance and reports the following findings: 1) There was a significant increase in telomere length and decrease in mtDNA levels at 6 and 12 months post-surgery. 2) Inflammatory marker hsCRP levels were significantly reduced while there was an increase in the adiponectin levels at 6 and 12 months after surgery. 3) Interestingly, age, weight, HbA1c and hsCRP levels were negatively correlated with telomere length at 12 months follow-up in those underwent metabolic surgery.

Unlike the routine inflammatory and oxidative stress markers that change with short-term lifestyle, the levels of telomere length and mtDNA represent robust, long-term biomarkers.We earlier reported an association of telomere shortening in Asian Indian patients with T2DM.[10] A recent meta-analysis showed a converse relationship between BMI and Telomere length in cross sectional studies.[16] Nathan et. al[17] reported an increase in telomere length following weight loss due to dietary modification and lengthening of telomere was shown associated with decrease in weight and fat mass. Interestingly, a recent Indian based GWAS study also revealed five genetic variants associated with telomere maintaining genes in T2DM.[18]

Obesity is associated with number of metabolic alterations including increased oxidative stress and chronic low-grade inflammation.[19] It is well conceived that adipose tissue promotes the process of aging and drives the development of chronic diseases, such as T2DM, non-alcoholic fatty liver disease (NAFLD), cancer, and cardiovascular diseases.[19] Indeed, a recent, large meta-analysis using cross-sectional data from 146,114 people reported an inverse relationship between BMI and telomere length.[20]

The increase in telomere length at 6 and 12 months post-metabolic surgery demonstrated in our study in obese-diabetic patients is a significant observation. Dersham et al[21] showed an increase in telomere length after 3-5 years of post-gastric surgery. Jongbloed et al[22] suggested that metabolic syndrome is a risk factor for accelerated aging of T cells and they have demonstrated an increase in the telomere length and decrease in T cell differentiation which was associated with percentage of body weight loss 6 months post bariatric surgery. Laimer et al[11] demonstrated an increase in telomere length after profound and sustained weight loss 10 years post bariatric surgery and this study emphasized that bariatric surgery ameliorates metabolic abnormalities after profound weight loss and these changes could overrule the influence of age and protect the DNA from the damage. Brando et al[23] also demonstrated an increase in telomere length following raise in fat free mass and decrease in waist circumference after 8 weeks of short term combined exercise.

In our study, metabolic surgery resulted in significant reduction in body weight, waist circumference, blood sugars, HbA1c and serum triglycerides. The post operative reduction in the availability of fuel (energy -glucose and TG) along with weight loss mighthave resulted in decrease in inflammatory markers like hsCRP and subsequent reductionin oxidative stress. There is a significant reduction in the circulating mtDNA copy number levels after metabolic surgery, which could reflect an underlying reduction of the mitochondrial oxidative stress levels.

While obesity has been shown to be associated with elevated urinary mtDNAcn levels, bariatric surgery has been demonstrated to reduce the levels of urinary mtDNAcn in obese individuals.[24] In our earlier study, we showed that metabolicsurgery resulted in significant improvement in beta cell function and insulin sensitivity along with reduction in anti-diabetes medications.[25]

Conclusion

To conclude, our study in India report an increase in telomere length and decrease in circulatory mtDNA copy number levels at 6 and 12 months post metabolic surgery in obese diabetic individuals. These robust biomarker alterations were more or less correlated to primary outcome benefits of metabolic surgery. One of the limitations of our study is the relatively small sample size as well as our estimations of telomere length andmtDNAcn levels which was done in serum samples. Future studies should focus on studying tissues — so as to demonstrate the underlying mechanisms of beneficial outcomes of metabolic surgery as well as to look at the clinical implications in the beneficial effect of telomere length and mtDNAcn as robust biomarkers.

Acknowledgements

This work is part of the PhD project of Dr. Sundaramoorthy Chandru undertaken at the University of Madras. We thank the participants for their cooperation.

Author Contributions

SC, PP, MB and VM, were involved in conception and design of this study. SC, PP, MB, RP, TAP, RMA, VM helped in the interpretation analysis and interpretation of data and revised all drafts of the article. VM and SC were involved in pre and post-operative assessment of individuals after metabolic surgery. RS were responsible for data analysis.All authors approved and read the manuscript.

Funding

This research did not receive any specific grant from funding agencies, in the public, commercial, or not-for-profit sectors.

References

1. Schnurr TM, Jakupović H, Carrasquilla GD, Ängquist L, Grarup N, Sørensen TIA, et al. Obesity, unfavourable lifestyle and genetic risk of type 2 diabetes: a case-cohort study. *Diabetologia* 2020; 63:1324-32.
2. Yu BP. Cellular defenses against damage from reactive oxygen species.*Physiol Rev* 1994; 74:139-62.
3. Yang S, Zhu H, Li Y, Lin H, Gabrielson K, Trush MA, et al. Mitochondrial adaptations to obesity-related oxidant stress. *Arch Biochem Biophys* 2000; 378:259-68.
4. Yuzefovych LV, Pastukh VM, Ruchko MV, Simmons JD, Richards WO, Rachek LI. Plasma mitochondrial DNA is elevated in obese type 2 diabetes mellitus patients and correlates positively with insulin resistance. *PLoS One* 2019 Oct 10;14:e0222278.
5. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. *Science* 2015; 350:1193-8.
6. Jeanclos E, Krolewski A, Skurnick J, Kimura M, Aviv H, Warram JH, Aviv A. Shortened telomere length in white blood cells of patients with IDDM. *Diabetes* 1998; 47:482-6.
7. Kim S, Parks CG, DeRoo LA, Chen H, Taylor JA, Cawthon RM, et al. Obesityand weight gain in adulthood and telomere length. *Cancer Epidemiol Biomarkers Prev* 2009; 18:816-20.
8. Verhulst S, Dalgård C, Labat C, Kark JD, Kimura M, Christensen K et al .A short leucocyte telomere length is associated with development of insulin resistance. *Diabetologia* 2016; 59:1258-65.
9. Horn RC, Gelatti GT, Mori NC, Tissiani AC, Mayer MS, Pereira EA et al. Obesity, bariatric surgery and oxidative stress. *Rev Assoc Med Bras* (1992). 2017; 63:229-35.
10. Monickaraj F, Aravind S, Gokulakrishnan K, Sathishkumar C, Prabu P, PrabuD, et al. Accelerated aging as evidenced by increased telomere shortening and mitochondrial DNA depletion in patients with type 2 diabetes. *Mol Cell Biochem* 2012; 365:343-50.
11. Laimer M, Melmer A, Lamina C, Raschenberger J, Adamovski P, Engl J, et al.Telomere length increase after weight loss induced by bariatric surgery:results from a 10-year prospective study. *Int J Obes (Lond)* 2016; 40:773-8.
12. Harrison GG, Buskirk ER, Lindsay Carter ER, Johnston FE, Lohman TG, Pollock ML, et al. Skinfold thickness and measurement technique. In: LohmanTG, Roche AF, Martorell R, editors. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988. pp. 55–70.
13. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18:499-502.
14. Marion L. Vetter, Scott Ritter, Thomas A. Wadden, and David B. Sarwer. Comparison of Bariatric Surgical Procedures for Diabetes Remission: Efficacy and Mechanisms, Diabetes Spectrum Volume 25, Number 4, 2012
15. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res* 2009; 37:e21.
16. Müezzinler A, Zaineddin AK, Brenner H. Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis. *Obes Rev* 2014; 15:192-201.
17. O'Callaghan NJ, Clifton PM, Noakes M, Fenech M. Weight loss in obese menis associated with increased telomere length and decreased abasic sites in rectal mucosa. *Rejuvenation Res* 2009; 12:169-76.
18. Sethi I, Sharma V, Sharma I, Singh G, Bhat GR, Bhanwer AJS, et al. TelomereMaintenance Genes are associated with Type 2 Diabetes Susceptibility in Northwest Indian Population Group. *Sci Rep* 2020; 10:6444.
19. Meinilä J, Perälä MM, Kautiainen H, Männistö S, Kanerva N, Shivappa N et al. Healthy diets and telomere length and attrition during a 10-year follow-up. *Eur J Clin Nutr* 2019; 73:1352-60.
20. Gielen M, Hageman GJ, Antoniou EE, Nordfjall K, Mangino M, Balasubramanyam M et al. Body mass index is negatively associated with telomere length: a collaborative cross-sectional meta-analysis of 87 observational studies. *Am J Clin Nutr* 2018; 108:453-75.
21. Dershem R, Chu X, Wood GC, Benotti P, Still CD, Rolston DD. Changes in telomere length 3-5 years after gastric bypass surgery. *Int J Obes (Lond)* 2017; 41:1718-20.
22. Jongbloed F, Meijers RWJ, IJzermans JNM, Klaassen RA, Dollé MET, van den Berg S et al. Effects of bariatric surgery on telomere length and T-cell aging. *Int J Obes (Lond)* 2019; 43:2189-99.
23. Brandao CFC, Nonino CB, de Carvalho FG, Nicoletti CF, Noronha NY, San Martin R et al. The effects of short-term combined exercise training on telomere length in obese women: a prospective, interventional study. *Sports Med Open* 2020; 6:5.
24. Lee H, Oh S, Yang W, Park R, Kim H, Jeon JS, et al. Bariatric Surgery Reduces Elevated Urinary Mitochondrial DNA Copy Number in Patients With Obesity. *J Clin Endocrinol Metab* 2019; 104:2257-66.
25. Chandru S, Pramodkumar TA, Pradeepa R, Muthukumar S, Balasubramanyam M, Bhuvaneshwari R, et al. Outcomes of metabolic surgeryin obese patients with type 2 diabetes with respect to impact on beta cell function, insulin sensitivity and diabetes remission - A study from south India.*Diabetes Metab Syndr* 2020; 14:1829-35.