Influence of endothelial dysfunction on telomere length in subjects with metabolic syndrome: LIPGENE study

Lorena González-Guardia · Elena María Yubero-Serrano · Oriol Rangel-Zuñiga · Carmen Marin · Antonio Camargo · Pablo Pérez-Martínez · Javier Delgado-Lista · Francisco Gómez-Delgado · Antonio Garcia-Rios · Francisco José Tinahones · Helen M. Roche · Francisco Pérez-Jiménez · José López-Miranda

Received: 23 September 2013 / Accepted: 26 June 2014 / Published online: 11 July 2014
© American Aging Association 2014

Abstract Previous evidences support that increased oxidative stress (OxS) may play an important role in metabolic syndrome (MetS) and both are closely linked to vascular dysfunction. This study determined whether there is a relationship between endothelial function and relative telomere length (RTL) in MetS subjects. In this cross-sectional study from the LIPGENE cohort, a total of 88 subjects (36 men and 52 women) were divided into four groups by quartiles of telomere length. We measured ischemic reactive hyperemia (IRH), total nitrite (NO) and protein carbonyl (PC) plasma levels, F2-isoprostanes urinary levels, and superoxide dismutase (SOD) and

glutathione peroxidase (GPx) plasma activities. IRH and NO plasma levels were higher in subjects with longer RTL (quartiles 3 and 4), while PC plasma levels, F2-isoprostanes urinary levels, and GPx and SOD plasma activities were lower in quartile 4 subjects (longest RTL). Additionally, MetS subjects with longer RTL had greater homeostatic model assessment- β level and lower triglycerides plasma levels. Our results suggest that endothelial dysfunction, associated with high levels of OxS, could be entailed in an increment of telomere attrition. Thus, further support of the molecular and cellular mechanisms involved in vascular dysfunction may contribute to the development

Lorena González-Guardia and Elena María Yubero-Serrano contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s11357-014-9681-9) contains supplementary material, which is available to authorized users.

L. González-Guardia · O. Rangel-Zuñiga · C. Marin · A. Camargo · P. Pérez-Martínez · J. Delgado-Lista · F. Gómez-Delgado · A. Garcia-Rios · F. Pérez-Jiménez · J. López-Miranda (✉)
Lipids and Atherosclerosis Unit, IMIBIC/Reina Sofia University Hospital/University of Cordoba, Avda. Menendez Pidal, s/n., 14004 Córdoba, Spain
e-mail: jlomezmir@uco.es

L. González-Guardia · O. Rangel-Zuñiga · C. Marin · A. Camargo · P. Pérez-Martínez · F. Gómez-Delgado · A. Garcia-Rios · F. J. Tinahones · F. Pérez-Jiménez · J. López-Miranda
CIBER Fisiopatología Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain

E. M. Yubero-Serrano
Division of Experimental Diabetes and Aging, Department of Geriatrics and Palliative Care, Mount Sinai School of Medicine, New York, USA

F. J. Tinahones
Biomedical Research Laboratory, Endocrinology Department, Hospital Virgen de la Victoria, Malaga, Spain

H. M. Roche
Nutrigenomics Research Group, School of Public Health and Population Science, UCD Conway Institute, University College Dublin, Dublin, Ireland

of strategies to decelerate vascular aging or prevent cardiovascular disease.

Keywords Endothelial dysfunction · LIPGENE study · Metabolic syndrome · Oxidative stress · Telomeres

Introduction

According to the criteria defined by the Third Report of the National Cholesterol Education Program Adult Treatment Panel III (National Cholesterol Education Program (NCEP) Expert Panel on Detection E 2002), metabolic syndrome (MetS) refers to the clustering of three or more characteristics such as hyperglycemia, hypertriglyceridemia, low level of high-density lipoprotein cholesterol (HDL-C), hypertension, and abdominal obesity (Duvnjak and Duvnjak 2009). Although insulin resistance is considered to be at the core of the MetS, one of the main mechanisms underlying this pathology is oxidative stress (OxS) (Pena-Orihuela et al. 2013). In fact, it has been recently published that the degree of OxS is influenced by the number of components of the metabolic syndrome (Yubero-Serrano et al. 2013).

OxS is often defined as the imbalance between reactive oxygen species (ROS) and the antioxidant capacity of an organism (Jones 2006). In this regard, antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) play a substantial role in the first line of defense for the detoxification of products resulting from OxS (Ray and Husain 2002; Johnson and Giulivi 2005). Additionally, protein carbonyl (PC) and F2-isoprostanes are considered as biomarkers of severe OxS, characterized by an irreversible damage to the protein structure and function (Hopps and Caimi 2013) and an increase in free radical-induced lipid peroxidation (Roberts and Morrow 2000), respectively.

It has been established that an increase in OxS levels, coupled with an impairment in nitric oxide (NO) availability, may cause alteration of endothelial cells resulting in endothelial dysfunction (Ferroni et al. 2006). These findings would be reflected in decreased ischemic reactive hyperemia (IRH), which is a method for measuring changes in acute endothelial reactivity (Fernandez et al. 2010).

Telomeres are DNA–protein complexes that cap and preserve the integrity of the chromosomal ends. They consist of several thousands of repetitive sequences of TTAGGG that are naturally shortened with each cell division due to the end replication problem (Nakashima et al.

2004; Saliques et al. 2010). Progressive attrition of telomeres leads to a critical length that triggers the cell arrest phenomenon known as senescence (Khan et al. 2012), and due to the universal fact that telomere shorten with age, its study is crucial for understanding mechanisms of age-related diseases (Armanios 2013). Notably, it has been reported that telomere shortening and oxidative DNA damage in endothelial progenitor cells is higher in coronary artery disease patients with MetS than in those without MetS (Sato et al. 2008). In addition, endothelial damage and atherosclerosis have been shown to be associated with telomere shortening in white blood cells more tightly than chronological aging (Nakashima et al. 2004). Nonetheless, there is still a lack of data in this field. It has not been described yet an explicit correlation between endothelial function (measured in terms of IRH) and telomere length in MetS subjects.

Taking this into account, the purpose of this study was to determine whether there is a relationship between endothelial function, through high OxS, and relative telomere length (RTL) in a cross-sectional study of MetS subjects of four groups divided by quartiles of telomere length.

Materials and methods

Subjects and design

This study was conducted within the framework of the LIPGENE study (“Diet, genomics and the metabolic syndrome: an integrated nutrition, agro-food, social and economic analysis”), a Framework VI Integrated Project funded by the European Union (Tierney et al. 2011).

Subject eligibility was determined using a modified version of the NCEP criteria for MetS, according to published criteria (2001).

Subjects were required to meet at least two of the following five criteria: waist circumference >102 cm (men) or >88 cm (women); fasting glucose 5.5–7.0 mmol/l; triglycerides ≥ 1.5 mmol/l; high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/l (men) or <1.3 mmol/l (women); blood pressure (BP) $\geq 130/85$ mmHg or treatment of previously diagnosed hypertension. We used a subgroup of pre-intervention data for 88 subjects (36 men and 52 women), which conformed to the LIPGENE inclusion and exclusion criteria (“Online Resource 1”) (Perez-Martinez et al. 2010). The statistical sample size calculation indicated that at least 12 participants per group would be needed to detect mean

differences equal or larger than 60 % in the IRH parameter among quartiles of RTL at a significance level of 95 % and a power analysis of 90 %.

All participants provided written informed consent and underwent a comprehensive medical history, physical examination, and clinical chemistry analysis before enrollment. Participants displayed no signs of cardiac dysfunction or hepatic, renal, and thyroid diseases and were requested to maintain their regular physical activity and lifestyle. Participants were also asked to record in a diary any event that could affect the outcome of the study, such as stress, changes in smoking habits and alcohol consumption, or intake of foods not included in the experimental design. None of the participants showed evidence of high alcohol consumption or a family history of early-onset cardiovascular disease, nor were any active smokers. All patients were free from cardiovascular complications at the time of the enrollment. The study was carried out in the Lipid and Atherosclerosis Unit at the Reina Sofia University Hospital, from February 2005 to April 2006. The experimental protocol was approved by the local ethics committee according to the Helsinki Declaration. The study was registered with the US National Library of Medicine Clinical Trials registry (NCT00429195).

Anthropometric measurements

After recording clinical histories and conducting physical examinations, we obtained the following anthropometric measurements for each individual: weight, height, body mass index, and waist circumference. Weight was measured while the subject was wearing light indoor clothing, without shoes and after voiding. Height was obtained with a stadiometer graduated in millimeters. The subject was barefoot with the back and head in contact with the stadiometer in the Frankfurt horizontal plane. Body mass index was calculated by dividing weight (kg) by height squared (m^2). Waist circumference (cm) was measured to the nearest 0.5 cm with a tape measure at the umbilical scar level. A non-stretchable tape measure was used to measure waist circumference. The measurement was taken directly on the skin with the subject in a standing position with the abdomen relaxed, the arms at the sides, and the feet together. We used the homeostatic model assessment index for insulin resistance ($HOMA_{IR}$: fasting insulin ($mU\ l^{-1}$)/fasting glucose ($mmol\ l^{-1}$)/22.5) and $HOMA\beta$ -cell function as the index of insulin secretory function derived from fasting plasma glucose and insulin concentrations, calculated as $20 \times$ fasting insulin ($mU\ l^{-1}$)/fasting glucose

($mmol\ l^{-1}$)-3.5 (Matthews et al. 1985). Insulin sensitivity was estimated by a quantitative insulin sensitivity check index (QUICKI) ($1/[\log\ insulin\ (mU\ l^{-1}) + \log\ baseline\ glucose\ (mg\ dl^{-1})]$) (Conwell et al. 2004). BP was measured using an automatic BP device. In accordance with the European Society of Hypertension Guidelines (Guidelines and Committee 2003), BP measurement was obtained with an appropriately sized cuff positioned at the heart level and after the patient had been relaxed for at least 5 min. The same arm was used for each measurement, and the average of two measurements was used for data processing.

Biochemical determinations

Plasma samples

Blood was collected in tubes containing ethylene diaminetetraacetic acid (EDTA) to yield a final concentration of 0.1 % EDTA. Plasma was separated from red cells by centrifugation at $1,500 \times g$ for 15 min at $4\ ^\circ C$ within 1 h of extraction. Plasma was immediately aliquoted and stored at $-80\ ^\circ C$ until analysis.

Biochemical analysis

The lipid variables were analyzed with a modular autoanalyzer (DDPPII Hitachi; Roche, Basel, Switzerland) with the use of Boehringer–Mannheim reagents. Triglycerides (TG) in plasma was assayed by means of enzymatic procedures (Bucolo and David 1973). HDL-C was measured by analyzing the supernatant obtained following precipitation of a plasma aliquot in dextran sulfate- Mg^{2+} , as described by Warnick et al. (1982). Plasma glucose concentrations were measured with an Architect-CG16000 analyzer (Abbott Diagnostics, Tokyo, Japan) by the hexokinase method. Plasma insulin concentrations were measured by chemoluminescence with an Architect-I2000 analyzer (Abbott Diagnostics, Tokyo, Japan). High-sensitivity C-reactive protein concentrations were measured according to Rifai et al. (1999).

Relative telomere length measurement

DNA isolation

DNA was extracted from buffy coats using the Gentra Puregene Blood kit (Gentra Systems Inc., Minneapolis, MN, USA), and low yielding samples ($<10\ ng$) were

subjected to whole genome amplification using the REPLI-g kit (Qiagen Ltd. West Sussex, UK).

Quantitative PCR analysis of telomere length

RTL was determined using the Cawthon method where the measurements are performed by qPCR (Cawthon 2002). We estimated the relative ratio of telomere repeat copy number (T) normalized against a single copy gene copy number (*Homo sapiens* ribosomal protein L13a gene RPL13a; S) for all the samples. Results for each PCR were relativized to a standard curve built using a reference DNA sample. The standard curves for telomere and genomic PCRs consisted of eight standards of reference DNA (1–25 ng). The correlation between relative T/S ratios measured by qPCR and relative telomere DNA restriction fragment length by Southern blotting has been confirmed previously to be highly consistent (Cawthon 2002; Epel et al. 2004).

All PCRs were performed in duplicate with the use of iQ5-BIORAD thermal cycler and SensiFAST™ SYBR Lo-ROX kit (Bioline). The coefficient of variation (%CV) was 9.32 % for the telomere repeat copy number and 6.76 % for the single copy gene copy number. The thermal cycler profile for both amplicons began with a 95 °C incubation for 3 min to activate the polymerase, followed by 40 cycles of 95 °C for 5 s, 54 °C for 15 s. The reaction mix composition was identical except for the oligonucleotide primers: 20 ng template DNA, 1× SensiFAST™ SYBR Lo-ROX, 200 nM reverse primer, 200 nM forward primer. The primer sequences were (written 5'→3'):

RPL13aF, CCTGGAGGAGAAGAGGAAAGAGA;
RPL13aR, TTGAGGACCTCTGTGTATTTGTCAA
teloF, CGGTTTGGTTGGGTTTGGGTTTGGGTTT
GGGTTTGGGTT;
teloR, GGCTTGCCTTACCCTTACCCTTACCC
TTACCCTTACCCT (O'Callaghan and Fenech 2011).

Study of endothelial function using laser Doppler

The Laser-Doppler linear Periflux 5000 (Perimed S.A., Stockholm, Sweden) was used to measure IRH. We found an inter-study variability of 8.85 % and an intra-study variability of 8.7 %.

Briefly, capillary flow of the second finger of the dominant arm of the patient was assessed for one minute before (t0) and after applying 4 min (td) of ischemia to the arm using a sphygmomanometer.

The ischemic reactive hyperemia was obtained via $IRH = (AUC_{td} - AUC_{t0}) \times 100 / AUC_{t0}$.

Determination of oxidative stress biomarkers

Nitric oxide (NO) is a free gas produced endogenously by a variety of mammalian cells. This molecule induces vasodilatation and inhibits platelet aggregation and adhesion to the vascular endothelium. Total nitrite (nitrite and nitrate) was used as an indicator of NO production and was assayed using the Griess method (Ricart-Jane et al. 2002), with an intra-assay coefficient of variation of 6.1 % and an inter-assay coefficient of variation of 7.7 %. The reaction was monitored at 540 nm (UV-1603 spectrophotometer, Shimadzu).

Protein carbonyl (PC) content was carried out in plasma samples using the method of Levine et al. (1990). Intra-assay coefficient of variation was 4.7 %, and inter-assay coefficient of variation was 8.0 %. Carbonyls were evaluated in a spectrophotometer (UV-1603 Shimadzu, Kyoto, Japan) at 360 nm.

The F2-isoprostane content was measured in urine samples by using the ELISA kit BIOXYTECH 8-Isoprostane Assay Immunoassay for 8-epi-Prostaglandin F2a (OXIS International, Portland, OR).

Antioxidant enzyme activities

Total SOD (E.C: 1.15.1.1) activity was determined by colorimetric assay in plasma at wavelength of 525 nm according to the method described by McCord and Fridovich et al. (Nebot et al. 1993), with an intra-assay coefficient of variation of 7.3 % and an inter-assay coefficient of variation of 8.7 %. GPx (E.C.: 1.11.1.9) activity was evaluated in plasma by the Flohé and Gunzler method (Flohe and Gunzler 1984; Zhu et al. 2008), with an intra-assay coefficient of variation of 6.5 % and an inter-assay coefficient of variation of 7.2 %. The GPx assay is based on the oxidation of NADPH to NAD⁺, catalyzed by a limiting concentration of glutathione reductase, with maximum absorbance at 340 nm. The absorbance was evaluated in a Shimadzu UV-1603 spectrophotometer (Kyoto, Japan).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS 17.0 for Windows Inc., Chicago, IL, USA) was used for the statistical comparisons. RTL was analyzed as quartiles

after removal of experimental outliers. Telomere length was categorized into quartiles (shortest to longest) and analyzed as a categorical variable. This resulted in the following distribution of RTL: quartile 1, $RTL \leq 1.10$; quartile 2, $1.10 < RTL \leq 1.26$; quartile 3, $1.26 < RTL \leq 1.56$; quartile 4, $RTL > 1.56$.

Univariate analysis of variance adjusted for age was performed to compare variables across quartiles of RTL with Bonferroni post hoc test used for multiple comparisons. Differences were considered to be significant when $p < 0.05$. All data presented in the text and tables are expressed as the means $\pm$ standard error (SE).

Results

Biochemical and anthropometric characteristics and metabolic assessment

The anthropometric, biochemical, BP, and metabolic parameters of the MetS subjects are shown in Table 1.

Plasma TG was higher in the subjects with the shortest RTL (quartile 1) compared to those in quartile 4 with the largest RTL ($p = 0.031$).

Baseline HOMA β was greater in the subjects grouped in the quartile 4 with the largest RTL compared with the subjects in the quartiles 1, 2, and 3 ($p = 0.003$). We did not find significant differences with respect to the remaining parameters between the four groups of MetS subjects according to the RTL (Table 1).

Study of endothelial function

NO plasma levels were higher in the quartiles 3 ($p = 0.012$) and 4 ($p = 0.016$) than the quartile 1 (shortest RTL) (Fig. 1a). In addition, IRH shows the same trend as the NO levels with greater percentage of change from baseline in the quartiles 3 (longest RTL) ($p = 0.015$) and 4 ($p = 0.011$) than quartile 1 (Fig. 1b).

Table 1 Baseline characteristics of the study population according to quartiles of RTL^{1,2,3}

	Quartile 1 (<i>n</i> =22)	Quartile 2 (<i>n</i> =24)	Quartile 3 (<i>n</i> =20)	Quartile 4 (<i>n</i> =22)	<i>p</i> value
Age (years)	59.73 $\pm$ 1.87	59.71 $\pm$ 1.55	58.60 $\pm$ 1.60	56.59 $\pm$ 1.94	0.540
Sex (female/male)	14/8	14/10	12/8	12/10	
HOMA _{IR}	3.37 $\pm$ 0.07	3.97 $\pm$ 0.10	3.42 $\pm$ 0.11	4.32 $\pm$ 0.12	0.700
HOMA β	89.94 $\pm$ 9.31 a	91.77 $\pm$ 8.93 a	94.21 $\pm$ 9.69 a	139.14 $\pm$ 9.49 b	0.003
Weight (kg)	87.78 $\pm$ 2.74	86.50 $\pm$ 2.63	89.66 $\pm$ 2.85	96.06 $\pm$ 2.79	0.940
Height (m)	1.57 $\pm$ 0.03	1.62 $\pm$ 0.02	1.54 $\pm$ 0.05	1.56 $\pm$ 0.04	0.129
BMI	34.28 $\pm$ 0.88	33.68 $\pm$ 0.84	34.28 $\pm$ 0.92	35.28 $\pm$ 0.90	0.800
Waist circumference (cm)	106.34 $\pm$ 2.18	103.54 $\pm$ 2.09	108.40 $\pm$ 2.27	109.71 $\pm$ 2.22	0.937
Insulin (mU/l)	12.22 $\pm$ 2.10	14.71 $\pm$ 2.05	12.69 $\pm$ 2.19	15.04 $\pm$ 2.14	0.657
Glucose (nmol/l)	6.29 $\pm$ 0.22	6.20 $\pm$ 0.22	5.93 $\pm$ 0.23	5.03 $\pm$ 0.23	0.143
TG (nmol/l)	1.89 $\pm$ 0.14 a	1.51 $\pm$ 0.13 a, b	1.55 $\pm$ 0.15 a, b	1.27 $\pm$ 0.14 b	0.031
LDL-C (nmol/l)	17.38 $\pm$ 8.63	34.02 $\pm$ 8.27	21.30 $\pm$ 8.78	26.16 $\pm$ 8.97	0.647
HDL-C (nmol/l)	1.11 $\pm$ 0.05	1.22 $\pm$ 0.05	1.08 $\pm$ 0.06	1.09 $\pm$ 0.06	0.325
hsCRP (mg/l)	6.63 $\pm$ 1.17	5.24 $\pm$ 1.12	7.09 $\pm$ 1.19	6.52 $\pm$ 1.16	0.573
SBP (mmHg)	142 $\pm$ 3	144 $\pm$ 3	138 $\pm$ 3	142 $\pm$ 3	0.306
DBP (mmHg)	87 $\pm$ 3	87 $\pm$ 2	88 $\pm$ 3	89 $\pm$ 3	0.900

Data are means $\pm$ SE. Means in a column with different letters are significantly different, $p < 0.05$ (univariate analysis of variance adjusted for age). Quartile 1: $RTL \leq 1.10$, quartile 2: $1.10 < RTL \leq 1.26$, quartile 3: $1.26 < RTL \leq 1.56$, quartile 4: $RTL > 1.56$

HOMA_{IR} homeostatic model assessment index for insulin resistance, HOMA β homeostatic model assessment index of insulin secretory function, BMI body mass index, TG triglycerides, LDL-C low-density lipoprotein-cholesterol, HDL-C high-density lipoprotein-cholesterol, SBP systolic blood pressure, DBP diastolic blood pressure, hsCRP high sensitivity C-reactive protein

Fig. 1 Total nitrite levels in plasma (a) and ischemic reactive hyperemia (b) across quartiles of RTL. Data were analyzed using univariate analysis of variance adjusted for age. All values represent the means $\pm$ SE Differences were considered to be significant when $*p<0.05$

Antioxidant enzymes activities and biomarkers of oxidative stress

We found that GPx plasma activity of subjects grouped in the quartile 4 with longer RTL was lower compared to quartile 1 with shorter RTL ($p=0.003$), with intermediate activity levels for quartiles 2 and 3 (Fig. 2a). Although differences of SOD plasma activity between quartiles were not statistically significant, there was a marked trend that shows an increase in the activity of the enzyme in the quartile 1 with respect to the other RTL groups (Fig. 2b).

PC plasma levels and F2-isoprostanes urinary levels were higher in the subjects grouped in the quartile 1 with the shortest RTL compared with the subjects in quartile 4 ($p=0.036$ and $p=0.014$; Fig. 3a, b, respectively).

Discussion

MetS comprises a cluster of cardiovascular risk factors (low HDL-C, elevated BP, fasting glucose and TG, and abdominal obesity) (Reaven 2002) leading to accelerated atherosclerosis and an increased risk of type 2 diabetes. Additionally, MetS is associated with major cardiovascular events and a high mortality rate (McNeill et al. 2005; Van Guilder et al. 2006).

Although insulin resistance is considered to be at the core of the MetS in terms of molecular mechanisms, it is exponentially increasing the number of studies that point out the importance of OxS as a deep influence for MetS progression. In fact, in the same population of this study, we have previously demonstrated that MetS subjects with more MetS components (four or five components) may have a higher OxS level (determined by

Fig. 2 Glutathione peroxidase (GPx) plasma activity (a) and superoxide dismutase (SOD) plasma activity (b) across quartiles of RTL. Data were analyzed using univariate analysis of variance

adjusted for age. All values represent the means $\pm$ SE Differences were considered to be significant when $*p<0.05$

Fig. 3 Protein carbonyl plasma levels (**a**) and isoprostanes urinary levels (**b**) across quartiles of RTL. Data were analyzed using univariate analysis of variance adjusted for age. All values represent the means $\pm$ SE. Differences were considered to be significant when $*p < 0.05$

SOD and GPx plasma activities, IRH and total nitrite plasma levels, among others parameters) (Yubero-Serrano et al. 2013). Thus, the study of the redox state in early MetS patients may provide a starting point for understanding the pathways that contribute to both the development of MetS and its subsequent complications.

Taking this into account, the present cross-sectional study displays a gradual and inverse relationship between high OxS levels and endothelial function with regard to RTL in MetS patients. We found that the shorter telomere length, the greater the degree of OxS, which leads to increased plasma activity of antioxidant enzymes (GPx, SOD), biomarker of OxS levels (PC and F2-isoprostanes), and decreased IRH and total NO levels.

It has been documented that increased production of ROS results in damage to the endothelium, and along with others mechanisms involved in vascular aging process such as activation of inflammatory mediators, impairment of the NO pathway, telomeres length and telomerase activity, all together contribute to the dysregulation of vascular homeostasis (Marin et al. 2013).

NO is an endogenous vasodilator that promotes the increase of blood flow, decreases vascular resistance, inhibits platelet aggregation, and also acts protecting endothelial cells from senescence and apoptosis (Ferroni et al. 2006; Toda 2012). A strong relationship has been found between telomere attrition and cell senescence in HUVECs, suggesting that DNA damage manifested as telomere shortening is likely to play a central role in the development of endothelial dysfunction in the pathogenesis of vascular disease (Hastings et al. 2004). In this connection, MetS study subjects with the shortest RTL have the lowest level of plasmatic NO,

and in accordance with this, they show the lowest IRH as well.

Interestingly, those MetS subjects with the shortest RTL are the ones whose antioxidant enzyme activities are higher, in which plasma GPx activity is increased and plasma SOD activity presents the same trend. A plausible explanation to these observations is the fact that in response to OxS and to prevent oxidative damage, cells attempt to strengthen their antioxidant arsenal as the first line of defense. For this purpose, antioxidant enzymes such as SOD, GPx, and catalase increase their activity to balance the excessive generated OxS (Yubero-Serrano et al. 2013). Furthermore, we also found an increase in PC plasma levels and F2-isoprostanes urinary levels in the subjects with the shortest RTL. PC are an indicator of severe OxS, characterized by an irreversible damage to the protein structure and function and frequently associated with obesity, insulin resistance, diabetes mellitus, and MetS (Hopps and Caimi 2013). Likewise, a large body of evidences have indicated that measurement of bioactive F2-isoprostanes in body fluids offers a unique noninvasive analytic utensil to study the role of free radicals in physiology, OxS-related diseases, and experimental acute or chronic inflammatory conditions (Basu 2008; Tucker et al. 2013).

Our present observations are consistent with a study in coronary artery disease patients with MetS, where telomere shortening and oxidative stress damage in endothelial progenitor cells were higher than in those without MetS. They suggest that telomere attrition in response to OxS may induce enhanced endothelial damage and contribute to increase the risk of subsequent cardiovascular events (Satoh et al. 2008).

In this study, we measured telomere length in circulating leucocytes due to the fact that this method is less invasive than obtaining human vascular tissue from participants. Although we did not study telomeres length in vascular cells, it has been demonstrated that circulating blood leucocyte DNA content is predictive of vascular telomere content and also is an accurate surrogate for vascular aging in population studies (Nakashima et al. 2004; Wilson et al. 2008).

Furthermore, systemic OxS is increased in subjects with MetS and is strongly associated with visceral fat accumulation (Fujita et al. 2006; Yubero-Serrano et al. 2013). In our study, we found that TG levels were higher and HOMA β was lower in those people with shorter telomeres and increased OxS levels. The same findings have been shown in type 2 diabetes mellitus patients where telomere length was inversely correlated to TG levels and considerably explained by insulin resistance, which in turn promoted an increase in ROS levels (Harte et al. 2012).

We are aware that this cross-sectional study has some limitations since these findings do not prove a cause-and-effect relationship and the use of a small sample population. In this regard, larger and prospective studies are needed to elucidate which of the parameters studied herein has the strongest effect above the others.

In summary, our results show that there is a link among endothelial function, OxS, and RTL in MetS subjects, in a manner that high levels of OxS may be related to endothelial dysfunction processes and telomere attrition. Thus, further support of the molecular and cellular mechanisms involved in vascular dysfunction may contribute to the development of strategies to decelerate vascular aging or prevent cardiovascular disease.

Acknowledgments This research was supported partly by grants from the Ministerio de Ciencia e Innovacion (AGL2006-01979, AGL2009-12270 to JL-M), ('CB06/03/0047-CIBER Fisiopatologia de la Obesidad y Nutricion' is an ISCIII grant awarded to FP-J), Consejería de Innovacion, Ciencia y Empresa, Junta de Andalucía (P06-CTS-01425 and CVI-7450 to JL-M), and Consejería de Salud, Junta de Andalucía (06/128, 07/43, PI0193/2009 to JL-M, 06/129 to FP-J).

References

- (2001) Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Jama* 285(19): 2486-97
- Armanios M (2013) Telomeres and age-related disease: how telomere biology informs clinical paradigms. *J Clin Invest* 123(3):996-1002
- Basu S (2008) F2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. *Antioxid Redox Signal* 10(8):1405-34
- Bucolo G, David H (1973) Quantitative determination of serum triglycerides by the use of enzymes. *Clin Chem* 19(5):476-82
- Cawthon RM (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Res* 30(10):e47
- Conwell LS, Trost SG et al (2004) Indexes of insulin resistance and secretion in obese children and adolescents: a validation study. *Diabetes Care* 27(2):314-9
- Duvnjak L, Duvnjak M (2009) The metabolic syndrome—an ongoing story. *J Physiol Pharmacol* 60(Suppl 7):19-24
- Epel ES, Blackburn EH et al (2004) Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A* 101(49):17312-5
- Fernandez JM, Da Silva-Grigoletto ME et al (2010) Pre-exercise intake of different carbohydrates modifies ischemic reactive hyperemia after a session of anaerobic, but not after aerobic exercise. *J Strength Cond Res* 24(6):1623-32
- Ferroni P, Basili S et al (2006) Endothelial dysfunction and oxidative stress in arterial hypertension. *Nutr Metab Cardiovasc Dis* 16(3):222-33
- Flohe L, Gunzler WA (1984) Assays of glutathione peroxidase. *Methods Enzymol* 105:114-21
- Fujita K, Nishizawa H et al (2006) Systemic oxidative stress is associated with visceral fat accumulation and the metabolic syndrome. *Circ J* 70(11):1437-42
- Guidelines, E. S. o. H.-E. S. o. C. and Committee (2003) 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 21(6):1011-53
- Harte AL, da Silva NF et al (2012) Telomere length attrition, a marker of biological senescence, is inversely correlated with triglycerides and cholesterol in South Asian males with type 2 diabetes mellitus. *Exp Diabetes Res* 2012:895185
- Hastings R, Qureshi M et al (2004) Telomere attrition and accumulation of senescent cells in cultured human endothelial cells. *Cell Prolif* 37(4):317-24
- Hopps E, Caimi G (2013) Protein oxidation in metabolic syndrome. *Clin Invest Med* 36(1):E1-8
- Johnson F, Giulivi C (2005) Superoxide dismutases and their impact upon human health. *Mol Aspects Med* 26(4-5): 340-52
- Jones DP (2006) Extracellular redox state: refining the definition of oxidative stress in aging. *Rejuvenation Res* 9(2):169-81
- Khan S, Chuturgoon AA et al (2012) Telomeres and atherosclerosis. *Cardiovasc J Afr* 23(10):563-71
- Levine RL, Garland D et al (1990) Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 186:464-78
- Marin C, Yubero-Serrano EM et al (2013) Endothelial aging associated with oxidative stress can be modulated by a healthy Mediterranean diet. *Int J Mol Sci* 14(5):8869-89
- Matthews DR, Hosker JP et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting

- plasma glucose and insulin concentrations in man. *Diabetologia* 28(7):412–9
- McNeill AM, Rosamond WD et al (2005) The metabolic syndrome and 11-year risk of incident cardiovascular disease in the atherosclerosis risk in communities study. *Diabetes Care* 28(2):385–90
- Nakashima H, Ozono R et al (2004) Telomere attrition in white blood cell correlating with cardiovascular damage. *Hypertens Res* 27(5):319–25
- National Cholesterol Education Program (NCEP) Expert Panel on Detection E, a. T. o. H. B. C. i. A. A. T. P. I. (2002) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. *Circulation* 106 (25): 3143–421
- Nebot C, Moutet M et al (1993) Spectrophotometric assay of superoxide dismutase activity based on the activated autoxidation of a tetracyclic catechol. *Anal Biochem* 214(2):442–51
- O'Callaghan NJ, Fenech M (2011) A quantitative PCR method for measuring absolute telomere length. *Biol Proced Online* 13:3
- Pena-Orihuela P, Camargo A. et al. (2013) Antioxidant system response is modified by dietary fat in adipose tissue of metabolic syndrome patients. *J Nutr Biochem*
- Perez-Martinez P, Moreno-Conde M et al (2010) Dietary fat differentially influences regulatory endothelial function during the postprandial state in patients with metabolic syndrome: from the LIPGENE study. *Atherosclerosis* 209(2):533–8
- Ray G, Husain SA (2002) Oxidants, antioxidants and carcinogenesis. *Indian J Exp Biol* 40(11):1213–32
- Reaven G (2002) Metabolic syndrome: pathophysiology and implications for management of cardiovascular disease. *Circulation* 106(3):286–8
- Ricart-Jane D, Llobera M et al (2002) Anticoagulants and other preanalytical factors interfere in plasma nitrate/nitrite quantification by the Griess method. *Nitric Oxide* 6(2):178–85
- Rifai N, Tracy RP et al (1999) Clinical efficacy of an automated high-sensitivity C-reactive protein assay. *Clin Chem* 45(12):2136–41
- Roberts LJ, Morrow JD (2000) Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med* 28(4):505–13
- Saliques S, Zeller M et al (2010) Telomere length and cardiovascular disease. *Arch Cardiovasc Dis* 103(8–9):454–9
- Satoh M, Ishikawa Y et al (2008) Association between oxidative DNA damage and telomere shortening in circulating endothelial progenitor cells obtained from metabolic syndrome patients with coronary artery disease. *Atherosclerosis* 198(2): 347–53
- Tierney AC, McMonagle J et al (2011) Effects of dietary fat modification on insulin sensitivity and on other risk factors of the metabolic syndrome—LIPGENE: a European randomized dietary intervention study. *Int J Obes (Lond)* 35(6):800–9
- Toda N (2012) Age-related changes in endothelial function and blood flow regulation. *Pharmacol Ther* 133(2):159–76
- Tucker PS, Dalbo VJ et al (2013) Clinical and research markers of oxidative stress in chronic kidney disease. *Biomarkers* 18(2): 103–15
- Van Guilder GP, Hoetzer GL et al (2006) Influence of metabolic syndrome on biomarkers of oxidative stress and inflammation in obese adults. *Obesity (Silver Spring)* 14(12):2127–31
- Warnick GR, Benderson J et al (1982) Dextran sulfate-Mg²⁺ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin Chem* 28(6):1379–88
- Wilson WR, Herbert KE et al (2008) Blood leucocyte telomere DNA content predicts vascular telomere DNA content in humans with and without vascular disease. *Eur Heart J* 29(21):2689–94
- Yubero-Serrano EM, Delgado-Lista J et al (2013) Oxidative stress is associated with the number of components of metabolic syndrome: LIPGENE study. *Exp Mol Med* 45:e28
- Zhu H, Zhang L et al (2008) Coordinated upregulation of a series of endogenous antioxidants and phase 2 enzymes as a novel strategy for protecting renal tubular cells from oxidative and electrophilic stress. *Exp Biol Med (Maywood)* 233(6):753–65