Original Research

Are insulin-resistance and oxidative stress cause or consequence of aging

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Impact statement

Insulin resistance is associated with oxidative stress leading to cardiovascular diseases. However, little research has been performed examining elderly individuals with or without insulin-resistance. We demonstrate that antioxidant defense systems alone is not able to abrogate insulin action in elderly individuals at high risk for atherosclerosis, whereas the combined oxidant-antioxidant markers (thiobarbituric acid-reacting substances (TBARS), Cu,Zn-superoxide dismutase (SOD-1), and total antioxidant status (TAS)) might be more efficient and perhaps produce better clinical outcome. In fact, a decrease in oxidative stress and strong interaction between antioxidant defense can be seen only among insulin-resistant elderly individuals. This is, in our opinion, valuable information for clinicians, since insulin-resistance is considered strong cardiovascular risk factor.

Abstract

Insulin resistance (IR) may be associated with oxidative stress and leads to cardiovascular disorders. Current research focuses on interplay between insulin-resistance indices and oxidant-antioxidant markers in elderly individuals with or without insulin-resistance. The assessment involved anthropometric data (weight, height, BMI, percentage of body fat (FAT)) and biochemical tests (glucose, lipids, serum insulin and plasma oxidant-antioxidant markers: Thiobarbituric Acid-Reacting Substances (TBARS), Cu,Zn-superoxide dismutase (SOD-1) and total antioxidant status). Insulin resistance index (IR) assuming a cut-off point of 0.3 allows to divides groups into: insulin sensitive group (InsS) IR $< 0{,}3$ ($n = 35$, median age 69.0 years) and insulin-resistant group (InsR) IR ≥ 0.3 ($n = 51$, median age 71.0 years). Lipids and antioxidant defense system markers did not differentiate the investigated groups. In the InsR elderly group, the FAT was increased ($P < 0.000003$) and TBARS ($P = 0.008$) concentration decreased in comparison with InsS group. A positive correlation for SOD-1 and total antioxidant status ($P < 0.05$; $r = 0.434$) and a negative correlation for TBARS and age ($P < 0.05$ with $r = -0.421$) were calculated in InsR individuals. In elderly individuals, oxidative stress persists irrespective of insulin-resistance status. We suggest that increased oxidative stress may be consequence of old age. An insulin action identifies those at high risk for atherosclerosis, via congruent associations with oxidative stress and extra- and intracellular antioxidant defense systems. Thus, we maintain that insulin-resistance is not the cause of aging.

Keywords: Aging, oxidative stress, thiobarbituric acid-reacting substances, superoxide dismutase, total antioxidant status, insulin resistance

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Introduction

Insulin-resistance is currently considered as a "nontraditional" risk factor for cardiovascular disease (CVD), and has been shown to trigger CVD by biochemical elements: hyperglycemia, dyslipidemia, protein metabolism abnormalities, oxidant-antioxidant systems and endothelial dysfunction, and clinical components: diabetes and hypertension.[1,2]

The underlying defect in over 90% individuals with hyperglycemia is insulin-resistance. The progression of insulin-resistance to diabetes parallels the progression of endothelial dysfunction to atherosclerosis and functional heart dysfunction. Moreover, Jørgensen *et al.*[3] found that in patients with hyperglycemia, structural and functional alterations in heart left ventricle persist and are enhanced with increasing diabetes duration despite reductions in

overall risk of CVD.[3] Furthermore, chronic hyperglycemia increases oxidative stress at the molecular, cellular, and tissue level. Therefore, the question is whether hyperglycemia and/or insulin-resistance are the underlying problem especially in elderly individuals.

Insulin-resistance promotes dyslipidemia, which is a combination of decreased concentration of high-density lipoproteins cholesterol (HDL-C) and elevated levels of triglycerides (TAG).[4] On the other hand TAG overproduction may induce oxidative stress at the cellular and systemic level. As a result, these systemic conditions can impair insulin signaling and further promote atherosclerosis and accelerated aging.

The increasing evidences show how traditional risk factors translate into oxidative stress and promote atherosclerosis.[5] The lipids accumulation, observed in insulin-resistance and obesity, contributes in overproduction of reactive oxygen species (ROS), which results in increased oxidative stress and in turn leads to endothelial dysfunction and atherosclerosis. Moreover, the oxidative damage to lipids, which results from an imbalance of the pro- and antioxidant equilibrium, is involved in the atherosclerotic disease.[6] The widely used biomarker for detecting lipoproteins oxidation is assessment of thiobarbituric acid reactive substances (TBARS).[7] Furthermore, antioxidant rescue systems, measured by total antioxidant status (TAS) for the extracellular space and by the activity of Cu,Zn-superoxide dismutase (SOD-1) for the intracellular space, are engaged against ROS, which are continuously created due to soundness metabolism or pathology.[8]

The increasing number of CVD events occurs more often among elderly individuals without previous CVD in comparison with those with previous CVD events.[9] Furthermore, elderly people are very often excluded from basic and interventional trials due to high mortality and morbidity. Thus, the exploration of the usefulness of widely measured and available, simple, feasible and economical agents with respect to the identification of the elderly individuals at risk for CVD and atherosclerosis are urgently needed.[10]

On top of it, aging per se plays an important role in the development of insulin-resistance and oxidative stress.[11,12] Among the damage theories, the accumulation of the ROS, which leads to accumulative damage of DNA, protein, and lipid molecules, is one of the major contributors to aging process.[13] Oxidative stress is causative from enzymes damage and metabolite cumulation, and thus promotes the non-canonical metabolic activity of the cells and plays a potential role in age-related diseases and pathology.[14] Oxidative damage leads through lipid peroxidation products accumulation to loss of cell membrane properties.[15] Moreover, during senescence, there are seen decreased prevalence of peripheral insulin sensitivity and age-related pancreatic beta cell dysfunction.[16,17]

Thus, in the light of getting a better insight into the complex age-associated changes in insulin-resistancy and oxidant-antioxidant balance, we have studied an interplay between insulin, insulin-resistance indices, and oxidant-antioxidant markers in elderly persons with or without insulin-resistance.

Materials and methods

The Independent Bioethics Committees of Poznan University of Medical Sciences in Poznan (number: 595/11) and of Medical University of Silesia in Katowice (number: KNW/0022/KB1/38/IV/16/17/18/19/20), Poland reviewed and approved the study protocol. It was carried out in compliance with the Declaration of Helsinki of 1975 for Human Research revised in 2008. Prior to elderly individuals' participation in the study, thorough information was provided and written informed consent was obtained from each participant.

Inclusion criteria

We invited to the study 519 elderly Caucasians ($\geq$60 years old), both genders, who declared no acute or chronic diseases and used to use no special diet neither drugs nor supplements.

Exclusion criteria

Smoking and alcohol status: Participants were asked whether they smoke or drink alcohol—than current smokers and alcohol consumers were excluded from the study.

Moreover participants who were previously diagnosed CVD (hypertension or coronary artery disease) or had positive clinical history of diabetes, stroke, inflammatory disease, malignant tumors, liver cirrhosis, or decreased renal function (measured by eGFR $<$60 mL/min/1.73 m^2), and mental disorder were excluded from the study.

In addition, participants who used to used substances with antioxidant power were excluded (including drugs and over the counter (OTC) vitamins and supplements).

Baseline assessment

Following recruitment, participants undergoes a baseline assessment. Complete physical examination, including anthropometric measures and arterial blood pressure, was done. The participants in light-weight clothes and without shoes had been measured weight and height to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was obtained from the formula: [BMI] = weight [kg]/square of the height [m^2]. Waist circumference (WC) was measured at the umbilicus using a non-elastic tape (to the nearest 0.1 cm), with the participants standing at the end of normal expiration. Percentage of body fat (FAT) was measured by bio-electrical impedance analysis (BIA) using BodyStat equipment (Bodystat 1500, Bodystat Limited, Douglas, British Isles).

Systolic and diastolic blood pressure (SBP and DBP) was measured three times at 2-min intervals after at least 5 min of rest using a validated sphygmomanometer (M10-IT, Omron Health Care, Kyoto, Japan), following the recommendations of the European Society of Hypertension.[18] The examined individuals were asked to avoid exercise and caffeinated beverages for at least 30 min before the measurement. The mean of three SBP and DBP measurements was calculated and used in all analyses.

Blood sampling and biochemical analysis

Following the WHO recommendation,[19] the 92 persons were qualified for an oral glucose tolerance test (OGTT). The OGTT allows us to exclude from the next steps of the investigation those who were diagnosed diabetes ($n = 6$).

Blood samples were obtained from an antecubital vein into vacutainer tubes. Fasting blood samples were collected in the morning after at least 12 h of fasting for all participants.

Glucose and glycated hemoglobin

Glucose. Plasma glucose concentrations during OGTT (G0′ at 0 min and G120′ at 120 min) were assessed by enzymatic method, with hexokinase (bioMerieux, Marcy I'Etoile, France), and the UV-160A Shimadzu spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Mean of glucose concentration (mean G) was calculated from the fasting glucose concentration 0′ and after 120 min of OGTT (G0′+ G120′)/2.

Glycated hemoglobin (HbA_{1c}). HbA_{1c} in whole blood was measured by NGSP (National Glycohemoglobin Standardization Program) certified method using high-performance liquid chromatography (HPLC) on the D-10 equipment of Bio-Rad (Hercules, CA, USA).

The sensitivity was 0.05% with intra-assay coefficient of variation (CV) of 2.35% and inter-assay CV of 2.66%.

Lipids assays

Concentration of total plasma cholesterol (T-C), high-density lipoproteins cholesterol (HDL-C), and triglycerides (TAG) were assessed by enzymatic methods (bioMerieux, Marcy I'Etoile, France), and the UV-160A Shimadzu spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The monitoring accuracy of the determinations was used by RANDOX Assayed Human Multi-Sera Level 1 (as normal) and RANDOX Assayed Human Multi-Sera Level 2 (as pathological) (Randox, Crumlin, United Kingdom).

Plasma low-density lipoproteins cholesterol (LDL-C) concentration was calculated according to Friedewald formula: [LDL-C] = [T-C] – [HDL-C] – [0.45·TAG], if TAG <4.56 mmol/L.

Plasma cholesterol of non-HDL (non-HDL-C) fraction was calculated from the formula: [non-HDL-C](mmol/L) = [TC] (mmol/L) – [HDL-C] (mmol/L).

Insulin and insulin-resistance indices

Insulin concentration during OGTT (Ins0′ – at 0 min and Ins120′ at 120 min) was assessed by enzyme-linked immunosorbent assay (ELISA) using reagents from BioSource (Nivelles, Belgium) and SunriseTM microplate reader from Tecan Group Ltd (Männedorf, Switzerland), with an analytical sensitivity of 0.15 mU/L.

Coefficients of variation (CV), intra-series, and inter-series were respectively 3.8% and 5.8%. The references levels for the method were 5–19 mU/L.

Mean insulin (mean Ins) was obtained from the fasting insulin concentration 0′ and after 120 min of OGTT (Ins0′+Ins120′)/2.

Insulin-resistance indices

Insulin resistance was defined as IR index value ≥ 0.3 and allowed to classifying subjects for insulin sensitive group (InsS-group) IR < 0.3 ($n = 35$, median age 69.0 years) and insulin-resistant group (InsR-group) IR ≥ 0.3 ($n = 51$, median age 71.0 years).

Insulin-resistance (IR) index was calculated by formula: IR = Ins0′ [mU/L]/glu0′ [mg/dL]
where Ins0′ and glu0′ represent the fasting insulin and fasting glucose, respectively, before OGTT.

The Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was calculated by formula: HOMA-IR = [(fasting insulin [mU/L] × fasting glucose [mmol/L])/22.5].

The Quantitative Insulin Sensitivity Check Index (QUICKI) was calculated using the following formula: QUICKI = 1/[log fasting insulin (mU/L) + log fasting glucose (mmol/L)].

The insulin sensitivity index obtained from the OGTT proposed by Matsuda was calculated as follows: Matsuda = 10,000/$\sqrt{}$[(G0′ × Ins0′) × (mean G) × (mean Ins)]. Ins0′ and G0′ represent the insulin and glucose concentrations, respectively, at time 0 (fasting) and mean Ins and mean G represent the mean insulin and glucose concentrations, respectively, obtained during the OGTT.

Oxidant-antioxidant markers

To analyze oxidant-antioxidant markers, we obtained concentration of plasma thiobarbituric acid-reacting substances (TBARS), reflecting plasma lipid peroxidation products, and plasma total antioxidant status (TAS) and activity of erythrocyte cytoplasmatic superoxide dismutase Cu-, Zn-SOD (EC: 1.15.1.1) (SOD-1) as described previously in manuscript by Dziegielewska *et al*.[8] Coefficients of variation (CV), intra-series, and inter-series were respectively for TBARS 2.80% and 4.70%, SOD-1 1.6% and 2.7% and TAS 2.50% and 4.80%.

Statistical analysis

The statistical analysis was done by Statistica for Windows (version 13.0). Depending on the distribution of data (checked by the Shapiro–Wilk test), participant characteristics were shown as medians and lower and upper quartiles. To assess the differences between the investigated elderly IR groups, a nonparametric Mann–Whitney *U* test was used. The bivariate correlation coefficients between the investigated parameters are expressed as R Spearman correlation coefficient. A *P*-value < 0.05 or lower was considered statistically significant.

Results

The clinical and biochemical results among the elderly investigated population are summarized in Table 1. The insulin concentration and insulin-resistance indices in all

Table 1. Basic clinical and biochemical characteristics of the studied groups.

	All elderly participants free of diabetes $n = 86$	Elderly InsS group $n = 35$	Elderly InsR group $n = 51$	Significant difference
females/males [*n*]	58/28	20/15	38/13	
Age [years]	70.0 (67.0–74.0)	69.0 (67.0–71.0)	71.0 (67.0–71.1)	NS
Weight [kg]	73.5 (68.0–83.0)	75.0 (69.0–79.0)	73.0 (67.0–84.4)	NS
Height [m]	1.60 (1.54–1.68)	1.62 (1.57–1.72)	1.59 (1.50–1.65)	NS
BMI [kg/m^2]	28.8 (26.0–31.0)	26.4 (24.2–30.0)	29.7 (26.6–31.5)	0.03
Waist [cm]	92.5 (86.0–100.5)	93.0 (86.0–98.0)	92.0 (86.0–101.0)	NS
Waist females [cm]	91.0 (82.0–95.0)	86.0 (80.0–94.5)	92.0 (83.0–98.0)	NS
Waist males [cm]	100.0 (92.0–110.0)	96.0 (92.0–108.0)	100.5 (91.0–114.0)	NS
FAT [%]	44.2 (28.1–47.6)	22.8 (12.9–38.2)	45.5 (43.0–49.2)	0.000003
FAT females [%]	45.5 (40.3–49.2)	37.2 (10.8–46.4)	47.4 (45.1–49.5)	0.002
FAT males [%]	26.7 (19.5–42.0)	19.5 (13.7–22.8)	42.8 (36.7–45.4)	0.0002
SBP [mmHg]	140.0 (130.0–150.0)	140.0 (125.0–140.0)	140.0 (130.0–160.0)	NS
DBP [mmHg]	80.0 (75.0–87.5)	80.0 (75.0–85.0)	80.0 (75.0–90.0)	NS
G 0′ [mmol/L]	5.8 (5.2–6.3)	6.0 (5.4–6.4)	5.7 (5.1–6.3)	NS
G 120′ [mmol/L]	6.8 (5.6–7.8)	6.9 (5.7–7.2)	5.9 (5.4–8.3)	NS
Mean G [mmol/L]	6.2 (5.4–7.1)	6.4 (5.8–6.8)	5.9 (5.3–7.3)	NS
HbA_{1c} [%]	6.0 (5.6–6.4)	6.2 (5.8–6.5)	5.9 (5.5–6.4)	NS

Note: Data are presented as median with interquartile range (lower–upper).
InsS: insulin sensitive group; InsR insulin resistant group; NS: not significant; BMI: body mass index; FAT: fat tissue; SBP: systolic blood pressure; DBP: diastolic blood pressure; G0′: fasting glucose; G120′: glucose at 120 min during oral glucose tolerance test; Mean G: mean glucose concentration from the oral glucose tolerance test; HbA_{1c}: glycated hemoglobin; *n*: number.

the elderly participants, InsS, and InsR groups are presented in Table 2. There were no gender differences in the IR and IS groups, due to insulin concentration and insulin resistance indices. Therefore, both genders were investigated together. Table 3 shows lipid profile and oxidant-antioxidant markers in the InsS, InsR, and all investigated peoples. Lipids and antioxidant defense system markers did not differentiate the groups: InsS and InsR elderly, otherwise good health individuals. In the InsR elderly group, the FAT was increased ($P < 0.000003$) and TBARS concentration decreased ($P = 0.008$) in comparison with InsS group.

Correlation analysis considering oxidant-antioxidant stress markers and other parameters, in both investigated groups, was calculated (Table 4). All correlation was done at $P < 0.05$.

In InsS elderly subjects, SOD-1 did not significantly correlate with any of the investigated parameters. In that group, an inverse correlation was found for TAS and FAT ($r = -0.496$), TAS and glucose concentrations: fasting, during OGGT and mean glucose ($r = -0.584$, $r = -0.481$, and $r = -0.566$, respectively). In addition, in InsS group, a positive correlation for TBARS and glucose concentrations: fasting, during OGGT, and mean glucose were calculated ($r = 0.509$, $r = 0.576$ and $r = 0.562$, respectively).

In InsR elderly group, the bivariate correlation coefficients showed positive correlations for SOD-1 and DBP ($r = 0.388$) and SOD-1 and LDL-C ($r = 0.487$), SOD-1 and TAS ($r = 0.434$). Moreover, positive bivariate correlations was found for TAS and G120′ and TAS and mean glucose concentrations ($r = 0.516$ and $r = 0.415$, respectively), for TAS and Ins120′ and TAS and mean insulin levels ($r = 0.542$ and $r = 0.525$, respectively), and for TAS and LDL-C and TAS and non-HDL-C ($r = 0.475$ and $r = 0.385$, respectively). Weak negative correlation was found for TAS and Matsuda index ($r = -0.398$). Concerning oxidative stress marker, in the InsR group positive correlation, was found for TBARS and Ins120′ and TBARS and mean insulin levels ($r = 0.395$ and $r = 0.395$, respectively), for TBARS and HbA_{1c} and TBARS and TAG and TBARS and non-HDL-C ($r = 0.442$, $r = 0.368$ and $r = 0.367$, respectively). Negative correlation for TBARS and age and TBARS and HDL-C ($r = -0.421$ and $r = -0.400$, respectively) were calculated.

Discussion

Although insulin-resistance is usually defined as a value greater than the 75th percentile value for non-diabetic subjects according to the World Health Organization (WHO),[20] the cut-off values of different insulin-resistance indices reported in the literature vary widely.[21] The IR is the easiest insulin-resistance index to obtain and thus in the general practice may facilitate decision-making. We investigated elderly subjects due to IR index assuming a cut-off point of 0.3 and compared more insulin-resistance indices with oxidant-antioxidant markers. Some studies investigated oxidant-antioxidant balance due to obesity and HOMA-IR index among elderly who declare to be health.[22] However, to our knowledge, this is the first study to examine the oxidant-antioxidant balance associated with insulin-resistance in elderly otherwise clinically investigated to be health subjects.

Decline in tissue function is seen while we age and is increased in disease burden.[23–25] With aging it is seen modifications in cellular composition, endocrine signaling, and tissue distribution which may play principal role in the progression of insulin resistance, metabolic dysfunction, and oxidative stress.[26–28]

Table 2. Insulin and insulin resistance indices in investigated groups.

	All elderly participants free of diabetes $n = 86$	Elderly InsS group $n = 35$	Elderly InsR group $n = 51$	Significant difference
Ins 0′ [mU/L]	31.6 (17.3–36.2)	15.8 (14.0–22.5)	34.6 (31.9–40.8)	0.000000...
Ins 120′ [mU/L]	67.1 (33.6–118.7)	52.2 (23.1–125.3)	81.7 (48.7–112.2)	NS
Mean Ins [mU/L]	46.6 (31.7–71.4)	37.1 (22.3–70.0)	54.6 (39.1–72.9)	0.014
IR	0.31 (0.17–0.35)	0.16 (0.12–0.22)	0.35 (0.32–0.39)	by deff.
HOMA-IR	7.38 (4.44–9.95)	4.34 (3.02–5.77)	8.46 (7.23–11.22)	0.000000...
QUICKI	0.45 (0.42–0.50)	0.50 (0.47–0.55)	0.44 (0.42–0.45)	0.000000...
Matsuda	2.64 (1.90–3.68)	3.68 (3.18–5.65)	2.29 (1.73–2.81)	0.000073

Note: Data are presented as median with interquartile range (lower–upper).
InsS: insulin sensitive group; InsR: insulin resistant group; NS: not significant; Ins 0′: fasting insulin; Ins 120′: insulin concentration at 120 min during oral glucose tolerance test; Mean Ins: mean insulin concentration from the Oral Glucose Tolerance Test; HOMA-IR: homeostasis model assessment for insulin resistance; QUICKI: quantitative insulin sensitivity check index; Matsuda: the insulin sensitivity index obtained from an oral glucose tolerance test; by deff: by definition.

Table 3. Lipid profile and oxidant-antioxidant markers in the studied groups.

	All elderly participants free of diabetes $n = 86$	Elderly InsS group $n = 35$	Elderly InsR group $n = 51$	Significant difference
T-C [mmol/L]	5.17 (4.64–6.69)	5.15 (4.63–5.69)	5.12 (4.65–5.69)	NS
TAG [mmol/L]	1.36 (0.79–1.81)	1.04 (0.79–1.40)	1.29 (0.79–2.06)	NS
HDL-C [mmol/L]	1.66 (1.31–1.78)	1.70 (1.45–1.79)	1.53 (1.28–1.77)	NS
HDL-C females [mmol/L]	1.70 (1.32–1.82)	1.78 (1.68–1.97)	1.65 (1.28–1.81)	NS
HDL-C males [mmol/L]	1.48 (1.26–1.66)	1.45 (1.26–1.66)	1.48 (1.31–1.63)	NS
LDL-C [mmol/L]	2.90 (2.49–3.45)	3.00 (2.45–3.43)	2.89 (2.67–3.46)	NS
Non-HDL [mmol/L]	3.49 (2.94–4.00)	3.42 (2.85–3.67)	3.39 (3.03–4.00)	NS
TBARS [μmol/L]	2.0 (1.7–2.4)	2.2 (2.0–3.0)	1.9 (1.7–2.2)	$P = 0.008$
SOD-1 [U/g HGB]	1051.4 (865.8–1336.7)	980.4 (886.7–1221.9)	1120.8 (809.7–1376.6)	NS
TAS [mmol/L]	1.3 (1.2–1.6)	1.3 (1.1–1.9)	1.3 (1.3–1.5)	NS

Note: Data are presented as median with interquartile range (lower–upper).
InsS: insulin sensitive group; InsR: insulin resistant group; NS: not significant; T-C: total cholesterol; TAG: triacylglycerols; HDL-C: high density lipoproteins cholesterol; LDL-C: low density lipoproteins cholesterol; nonHDL: non high density lipoproteins; AI: atherosclerotic index; AIP: atherosclerotic index of plasma; TBARS: thiobarbituric acid-reacting substances; SOD-1: superoxide dismutase; TAS: total antioxidant status; HGB: hemoglobin.

The study of Kozakiewicz *et al*.[29] clearly showed age-related changes including increase in intensity of TBARS with the simultaneous decrease of crucial activity of antioxidative enzymes. The present work investigated only non-smoking, healthy elderly patients with or without insulin-resistancy, and we found that insulin-resistant otherwise with good health elderly persons had lower TBARS concentration in comparison with insulin sensitive ones.

The percentage of body fat in investigated elderly persons free of other diseases was higher in the insulin-resistant elderly group. This is with agreement with the work of Karakelides *et al*.[30] who demonstrated that age-related reductions in insulin sensitivity are likely due to an age-related increase in adiposity. Moreover Karakelides *et al*. suggested that insulin-resistance is rather not a consequence of advanced chronological age. However, McLaughin *et al*.[31] noted that insulin resistance may occur even in individuals with normal BMI, not only in obese individuals. Our elderly persons both insulin sensitive and insulin resistant had comparable waist circumference, but insulin-resistant subjects had higher BMI and percentage of body fat. Both groups did not exceed the BMI 35 kg/m^2. Yet, obesity, diabetes, or both enhance and preserve the insulin resistance, inflammatory state, and oxidative stress.[32]

Palmer and Kirkland[33] argued that age-related disorders are more widespread in obese than lean individuals and suggested that obesity makes susceptible to age-related disease and is, in some way, a state of premature aging. He asked if adipose tissue dysfunction, seen in both aging and obesity, might be the same nominative case. In this context, our study showed no significant difference in antioxidant defense in insulin-resistant increased body mass older persons in comparison with insulin-sensitive ones. Moreover, only in insulin-resistant elderly group, there was inverse relationship for TBARS and HDL-C. The low molecular weight antioxidants, which HDL-C is agent, have been shown to be modified in elderly, which may lead to the reinforcement of oxidative stress and in this way to the development of aging.[34]

Another result of note was the positive correlation of antioxidant defense systems: SOD-1 and TAS only in elderly with insulin-resistance. Since TAS reflects a combination of various antioxidant extracellular defenses and SOD-1 reflects intracellular antioxidant defense, our findings suggest that only during the development of the improper

Table 4. The correlations between oxidative stress markers and clinical and biochemical parameters in the studied subjects.

	InsS			InsR		
Variable	**SOD-1**	**TAS**	**TBARS**	**SOD-1**	**TAS**	**TBARS**
Age	−0.136	−0.306	−0.028	−0.174	−0.211	**−0.421**
Weight	−0.136	0.056	0.155	0.086	0.171	0.052
Height	0.019	0.244	−0.142	−0.068	−0.093	0.275
BMI	−0.158	−0.221	0.323	0.154	0.314	−0.158
Waist	−0.081	0.063	0.064	−0.002	0.201	0.163
FAT	−0.165	**−0.496**	0.310	−0.070	−0.072	−0.174
SBP	−0.171	−0.144	0.191	0.167	0.124	0.277
DBP	−0.161	0.104	−0.390	**0.388**	0.220	0.074
G0′	−0.290	**−0.584**	**0.509**	−0.191	0.140	0.216
G120′	−0.294	**−0.481**	**0.576**	0.148	**0.516**	0.252
Mean G	−0.342	**−0.566**	**0.562**	0.118	**0.415**	0.251
Ins 0′	−0.149	0.175	−0.094	−0.201	0.140	0.175
Ins 120′	−0.156	−0.186	0.079	0.296	**0.542**	**0.395**
Mean Ins	−0.139	−0.156	0.009	0.276	**0.525**	**0.395**
IR	−0.047	0.234	−0.305	−0.051	0.102	0.216
HOMA	−0.178	0.010	0.028	−0.194	0.128	0.201
QUICKI	0.178	−0.010	−0.028	0.194	−0.128	−0.201
Matsuda	0.205	0.187	−0.066	−0.055	**−0.398**	−0.354
HbA_{1c}	−0.223	−0.396	0.360	0.026	0.336	**0.442**
T-C	0.128	−0.068	−0.094	0.273	0.230	0.312
TAG	0.108	0.132	−0.135	−0.160	0.154	**0.368**
HDL-C	0.059	−0.238	−0.268	−0.009	−0.286	**−0.400**
LDL-C	0.099	0.024	0.078	**0.487**	**0.475**	0.277
Non-HDL-C	0.064	−0.006	0.013	0.327	**0.385**	**0.367**
SOD-1	–	0.408	−0.279	–	**0.434**	0.100
TAS	0.408	–	−0.416	**0.434**	–	0.242
TBARS	−0.279	−0.416	–	0.100	0.242	–

Note: Significance at $P < 0.05$ are highlighted.
InsS: insulin sensitive group; InsR: insulin resistant group; BMI: body mass index; FAT: fat tissue; SBP: systolic blood pressure; DBP: diastolic blood pressure; G0′: fasting glucose; G120′: glucose at 120 min during oral glucose tolerance test; Mean G: mean glucose concentration from the oral glucose tolerance test; HbA_{1c}: glycated hemoglobin; Ins 0′: fasting insulin; Ins 120′: insulin concentration at 120 min during oral glucose tolerance test; Mean Ins: mean insulin concentration from the oral glucose tolerance test; HOMA-IR: homeostasis model assessment for insulin resistance; QUICKI: quantitative insulin sensitivity check index; Matsuda: the insulin sensitivity index obtained from an oral glucose tolerance test; T-C: total cholesterol; TAG: triacylglycerols; HDL-C: high density lipoproteins cholesterol; LDL-C: low density lipoproteins cholesterol; nonHDL: non high density lipoproteins; TBARS: thiobarbituric acid-reacting substances; SOD-1: superoxide dismutase; TAS: total antioxidant status.

condition, such is insulin-resistance, intensive antioxidant protection is needed.[35]

Evidence indicates that elevated body fat and hypertension are responsible for increased oxidative stress in elderly persons.[36,37] So far, it should be noted that, there have not been reports documenting healthy elderly persons only with insulin-resistance and higher damage oxidative marker (TBARS) which correlates negatively with age. Moreover, it is particularly interesting that our investigated elderly population had higher TBARS in insulin-sensitive ones. It seems that alternative mechanisms other than insulin-resistance would be necessary to induce an increase in TBARS in healthy elderly peoples.

Concerning insulin-resistance, there is a defined relationship between raised fat mass, glycaemia, chronic low-grade systemic inflammation, and increased oxidative stress.[38,39] In that context, our elderly insulin-resistant individuals had positive correlation between TBARS and post load glucose concentration and insulin level as well with glycated hemoglobin. Moreover, in insulin-resistant ones, we found intensive total antioxidant defense activation with rising glucose, while in insulin-sensitive ones, the inverse relationship with TAS and glycaemia was noted. It indicates that separate mechanisms maintain stability in elderly insulin-sensitive individuals.

We suggest that in insulin-resistant elderly persons, the pathological events expansion go through induction of antioxidant defense systems on extra- and intracellular levels and increased influence of insulin action on oxidative stress.

Limitations of the study

This study is limited by some factors and should be interpreted with caution.

First of all, the research was done on elderly white population, thus it is impossible to generalize to world elderly population.

Second, the inclusion criteria were no special diet or supplements and exclusion criteria were no drugs with antioxidant capacity, but we did not calculate dietary antioxidant intake.

Third, it is known that lipids are valuable tool for stratification cardiovascular risk and we investigated crosstalk between oxidant-antioxidant markers and lipids in the context of insulin-resistance in elderly population. In older

persons such tool may facilitate decision-making of the general practitioner. Yet, lipids are quickly available for cardiovascular risk markers, and in the research we did not use more specific indicators of cardiovascular disease.

Therefore, greater number of subjects, not only white population, inclusion criteria of dietary intake, and more specific cardiovascular indicators are needed in the further studies to highlight the general population and confirm the possible clinical involvements and implications.

Conclusions

The present scientific data dispassionately and in an accessible way allow readers to make their own judgments to answer the question is insulin-resistance and oxidative stress cause or consequence of aging. In elderly individuals, oxidative stress persists irrespective of insulin-resistance status. We suggest that increased oxidative stress may be the consequence of old age. An insulin action identifies those at high risk for atherosclerosis, via congruent associations with oxidative stress and extra- and intra-cellular antioxidant defense systems. Thus, we maintain that insulin-resistance is not the cause of aging.

Authors' contributions: All authors contributed in writing the paper, reviewed the results and approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and/or integrity of any part of the work.

DECLARATION OF CONFLICTING INTERESTS

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