Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 18: 21–27
DOI 10.1007/5584_2015_166

Published online: 6 November 2015

Oxidative Stress and Nitric Oxide in Sedentary Older Adults with Intellectual and Developmental Disabilities

E. Carmeli, A. Bachar, O. Rom, and D. Aizenbud

Abstract

Individuals with moderate-to-profound intellectual and developmental disabilities (IDD) are characterized by significant cognitive deficits, abnormal muscle tone, poor posture and balance, and inactive lifestyle. Increased oxidative stress (OS) has been implicated in a variety of chronic diseases, inflammatory conditions, aging, and even following intense physical exercise. Nitric oxide (NO) is a highly reactive mediator that has been shown to play different roles in a variety of different biological process and in aging. The aim of the study was to investigate the serum levels of global OS and NO metabolites (NOx) in sedentary and non-sedentary older adults with IDD. Global OS was measured by CR 3000 instrument, FORM system, and NOx were measured by determination of serum nitrite levels. OS and NOx levels were significantly higher in sedentary IDD comparing non-sedentary controls. The increased of OS and NOx levels suggest their possible involvement in the phenomenon of 'accelerated aging' in IDD. Our findings can provide another aspect indicating both OS and NOx as possible biochemical markers and their potential application in minimizing their negative influence through future therapeutic strategies.

E. Carmeli (✉)
Department of Physical Therapy, University of Haifa, 199 Aba Khoushy Ave., Mount Carmel, Haifa, Israel
e-mail: ecarmeli@univ.haifa.ac.il

A. Bachar
Department of Nursing, Neve Ram Residential Care Center, Rechasim, Israel

O. Rom
Department of Anatomy and Cell Biology, Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel

D. Aizenbud
Department of Anatomy and Cell Biology, Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel

Department of Orthodontic and Craniofacial Anomalies, Rambam Health Care Campus, Haifa, Israel

Keywords
Aging • Cognition • Intellectual disabilities • Sedentary lifestyle • Oxidative stress • Nitric oxide

1 Introduction

In recent years, the life expectancy of individuals with intellectual and developmental disabilities (IDD) has increased considerably. However, their life expectancy still remains lower than that of the general population (Coppus 2013). Individuals with IDD often experience health issues associated with aging at earlier ages and at higher rates than the general population (Dykens 2013; Heller and Sorensen 2013). The 'premature' or 'accelerated aging' process of individuals with IDD can be reflected by an increased risk of chronic diseases such as cardiovascular diseases, hypertension, type II diabetes, dementia, depression, and health changes in musculoskeletal system (Carmeli et al. 2003; Heller and Sorensen 2013). Thus, older adults with IDD are prone to develop early chronic morbidities and a sedentary lifestyle, which result in frequent hospitalization, obesity, frailty, falls, and mortality (Carmeli et al. 2003).

The treatment of patients with chronic neurological conditions including IDD has become increasingly complex for various reasons. One of the main reasons is the presence of dual neurological diagnoses (i.e., epilepsy, Down syndrome, Alzheimer's disease, and schizophrenia) or the presences of the above mentioned comorbidities (Carmeli et al. 2003; Flanagan et al. 2007). A sedentary lifestyle, common among older adults with IDD, may increase the risk for some of these comorbidities such as obesity, osteoporosis, sarcopenia, and cardiovascular diseases (Heller and Sorensen 2013).

The majority of aged persons with IDD lives at residential care centers and benefit from a social, physical, psychological, and occupational support as well as recreation activities (Merrick et al. 2006). However, health promotion and morbidity prevention among aged individuals with IDD has received little attention from policy makers in public health and welfare authorities. Indeed, health promotion interventions for adults with IDD, which focus on physical activity and exercise, have been shown to have positive effects such as improved fitness, lower blood pressure, and weight loss (Heller and Sorensen 2013). In our recent publication we have found, quote: "*low participation in physical activity for persons with IDD*" (Carmeli et al. 2012).

Oxidative stress (OS) is thought to play a central role in neuro-pathologies, metabolic diseases, aging, strenuous physical activity, poor nutrition, and can also be a result of exposure to toxins (e.g., drugs, cigarette smoking, alcohol, etc.) or pathogens (Garcez et al. 2005; Valko et al. 2007). High levels of free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) are present in conditions of oxidative stress (Floyd et al. 2000). Nitric oxide (NO) is an inflammatory mediator and an important free radical. NO plays a crucial role in the homeostatic regulation of the cardiovascular, neuronal, and immune systems (Mangge et al. 2014). NO also serves as a second messenger in various physiological processes including neurotransmission in the CNS, maintenance of vasodilator tone, and arterial pressure (Czapski et al. 2007).

Despite these important physiological functions, oxidized metabolites of nitrogen (NOx) are well-known toxic agents, being one of the constituents of air pollution and cigarette smoke (Grisham et al. 1999; Krause 2007). In biological systems, NO decomposes to nitrite and nitrate. NO is produced by mammalian cells and is constitutively synthesized by endothelial NO synthase (eNOS) and by inducible neuronal NO synthetase (iNOS). Excessive iNOS levels can be found in the hypothalamus and in aging (Floyd and Hensley 2000; Ferrini et al. 2001). Increased iNOS levels are also evident in various chronic inflammatory diseases

Table 1 Characteristics of participants

	Sedentary group (12)		Active group (10)	
	Mean ± SD	Range	Mean ± SD	Range
Sex (F/M)	8/4	–	7/3	–
Age (yr)	57 ± 5	51–63	55 ± 4	50–60

such as arthritis, obesity, hepatitis, inflammatory bowel diseases, and septic and hemorrhagic shock. Therefore, NO relates to ROS and RNS that are involved in oxidative stress and brain damage (Floyd et al. 2000).

The aim of this study was to investigate the serum levels of OS and NOx in active and sedentary older adults with IDD. We hypothesized that higher levels of OS and NOx are present among sedentary older adults with IDD. Higher levels of OS and NOx may play a role in the on-going pathological processes among older adults with IDD, leading to deterioration of their function and accelerated aging.

2 Methods

2.1 Participants

This cross-sectional study received prior approval by the Institutional Ethic Committee of Residential Care Centers under the administrative control of the Israeli Ministry of Welfare. A written consent received from guardians. The sample consisted of permanent residents who lived in residential care centers. Inclusion criteria included: (1) moderate-to-profound IDD diagnosed within 1–3 years after birth by IQ-scores defined by the Wechsler Abbreviated Scale of Intelligence (Harcourt Assessment Inc., San Antonio) (Hays et al. 2002); (2) sedentary behavior in most of the day hours (i.e., bedbound or wheelchair); (3) aged 50 or older. Exclusion criteria included: (1) diagnosis of a specified moderate mental retardation such as fragile X and Down syndrome; (2) clinical history of neurological diseases (e.g., Parkinson's disease, stroke, Alzheimer's disease, neuropathy, or brain surgery); (3) presence of peripheral neurological symptoms; (4) medical history such as pulmonary disease, type II diabetes mellitus, chronic or acute heart failure, adrenal or kidney diseases, and smoking. None of the participants received narcotic medications or glucocorticoids during the time of the experiment.

From a population sample of 253 permanent residents with IDD, 23 met the inclusion criteria. Twelve potential participants were randomly selected and included in the study as the experimental group (sedentary group). Ten IDD individuals that were independent in activities of daily living and were characterized with a mild active lifestyle served as the control group (active group). The characteristics of the participants are shown in Table 1.

2.2 NOx Assay

NOx were measured in the serum obtained after night sleep. The assay followed the exact protocol as previously described (Carmeli et al. 2009). Sera from all participants were provided by a nurse practitioner. A total of 6 mL of venous blood samples were collected after an overnight fasting and were drawn into 'vacutainer' 10 mL tubes for metabolite assays, which did not contain any additives. Briefly, 40 mL of each sample were transferred to 96-well plates and mixed with 40 μL of the reduction solution (NADPH 1.25 ng/μL; FAD 10.4 ng/μL; KH_2PO_4 0.125 M) containing 0.5 U of NO_2 reductase for 2 h at 37 °C. After this time, 80 μL of Griess reagent (one part 0.1 % N-1-naphthyl-ethylene-diamine dihydrochloride in water and one part 1 % sulfanilamide in 3 % concentrated H_3PO_4) were added to each well. The mixture was incubated for 5 min at room temperature and read at 540 nm in a microplate reader. Concentrations were determined by a standard curve of sodium nitrite. The detection limit of the assay was 1 mM of nitrite.

2.3 Global Oxidative Stress Analysis

This assay followed the same protocol as previously describe (Carmeli et al. 2008). The analyses were carried out using CR 3000 instrument (FORM system, Catellani Group, Callegari S.p.A, Parma, Italy). All measurements were performed within 0.3–1 h post blood drawing. The CR 3000 instrument includes software and photometers (505 nm) with one or three reading cells for the determination of primary tests on whole capillary blood. The CR 3000 kits include ready filled cuvettes (4.8 pH buffered chromogen) that are bar coded (so that the reader automatically recognizes the test about to be performed and the *k*-factor), and capillaries for blood collection. The prepared reagents are able to promote the iron-catalyzed Haber-Weiss reaction. After an overnight fast (8–10 h), and between 08:00 and 09:00 a.m., a total of one drop of capillary blood samples, approximately 10 μl, were drawn from the subject's finger, and inserted into the prepared cuvette. The cuvette was then gently rocked several times until the sample was completely diluted. The sample was then placed in the square cuvette which contained the solid chromogen mixture, after closing the cuvette with its cap-on, it was shaken for 30 s until the reactive has dissolved. Then placed in the table centrifuge for 1 min (at room temperature), then placed in the reading cell.

Oxidants profile is measured by a colorimetric test based on the ability of transition metals, such as iron, to catalyze the breakdown of hydroperoxides into derivative radicals according to Fentons and Haber-Weiss' reactions shown below:

$$\text{R-OOH} + \text{Fe}^{2+} \xrightarrow{\text{A}} \text{R-O}^{\cdot} + \text{OH}^{-} + \text{Fe}^{3+}$$

$$\text{R-OOH} + \text{Fe}^{3+} \xrightarrow{\text{B}} \text{R-OO}^{\cdot} + \text{H}^{+} + \text{Fe}^{2+}$$

$$\text{RO}^{\cdot} + \text{ROO}^{\cdot} + 2\text{CrNH}_2 \xrightarrow{\text{C}} \text{RO}^{-} + \text{ROO}^{-} + \left[\text{Cr} - \text{NH}_2^{+\cdot}\right]$$

Reference values are

160–200 = 1.22–1.58 mmol/l H_2O_2 – Excellent

201–250 = 1.59–1.97 mmol/l H_2O_2 – Very good-Good

251–310 = 1.98–2.17 mmol/l H_2O_2 – Good – Fair

311–500 = 2.18–2.36 mmol/l H_2O_2 – Bad

2.4 Statistical Analysis

A group comparison was assessed using a two-tailed Mann-Whitney U test (for abnormally distributed values) and a *t*-test (for normal distributed values). Data were expressed as means ± SD. Analyses were performed using the SPSS statistical package (ver. 12 for Windows). Differences were considered significant if $p < 0.05$.

Due to a small sample size, the effect of size was used to quantify differences in OS and NOx measures between the participants. The size-effect for each measure was computed as standardized mean differences in the score change between the two groups, namely, $(d_1 - d_2)/\delta_p$, where d_1 and d_2 denote the scores of the sedentary and active groups, respectively, and δ_p is the pooled standard deviation of the score difference. For the sake of completeness, despite the small sample size, we also conducted a two sample *t*-test and Mann-Whitney *U* test for the mean score differences (between the final groups).

3 Results

3.1 Level of Global Oxidative Stress

The CR3000 results for global oxidative stress (OS) in IDD participants were significantly higher in the sedentary group when compared with the active control group (Fig. 1).

3.2 Level of Oxidized Metabolites of Nitrogen

Oxidized metabolites of nitrogen (NOx) were significantly higher in sedentary group when

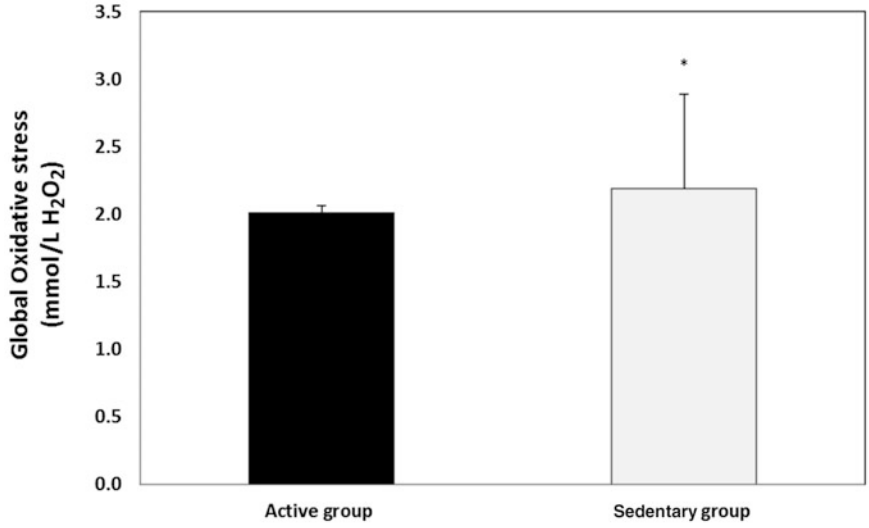

Fig. 1 Global oxidative stress in active *vs.* sedentary individuals with intellectual and developmental disabilities (IDD); *p $<$ 0.05 *vs.* the active group

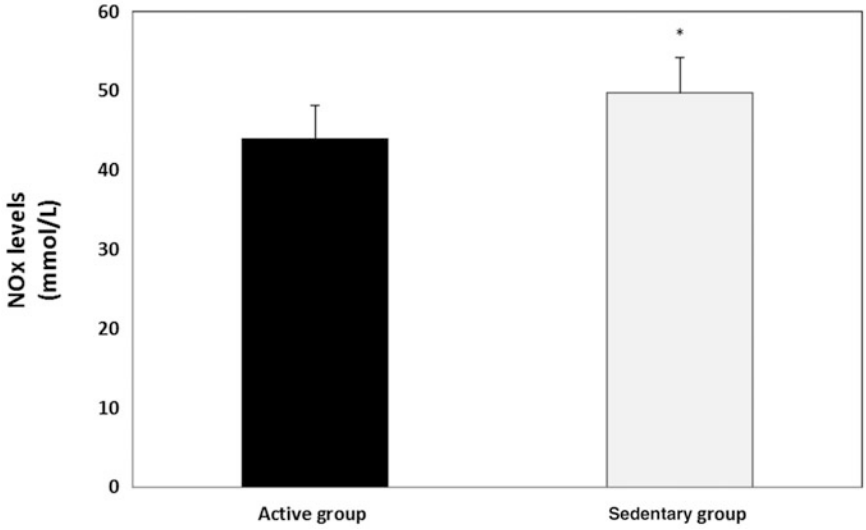

Fig. 2 NO_x in active *vs.* sedentary individuals with intellectual and developmental disabilities (IDD); *p $<$ 0.05 *vs.* the active group

compared with active control group (Fig. 2). The mean NOx level of females and males in both groups were not statistically different. In the sedentary group, the level of OS correlated significantly with NOx level of the same participants at the same point of time ($r = 0.78$, $p < 0.05$). Insignificant correlation was found between NOx and OS in the active control group ($r = 0.45$).

4 Discussion

Early aging, chronic morbidities, and a sedentary lifestyle are more common among older adults with IDD. Higher levels of OS and NOx may promote various pathological processes and accelerated aging in older adults with IDD. The aim of the present study was to assess the OS and

NOx levels in sera of active and sedentary older adults with IDD.

Despite research progress in the field of early-onset aging among IDD persons, our knowledge and understanding of what mechanisms are involved is still ambiguous. One of the main factors that attribute to accelerated aging, particularly among IDD persons, is a passive lifestyle or lack of physical activity. The consequences of physical activity on one hand, and physical inactivity on the other hand are widely known. Metabolic and physiologic adaptation to explain inactivity is generally supported by a model of metabolic homeostasis based on 'use it or lose it' assumption (Baskin et al. 2015). Following prolong inactive periods, certain biological pathways are induced such as apoptosis and inflammation, and often they are irreversible, especially in the aged (Russ and Lanza 2011).

Among the most prominent signal transduction inducers of apoptosis there are a few molecules accountable to OS. Because an analysis of a single molecule does not reflect the complexity of passive lifestyle, a multivariate approach is required to better illustrate the complex dynamic networks of physical inactivity. In the present study, high serum levels of circulatory NOx and systemic OS were revealed in sedentary older adults with IDD. Oxidative stress is thought to have a pivotal role not only in a number of chronic diseases, but also in aging and in physical inactivity (Lawler and Hindle 2011). In the present study, a high serum global OS concentration was demonstrated in healthy, yet sedentary IDD people. Therefore, we suggest that there is a direct mechanistic link among the systemic OS and inactivity status.

The role of NO in many diseases is controversial, yet most researchers agree that measuring NOx levels in the serum is related to NO production (Andrukhov et al. 2013). There are both direct effects of NO, mediated by the NO molecule itself, and indirect effects of NO, mediated by reactive nitrogen species produced by the interaction of NO with superoxide radicals. High serum concentrations of NOx induce toxicity by tyrosine residues and by inducing lipid peroxidation which might lead to vascular atherosclerosis in the brain, heart, and kidneys (Eiserich et al. 1998).

A high level of NOx was observed in muscles of young sedentary subjects (Saltin 2007). The levels of OS and NOx are progressively increasing with aging. Aging and inactivity have synergetic and harmful effects leading to biological and physiological damage. As reported by Nyberg et al. (2012), lifelong physical activity opposes this harmful effect. Thus, accumulation of NOx and free radicals with aging can be attenuated by maintaining a physically active lifestyle. Higher levels of OS and NOx in sedentary individuals with IDD in comparison with active individuals with IDD are explainable by the 'cytokine hypothesis of inactivity' (Dammann and Leviton 1998), or 'diseasome of physical inactivity' (Pedersen 2009). According to these hypotheses, disuse of skeletal muscles releases cytokines that induce inflammation, causing muscle atrophy and fat accumulation.

The main limitation of the present study relates to the controversy regarding the specificity of OS serum values. Moreover, if OS values are increased, it has been recommended that a more specific assay such as HPLC should be performed, which can be measured by fluorometry or spectrophotometry with higher sensitivity (Mehanna et al. 2011). Yet, the statistical deviation percentages in the present results confirmed that measuring OS with CR3000 is accurate enough. Because the source of NOx is not only endothelial cells (eNOS), but also macrophages (iNOS) and neuronal cells (nNOS), the serum level of NOx may reflect the status of eNOS and, to a small extent, that of iNOS.

5 Conclusions

Serum oxidative stress and NOx levels in sedentary older individuals with intellectual and developmental disabilities are elevated in comparison to active controls. These results indicate that inactive lifestyle may be comparable to a pathological condition of a disease. Future programs of health promotion and morbidity prevention in patients with intellectual and developmental disabilities should focus on healthy behaviors

such as physical activity, but also on nutritional regimes and anti-inflammatory drugs or supplements for either improving the anti-oxidant defense mechanism or decreasing the accumulation of free radicals.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

References

Andrukhov O, Haririan H, Bertl K, Rausch WD, Bantleon HP, Moritz A, Rausch-Fan X (2013) Nitric oxide production, systemic inflammation and lipid metabolism in periodontitis patients: possible gender aspect. J Clin Periodontol 40(10):916–923

Baskin KK, Winders BR, Olson EN (2015) Muscle as a 'Mediator' of systemic metabolism. Cell Metab 21 (2):237–248

Carmeli E, Merrick J, Kessel S, Masharawi Y, Carmeli V (2003) Elderly persons with intellectual disability: a study of clinical characteristics, functional status, and sensory capacity. Sci World J 3:298–307

Carmeli E, Harpaz Y, Kogan NN, Fogelman Y (2008) The effect of an endogenous antioxidant glabridin on oxidized LDL. J Basic Clin Physiol Pharmacol 19 (1):49–63

Carmeli E, Beiker R, Morad M (2009) Nitric oxide and interlukin-6 levels in intellectual disability adults with epilepsy. Res Dev Disabil 30(3):567–571

Carmeli E, Merrick J, Imam B, Levy R (2012) Exercises and sports participation in healthy older adults with intellectual disability – a pilot study. Health 4 (9A):769–774

Coppus AM (2013) People with intellectual disability: what do we know about adulthood and life expectancy? Dev Disabil Res Rev 18(1):6–16

Czapski GA, Cakala M, Chalimoniuk M, Gajkowska B, Strosznajder JB (2007) Role of nitric oxide in the brain during lipopolysaccharide-evoked systemic inflammation. J Neurosci Res 85:1694–1703

Dammann O, Leviton A (1998) Infection remote from the brain, neonatal white matter damage, and cerebral palsy in the preterm infant. Semin Pediatr Neurol 5:190–201

Dykens EM (2013) Aging in rare intellectual disability syndromes. Dev Disabil Res Rev 18(1):75–83

Eiserich JP, Patel RP, O'Donnell VB (1998) Pathophysiology of nitric oxide and related species: free radical reactions and modification of biomolecules. Mol Asp Med 19(4–5):221–357

Ferrini M, Wang C, Swerdloff RS, Sinha-Hikim AP, Rajfer J, Gonzalez-Cadavid NF (2001) Aging-related increased expression of inducible nitric oxide synthase and cytotoxicity markers in rat hypothalamic regions associated with male reproductive function. Neuroendocrinology 74(1):1–11

Flanagan J, Melillo KD, Abdallah L, Remington R (2007) Interpreting laboratory values in the rehabilitation setting. Rehabil Nurs 32:77–84

Floyd RA, Hensley K (2000) Nitrone inhibition of age-associated oxidative damage. Ann N Y Acad Sci 899:222–237

Floyd RA, Hensley K, Bing G (2000) Evidence for enhanced neuro-inflammatory processes in neurodegenerative diseases and the action of nitrones as potential therapeutics. J Neural Transm Suppl 60:387–414

Garcez ME, Peres W, Salvador M (2005) Oxidative stress and hematologic and biochemical parameters in individuals with Down syndrome. Mayo Clin Proc 80(12):1607–1611

Grisham MB, Jourd'Heuil D, Wink DA (1999) Nitric oxide. I. Physiological chemistry of nitric oxide and its metabolites: implications in inflammation. Am J Physiol 276(2 Pt 1):G315–G321

Hays JR, Reas DL, Shaw JB (2002) Concurrent validity of the Wechsler abbreviated scale of intelligence and the Kaufman brief intelligence test among psychiatric inpatients. Psychol Rep 90:355–359

Heller T, Sorensen A (2013) Promoting healthy aging in adults with developmental disabilities. Dev Disabil Res Rev 18(1):22–30

Krause KH (2007) Aging: a revisited theory based on free radicals generated by NOX family NADPH oxidases. Exp Gerontol 42(4):256–262

Lawler JM, Hindle A (2011) Living in a box or call of the wild? Revisiting lifetime inactivity and sarcopenia. Antioxid Redox Signal 15(9):2529–2541

Mangge H, Becker K, Fuchs D, Gostner JM (2014) Antioxidants, inflammation and cardiovascular disease. World J Cardiol 6(6):462–477

Mehanna C, Baudouin C, Brignole-Baudouin F (2011) Spectrofluorometry assays for oxidative stress and apoptosis, with cell viability on the same microplates: a multiparametric analysis and quality control. Toxicol in Vitro 25(5):1089–1096

Merrick J, Kandel I, Stawski M (2006) Trends in mental health services for people with intellectual disability in residential care in Israel 1998–2004. Isreal J Psychiatry Relat Sci 43:281–284

Nyberg M, Blackwell JR, Damsgaard R, Jones AM, Hellsten Y, Mortensen SP (2012) Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans. J Physiol 590(Pt 21):5361–5370

Pedersen BK (2009) The diseasome of physical inactivity-and the role of myokines in muscle-fat cross talk. J Physiol 587(Pt 23):5559–5568

Russ DW, Lanza IR (2011) The impact of old age on skeletal muscle energetics: supply and demand. Curr Aging Sci 4(3):234–247

Saltin B (2007) Exercise hyperaemia: magnitude and aspects on regulation in humans. J Physiol 583 (Pt 3):819–823

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39(1):44–84