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Unhealthy diet and ultrafine carbon black particles induce senescence and disease associated phenotypic changes

Nicole Büchner [a,1], Niloofar Ale-Agha [a,b,1], Sascha Jakob [a,1], Ulrich Sydlik [b], Kerstin Kunze [a], Klaus Unfried [b], Joachim Altschmied [a], Judith Haendeler [a,*]

[a] Molecular Aging Research, IUF — Leibniz Research Institute for Environmental Medicine at the University of Duesseldorf gGmbH, 40225 Duesseldorf, Germany
[b] Molecular Toxicology, IUF — Leibniz Research Institute for Environmental Medicine at the University of Duesseldorf gGmbH, 40225 Duesseldorf, Germany

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ABSTRACT

Diet and pollution are environmental factors known to compromise "healthy aging" of the cardiovascular and respiratory systems. The molecular consequences of this permanent burden in these cells are still unknown. Therefore, this study investigates the impact of unhealthy diet on aging-related signaling pathways of human, primary cardiovascular cells and of airborne particles on lung epithelial and human endothelial cells. Nutrition health reports have shown that the diet in industrialized countries contains more than 100 mg/dl low density lipoprotein (LDL) and a high fraction of added sugars, especially fructose. Several studies demonstrated that ultrafine particles can enter the circulation and thus may interact with endothelial cells directly. Both, dietary compounds and pollution derived particles, have been shown to increase the risk for cardiovascular diseases. To simulate an unhealthy diet, we supplemented cell culture media of human primary endothelial cells, smooth muscle cells and cardiomyocytes with LDL and replaced 1/3 of glucose with fructose. We observed hypertrophy in cardiomyocytes, enhanced proliferation in smooth muscle cells and increased senescence, loss of endothelial nitric oxide synthase and increased nuclear FoxO3A in endothelial cells. With respect to pollution we have used ultrafine carbon black particles (ufCB), one of the major constituents of industrial and exhaust emissions, in concentrations our lungs and vessels are constantly exposed to. These concentrations of ufCB increased reactive oxygen species in lung epithelial and vascular endothelial cells and reduced the S–NO content, a marker for NO-bioavailability, in endothelial cells. NO increases activation of Telomerase Reverse Transcriptase (TERT), an enzyme essential for telomere maintenance. TERT is required for proper endothelial cell function and is inactivated by Src kinase under conditions of oxidative stress. ufCB significantly increased Src kinase activation and reduced Telomerase activity in endothelial and lung epithelial cells. As a consequence, ufCB increased senescence of endothelial cells. To investigate whether ufCB show also effects in vivo, we instilled ufCB in concentrations not inducing inflammation into mice. Indeed, eNOS expression was reduced in the abdominal aorta of animals treated with ufCB.
Thus, a combination of fructose and LDL in the diet and ufCB, as a major constituent of air pollution, seem to accelerate respiratory and cardiovascular cellular changes, which may compromise "healthy aging" and can lead to cardiovascular and pulmonary diseases.

1. Introduction

Aging can be defined as the increasing inability of the elderly human body to adapt to changes in the environment. In the cardiovascular system characteristic morphologic, structural and biochemical alterations occur with age leading to a reduction in the maximal capacity of the cardiovascular system (Lakatta, 2002). Up to now the cellular and molecular mechanisms leading to these changes with age are not well understood, because most of the studies investigated cardiovascular diseases and only epidemiological studies correlated environmental factors with diseases and/or aging. Of note, environmental factors, mostly diet and exercise, have a strong influence on the cardiovascular health status and seem to delay or accelerate cardiovascular aging (Aoi, 2009; Stanner, 2009). Nutrition

* Corresponding author at: Molecular Cell & Aging Research, IUF — Leibniz Research Institute for Environmental Medicine at the University of Duesseldorf gGmbH, Auf'm Hennekamp 50, 40225 Duesseldorf, Germany. Tel.: +49 211 3389 291; fax: +49 211 3389 331.
E-mail addresses: Nicole.Buechner@uni-duesseldorf.de (N. Büchner), Niloofar.Ale-Agha@uni-duesseldorf.de (N. Ale-Agha), Sascha.Jakob@uni-duesseldorf.de (S. Jakob), ulrich.sydlik@uni-duesseldorf.de (U. Sydlik), Kerstin-Kunze@gmx.net (K. Kunze), klaus.unfried@uni-duesseldorf.de (K. Unfried), Joachim.Altschmied@uni-duesseldorf.de (J. Altschmied), juhae001@uni-duesseldorf.de (J. Haendeler).
[1] Authors contributed equally to the work.

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health reports and dietary guidelines have shown that in industrialized countries the low density lipoprotein (LDL) levels in the blood still exceed the reference value. This has been correlated with a higher risk for cardiovascular diseases. Therefore, it is advised, but not adhered to, not to consume too many saturated fatty acids. The same holds true for added sugars, because they contain mostly fructose, which, in contrast to glucose, is taken up independently of insulin. Thus, it seems mandatory to investigate the effects of LDL and fructose on human, adult primary cells to reveal molecular mechanisms directly modulated by components of unhealthy diet, which is common in industrialized countries. Besides the negative influences of diet, air pollution seems to affect not only the lungs but also the cardiovascular system (Brook, 2008; Brook et al., 2002). Air pollution is a complex mixture of compounds in gaseous and particle phases. Although particulate and gaseous pollutants coexist and may both cause adverse health effects, the most compelling evidence defines particulate matter (PM) as the major source for human diseases (Donaldson et al., 2005). Within PM, carbon black particles produced not only by traffic and industry, but also in every household and office, have the major part (Janssen et al., 2011). Although one would think that PM causes a health risk mostly to the lungs, the overall evidence indicates that the majority of the PM effects are upon the cardiovascular system (Brook et al., 2008; Chen and Schwartz, 2008; Donaldson et al., 2005). In fact, more people seem to die from cardiovascular than from pulmonary diseases during episodes of PM pollution (Pope et al., 1999). Effects on the cardiovascular system were strongly supported by investigations showing an association between PM and hospital admission for ischemic heart disease and congestive heart failure (Schwartz and Morris, 1995) and a correlation between exposure to PM pollution and cardiovascular morbidity (Hoek et al., 2001). A major issue, however, has been to determine how the inhalation of PM into the lungs could affect the cardiovascular system. Two major hypotheses, which are not mutually exclusive, have been proposed to account for the effects of inhaled particles. One hypothesis is, that once deposited in the lung, PM initiates a local inflammation, which causes a secondary systemic inflammation via oxidative stress that sets off or exacerbates cardiovascular dysfunctions. The second hypothesis involves the documented direct passage of particles into the blood stream after inhalation. Of note, only ultrafine particles, which have per definition an aerodynamic diameter of <100 nm, have been shown to directly enter the systemic circulation (Nemmar et al., 2001, 2002, 2004; Shimada et al., 2006). Therefore, it is tempting to speculate that these ultrafine particles have direct effects on remote target tissues, especially the vasculature. Moreover, unlike fine particles, these ultrafine particles are barely recognized by phagocytic cells, such as macrophages (Borm and Kreyling, 2004). Because of this low uptake by macrophages, ultrafine particles may directly interact with endothelial cells in the circulation or lung epithelial cells and thereby could induce signaling events independent of systemic inflammation. As described above, the major constituent of PM are carbon black particles, which include fine as well as ultrafine particles. However, up to now in all cell culture and animal studies with carbon black particles only concentrations were used, which were cytotoxic or induced inflammation (Sun et al., 2009; Suwa et al., 2002; Yamawaki and Iwai, 2006). Thus, it is still unclear how ultrafine carbon black particles in concentrations we are exposed to every day affect adult epithelial and endothelial cells directly and whether signaling pathways are modified, which are involved in aging and disease.

In the present study, we determined that two potentially unhealthy dietary components, high fructose and low density lipoprotein, induce phenotypic and molecular changes in human adult primary cardiovascular cells, which were previously described as hallmarks of cardiovascular disorders and aging. Moreover, we provide for the first time evidence that non-toxic, non-inflammatory concentrations of ultrafine carbon black particles, one of the major constituents of air pollution, trigger molecular pathways in adult endothelial and epithelial cells, which are indicative of aging and/or the onset or progression of diseases.

2. Materials and methods

2.1. Particles

Ultrafine carbon black particles (ufCB, Printex 90; 14 nm in diameter) were obtained from Degussa (Frankfurt, Germany). Stock ufCB suspensions (1 mg/ml) were freshly prepared in PBS by sonication for 60 min at 50–60 Hz, 120 W. Cells were treated with concentrations of ufCB as indicated.

2.2. Cell culture

Human umbilical vein endothelial cells (EC) were purchased from Lonza (Cologne, Germany) and cultured in endothelial basal medium supplemented with hydrocortisone (1 μg/ml), bovine brain extract (12 μg/ml), gentamicin (50 μg/ml), amphotericin B (50 ng/ml), epidermal growth factor (10 ng/ml), and 10% fetal calf serum as described previously (Haendeler et al., 2002; Hoffmann et al., 2001a). Human adult primary cardiomyocytes (CM) and arterial smooth muscle cells (SMC) were purchased as fresh isolates from PromoCell (Berlin, Germany) and cultured according the manufacturer's instructions. To simulate unhealthy diet EC, CM and SMC were treated with medium containing 1.1 mg/ml glucose, 0.4 mg/ml fructose and 1 mg/ml LDL (equals the clinical used concentration definition 100 mg/dl LDL) as well as with medium containing either 1.1 mg/ml glucose plus 0.4 mg/ml fructose or 1.5 mg/ml glucose plus 1 mg/ml LDL alone, or with control medium containing 1.5 mg/ml glucose. The T-antigen negative rat lung epithelial cell line RLE-6TN (LEC) was purchased from ATCC. This cell line was derived from alveolar type II cells isolated from F344 male rat. Although expression of the SV40-T antigen was negative by nuclear immunostaining and by PCR, these cells were derived by a spontaneous immortalization. This cell line was cultured as previously described (Sydlik et al., 2006).

2.3. Animal experiments

Female Balb/c mice (8 weeks old; Janvier SAS, Le Genest Saint Isle, France) were instilled by repetitive pharyngeal aspiration of 50 μl suspensions (PBS or ufCB 2.5 mg/kg in PBS) on days 0, 1, 2, and 3 under inhalation anesthesia (isoflurane, 5%, 1 min). Animals were killed by exsanguination under pentobarbital anesthesia on day 28. After flushing the circulation with PBS abdominal aortas were prepared, shock frozen, and stored at −80 °C until further use. Bronchoalveolar lavage (BAL) was performed by flushing the lungs with 4 × 1 ml sterile PBS. After centrifugation cells from all four lavages were pooled. Total BAL cell numbers were counted flow cytometrically. All animal experiments were performed after obtaining relevant permission according to German animal protection laws.

2.4. Viability measurements

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays were performed as described previously (Haendeler et al., 2009).

For measurement of lactate dehydrogenase (LDH) release the culture medium was harvested. A positive control leading to complete lysis of the cells with 0.9% Triton X-100 was included. LDH activity in the medium was determined by monitoring the reduction of pyruvate spectrophotometrically.