

Increased oxidative stress in younger as well as in older humans

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Abstract

Background: The free radical theory of aging is based upon the adverse effects of oxidative stress (OS), and indices of OS generally increase with advancing age. However, since OS may also be a normal physiological response in youth, when reactive oxygen species (ROS) act as signal transducers during normal growth and development, we compared markers of OS in normal humans over a wide spectrum of different ages. **Methods:** Fasting breath samples were collected from 102 healthy volunteers (age 9 to 89 years) and volatile organic compounds (VOCs) were assayed by gas chromatography and mass spectroscopy. The intensity of OS in each volunteer was estimated by the breath methylated alkane contour (BMAC), a three-dimensional display of the abundance of C4–C20 alkanes and monomethylated alkanes. The collective abundance of these VOCs in a breath sample was reduced to a single value, the volume under curve (VUC), and correlated with chronological age. **Results:** Compared to subjects aged 20–40 years, the mean BMAC VUC was significantly increased in subjects aged <20 ($p < 0.0001$) and >40 years ($p < 0.001$). A cubic function correlated BMAC VUC (x) with chronological age (y): $y = 33.7 - 3.29x + 0.072x^2 - 0.0004x^3$ ($r = 0.48$). **Conclusions:** Breath markers of OS were significantly increased both in younger and in older subjects, compared to those aged 20–40 years. Increased OS in older subjects was consistent with previous reports, but increased OS in younger subjects aged <20 years is a new observation; this may be a normal physiological response in youth.

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1. Introduction

The free radical theory of aging is based upon the adverse effects of oxidative stress (OS), and studies in humans and other organisms have shown that indices of OS generally increase with advancing age [1]. However, we are not aware of any previous study

comparing markers of OS in normal humans over a wide spectrum of different ages. We present here evidence that OS is increased in younger as well as in older normal humans, compared to adults aged between 20 and 40 years.

2. Materials and methods

We employed a set of breath markers of OS, the breath methylated alkane contour (BMAC), compris-

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Table 1
Demographics of study group

Age group (years)	<20	20–40	>40
N	7	46	49
Sex (m/f)	2/5	24/22	16/33
Smoker/nonsmoker/ex-smoker	0/7/0	7/33/2 *	4/20/19 **

Data not available for * two and ** six subjects.

ing the abundance of C4–C20 alkanes and monomethylated alkanes [2]. Their collective abundance in a breath sample was reduced to a single value, the volume under curve (VUC), and correlated with chronological age. Volatile organic compounds (VOCs) in 1.0-l samples of breath and room air were separately collected with a breath collection apparatus (BCA), a portable microprocessor-controlled device, and captured on sorbent traps containing highly purified activated carbon [2]. VOCs were assayed by automated thermal desorption combined with gas chromatography and mass spectroscopy. The human study has been described [2]; fasting breath samples were collected from 102 healthy volunteers aged from 9 to 89 years (Table 1). Subjects sat for approximately 20 min prior to the collections of breath and air in order to allow time for equilibration between VOCs in room air and in blood. For a given breath VOC:

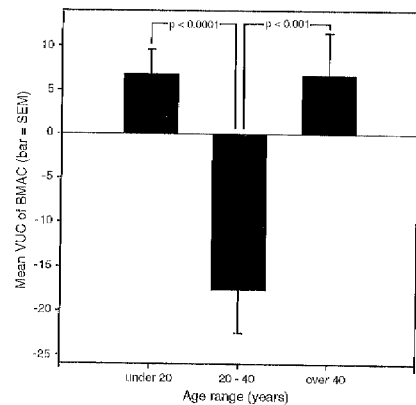
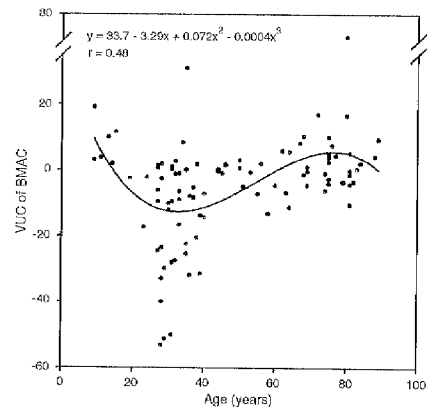
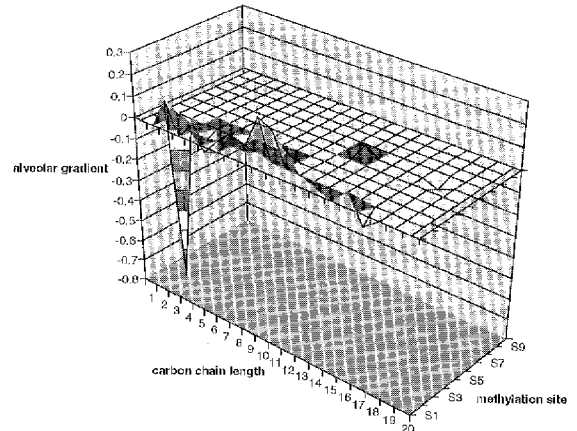
$$\text{alveolar gradient} = V_b/I_b - V_a/I_a,$$

where V_b = area under the curve (AUC) of chromatogram peak for that VOC, and I_b = AUC of internal standard used to calibrate the instrument (0.25 ml 2 ppm 1-bromo-4-fluoro-benzene [Supelco, Bellefonte, PA]). V_a and I_a denote corresponding AUCs derived from the associated air sample.

Fig. 1. Upper panel: Typical breath methylated alkane contour (BMAC) (normal 30-year-old female). Alkanes and monomethylated alkanes are displayed with x -axis = carbon chain length, z -axis = methylation site, and y -axis = alveolar gradient. This figure includes n -alkanes, showing them as methylated at C1 e.g. alkane with carbon chain length = 5 (propane) becomes C6 alkane hexane when methylated at C1. The alveolar gradient (abundance in breath minus abundance in room air) varies with rate of synthesis minus rate of clearance. Middle panel: Volume under curve (VUC) of BMAC versus chronological age. Data points indicate values for 102 healthy volunteers aged 9–89 years. Solid line indicates best-fit cubic function. Lower panel: VUC of BMAC in three groups: <20, 20–40, >40 years. Bars indicate mean and SEM; groups were compared by two-tailed t -tests.

3. Results

In each subject, alveolar gradients of C4–C20 n -alkanes and monomethylated alkanes were determined and displayed in a three-dimensional surface plot, the BMAC (Fig. 1). Seventy-three different C4–C20 alkanes and monomethylated alkanes were ob-



served in at least one of the breath samples. A cubic function correlated BMAC VUC with chronological age. Compared to subjects aged 20–40 years, the mean BMAC VUC was significantly increased in subjects aged under 20 and over 40 years.

4. Discussion

The observed increase in markers of OS in older subjects was consistent with previous reports [1]. Mammalian life depends upon oxygen as the final acceptor of electrons in mitochondrial electron transport, but the process also generates toxic metabolites. Reactive oxygen species (ROS) leak from mitochondria into the cytoplasm where they cause cellular damage by oxidizing a variety of biologically important molecules, including DNA, proteins and lipids. Peroxidation of polyunsaturated fatty acids generates alkanes observed in the BMAC. OS comprises the injurious effects of ROS, and according to the free radical theory, the resulting molecular damage is cumulative and ultimately lethal. OS also accelerates shortening of telomeres [3], the mitotic clock in human somatic cells, until eventually a critical deletion causes cellular senescence and death.

Elevated markers of OS in younger subjects aged <20 years is a new observation and its cause is unknown. This elevation may have been either true or apparent. An apparent elevation could possibly have resulted from reduced catabolism of OS markers by cytochrome P450 (CYP) mixed oxidase enzymes, but this appears unlikely in view of what is known of drug catabolism by hepatic CYP: activity increases soon after birth to reach a level equivalent to that in the young/mature adult, then gradually decreases with increasing age [5].

It is possible that elevated OS may be a normal physiological response in youth. There is increasing evidence that reactive oxygen species (ROS) act as signal transducers during normal growth and development. Growth factor or cytokine stimulation causes a rapid increase in intracellular ROS which appear essential for a host of downstream signaling events [6]. ROS play an essential role in the regulation of cell proliferation and differentiation, e.g. within the central and peripheral nervous system, ROS initiate and

promote the establishment of neuronal patterns and subsequent neurogenesis [7].

In the past, breath tests for markers of OS have generally been restricted to ethane and pentane, the VOCs most easily measured by gas chromatography. The advantage of the BMAC is that it incorporates a more comprehensive spectrum of markers of OS, although it does not include ethane because the assay is optimized for C4 to C20 VOCs. Monomethylated alkanes appear to be metabolites of alkanes formed by lipid peroxidation, and may be generated by trans-methylation enzymes in the microsomes [4]. The major strength of this study is that we employed a comprehensive set of breath markers of OS rather than a single marker such as pentane or ethane, thereby reducing the likelihood of error arising from chance alone. The BMAC comprises a set of 107 different VOCs, of which 73 were observed at least once in a breath sample. However, potential sources of error include the comparatively small number of subjects aged <20 years ($n=7$), and the lack of control for potential confounding variables such as diet, alcohol consumption and tobacco smoking.

We conclude that breath markers of OS were elevated in younger normal humans as well as in older subjects. In younger subjects, this may reflect normal physiologic changes during growth and development. However, since this group was small, these findings should be regarded as tentative. Further studies are needed to confirm and extend these findings in larger numbers of subjects over a greater range of ages, and controlling for potential confounding variables such as diet and alcohol consumption.

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References

- [1] Ashok BT, Ali R. The aging paradox: free radical theory of aging. *Exp Gerontol* 1999;34:293–303.

- [2] Phillips M, Cataneo RN, Greenberg J, Gunawardena R, Naidu A, Rahbari-Oskoui F. Effect of age on the breath methylated alkane contour, a display of apparent new markers of oxidative stress. *J Lab Clin Med* 2000;136:243–9.
- [3] Saretzki G, von Zglinicki T. Replicative senescence as a model of aging: the role of oxidative stress and telomere shortening—an overview. *Z Gerontol Geriatr* 1999;32:69–75.
- [4] Holloway CJ, Husmann-Holloway SH, Brunner G. Enzymatic methylation of alkane thiols. *Enzyme* 1979;24:307–12.
- [5] Tanaka E. In vivo age-related changes in hepatic drug-oxidizing capacity in humans. *J Clin Pharm Ther* 1998;23:247–55.
- [6] Finkel T. Reactive oxygen species and signal transduction. *IUBMB Life* 2001;52:3–6.
- [7] Verity MA. Oxidative damage and repair in the developing nervous system. *Neurotoxicology* 1994;15:81–91.