

Comparison of the antioxidant potential in urine, saliva and skin

Vergleich des antioxidativen Potentials in Urin, Speichel und Haut

Abstract

Aim: Free radicals, oxidative stress and their possible consequences for health are becoming increasingly important in modern medicine. Reactive species influence the organism, potentially causing oxidative cell damage. They can be produced by exogenous sources, or be a product of a variety of not only physiological metabolic processes, such as immune response, but also pathological processes. The antioxidant protection system protects the organism from oxidative damage caused by reactions producing an excess of free radicals. The analysis of antioxidant potential (AOP) is therefore becoming increasingly important for the diagnosis of individual vitality.

Method: The photochemoluminescence method was used to measure the AOP in urine and saliva, spectrometry was employed to measure the β -carotene content of the skin. In addition, it was investigated whether the AOP_{saliva} correlated with the AOPU_{urine} (uric-acid independent AOP) as well as the β -carotene content of the skin.

Results: The AOP was significantly higher in urine than in saliva, and both values were significantly positively correlated with each other. However, there was no significant correlation to the β -carotene content of the skin.

Discussion: The components of the AOPU_{urine} are accumulated over time (night), whereas AOP measurement in saliva is like a snapshot, which explains why AOPU_{urine} was significantly higher than AOP_{saliva}, although the two parameters are correlated with each other. β -carotene is a fat-soluble antioxidant, whereas in our study, only water-soluble antioxidants were determined in the urine. This explains why there is no positive correlation between β -carotene of the skin and AOP.

Conclusion: For the characterization of the AOP in epidemiological studies, we recommend determining the AOPU_{urine} and parallel to this, the β -carotene content of the skin.

Keywords: antioxidant potential, free radicals, oxidative stress, beta-carotene, reactive oxygen species, urine, saliva, photochemoluminescence, antioxidants

Zusammenfassung

Zielsetzung: Freie Radikale, oxidativer Stress und deren mögliche Konsequenzen für die Gesundheit gewinnen zunehmend an Bedeutung für die moderne Medizin. Neben potenziellen Verursachern oxidativer Zellschäden, die über exogene Quellen den Organismus beeinflussen, sind reaktive Spezies Produkt einer Vielzahl physiologischer Stoffwechselprozesse, der Immunreaktion, aber auch pathologischer Vorgänge. Als Gegenspieler zur Prävention oxidativer Schäden infolge überschießender radikalischer Reaktionen fungiert das antioxidative Schutzsystem. Die Analyse des Antioxidativen Potentials (AOP) gewinnt daher zunehmend an Bedeutung zur Diagnostik der individuellen Vitalität.

Methode: In dieser Studie wurden mittels Photochemolumineszenz das AOP in Urin und Speichel sowie spektrometrisch der β -Carotin-Gehalt der Haut bestimmt. Zusätzlich wurde untersucht, ob das AOP_{Speichel} mit

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dem AOPU_{urin} (Urat unabhängiges AOP) und dem β -Carotin-Gehalt der Haut korreliert.

Ergebnisse: Das AOP war im Urin signifikant höher als im Speichel, wobei beide Werte signifikant positiv miteinander korrelierten. Dagegen ergab sich bei beiden Parametern kein signifikanter Zusammenhang zum β -Carotin-Gehalt in der Haut.

Diskussion: Während im Urin Bestandteile des AOP über einen gewissen Zeitraum akkumulieren, stellt Speichel eine Momentaufnahme dar. Daher erklärt sich der signifikant höhere Gehalt von AOPU im Urin, obwohl die beiden Parameter miteinander korrelieren. Da β -Carotin ein fettlösliches Antioxidans ist, während im Urin nur wasserlösliche Antioxidantien bestimmt werden, war keine positive Korrelation vorhanden.

Schlussfolgerung: Für epidemiologische Untersuchungen zur Charakterisierung des AOP empfiehlt sich die Bestimmung des AOPU_{urin} und parallel dazu des β -Carotin-Gehalts der Haut.

Schlüsselwörter: antioxidatives Potential, freie Radikale, oxidativer Stress, beta-Carotin, reaktive Sauerstoffspezies, Urin, Speichel, Photochemolumineszenz, Antioxidantien

Introduction

Radical generation can be triggered exogenously (e.g., UV and ionizing radiation) and endogenously (e.g., mitochondrial respiratory chain and immune system) [1], [2]. An excess of free radicals due to increased formation or inadequate antioxidant mechanisms is harmful to the organism [3]. Because of their unpaired electrons and the associated instability and reactivity, radicals can cause damage in biological systems with the consequence of changing their structure and function. The undesirable effects include the inactivation of NO by direct chemical reaction with reactive oxygen species (ROS), and oxidative damage to cellular components such as DNA and proteins [4]. These effects are possibly related to the development of cardiovascular diseases, neurodegenerative diseases, cancer, and aging processes [5], [6], [7].

The antioxidant system is responsible for minimizing oxidative cell damage in the body caused by free radicals. Ideally, it creates a balance which permits the beneficial effects of free radicals while preventing the harmful ones. The system includes enzymatic antioxidants (e.g., superoxide dismutase) and non-enzymatic exogenous and endogenous antioxidants, which can be hydrophilic or lipophilic (e.g., ascorbic acid and glutathione, resp.) [8].

AOP in urine and saliva can be determined by different methods, i.e., photochemoluminescence (PCL) [6], [7]. Another method to determine the antioxidant status is the measurement of the β -carotene content of the skin [9]. The antioxidant balance of an individual is influenced by diet, sports, stress, among others [10], [11], [12]. Up to now, the AOP in saliva, urine and skin were not determined in parallel in the same person. Therefore, the focus of the study was to compare the AOP of the 3 sampled media.

Methods

Nine psychology students (7 women, 2 men, 20 to 25 years old) participated in the three-day study. Every morning, fasting samples of spot urine and saliva from each participant were collected to determine AOP_{saliva} and AOPU_{urine}. The samples were coded and then immediately frozen at -80°C . While in saliva only the AOP was analyzed, in the urine samples, both AOPU and the creatinine content were determined, where the latter was adjusted for volume excreted. Before analysis, the samples were allowed to thaw overnight in the refrigerator and were centrifuged after mixing briefly (5000 rpm) in order to separate suspended matter. The centrifuged urine was diluted 1:10 and incubated for 5 min with uricase to eliminate uric acid. Subsequently, the AOP was measured by using Photochem[®] and the ACW kit (Analytik Jena, Germany) [12], [13]. The chemicals had pro analysis quality. For all investigations, high-purity water was used (Reinstwasser-System, SG Wasseraufbereitung und Regenerierstation GmbH, Barsbüttel, Germany). For creatinine, we used the creatinine-DRI[®] Test Detect (Micro Genetics GmbH, Passau, Germany). The AOP was calculated using the following formula:

$$\text{AOP (mg/g creatinine)} = \text{AOP (mg/dl)} \times 1000 / \text{creatinine (mg/dl)}$$

Parallel to the daily sampling, we determined the β -carotene content (scale 0–10) of the skin by using biozoom[®] (Opsolution Nanophotonics GmbH, Kassel, Germany) [14]. The participants were instructed not to apply any skin cream in each morning before measurement. Statistical analysis was performed with the program PASW[®] Statistics 18 (IBM). The parameters were intended for descriptive statistics, and Pearson's correlation was calculated and tested for significance.

Table 1: Descriptive statistics of the study (n=9)

Parameter		AOP (mg/g) creatinin in urine	AOP (mg/dl) in saliva	β -carotene content
Mean value		39.70	2.05	4
Standard error of average		2.70	0.52	0.19
Median		37.53	0.81	4
Standard deviation		14.015	2.68	1
Variance		196.43	7.18	1
Minimum		17.61	0.29	2
Maximum		68.30	10.55	6
Percentile	5	18.43	0.29	2.40
	95	66.97	9.75	6

Table 2: Correlation analysis of the parameters AOP_{saliva}, AOPU_{urine} and β -carotene (skin)

		Average of AOPU _{urine} (mg/g creatinine)	Average of AOP _{saliva} (mg/dl)
Average of saliva-AOP (mg/dl)	Correlation according to Pearson	0.720*	1
	significance (2-sided)	0.029	
	N	9	9
β -carotene _{skin}	correlation according to Pearson	0.165	-0.126
	significance (2-sided)	0.672	0.747
	N	9	9

Results

The AOPU_{urine} was significantly higher than in saliva. The mean value of β -carotene, 4.0, was slightly lower than expected (Table 1).

The data analysis shows a significant positive correlation between the parameters AOPU_{urine} and AOP_{saliva} at $p < 0.05$ (Table 2). In contrast, the values of β -carotene_{skin} with those of AOPU_{urine} show a weak positive correlation, but this was not significant. The AOP_{saliva} shows a slight negative correlation with the values of skin measurement, but the difference was not significant (Table 2).

Discussion

Antioxidant capacity was determined in materials which are non-invasively accessible, that is, in saliva, urine and skin. The intention was to use conditions suitable for future epidemiological studies. The ideal would be to collect 24-h urine. Since this was not logistically realizable, spontaneous urine was used and normalized by creatinine content in urine; this method has been proven in studies on iodine deficiency screening [15]. Influences of age, sex, lifestyle and nutrition were not considered in the pilot study, because the purpose was to compare the meas-

ured parameters. While urine is an excretion with accumulation of the excreted substances over time, saliva is a secretion, in which components can be exogenously influenced and endogenously metabolized, thus the composition can change quickly. This may explain the finding that the AOPU_{urine} is at least 10 times higher than AOP_{saliva}. However, these two parameters were correlated significantly with each other. This result supports the hypothesis that urine and saliva are suitable for determining the AOP. Since saliva production is difficult to standardize and more susceptible to external influences than urine, the standard deviation in saliva is about 5.2 higher than in urine.

β -carotene is a fat-soluble antioxidant. In the urine, only water-soluble antioxidants were analyzed. This may be the reason why there is no positive correlation between β -carotene of the skin and the AOP of saliva and urine.

Conclusions

Our results suggest that in epidemiological studies, the AOPU_{urine} and, in parallel, the β -carotene content of the skin are suitable for characterizing the antioxidative status, whereas the AOP_{saliva} is dispensable.

Notes

Competing interests

The authors declare that they have no competing interests.

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