Natural zeolites chabazite/phillipsite/analcime increase blood levels of antioxidant enzymes

Giada Dogliotti,1 Alexis E. Malavazos,2 Sonia Giacometti,3 Umberto Solimene,1,4 Mauro Fanelli,5 Massimiliano M. Corsi1,4 and Elena Dozio1,*

1Dipartimento di Morfologia Umana a Scienze Biomediche “Città Studi”, University of Milan, Via Mangiagalli 31, 20133 Milan, Italy
2Unità Operativa di Diabetologia e Malattie Metaboliche and Unità Operativa Laboratorio di Patologia Clinica, Dipartimento dei Servizi di Diagnosi e Cura – Medicina di Laboratorio, IRCCS Policlinico San Donato, Piazza E. Malan, 20097 San Donato Milanese, Milan, Italy
3Dipartimento di Biologia, University of Milan, Via Celoria 26, 20133 Milan, Italy
4Centro di Ricerche in Bioclimatologia Medica, University of Milan, Via Cicognara 7, 20129 Milan, Italy
5Geomedical srl, 01010 Marta, Viterbo, Italy

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Imbalance between reactive oxygen species generation and antioxidant capacity induces a condition known as oxidative stress which is implicated in numerous pathological processes. In this study we evaluated whether natural zeolites chabazite/phillipsite/analcime may affect the levels of different antioxidant enzymes (glutathione peroxidase, superoxide dismutase, glutathione reductase), total antioxidant status and oxidative stress in 25 clinically healthy men, both non-smokers and smokers. Measurements were performed on whole blood or on plasma samples before (T0) and after 4-weeks zeolites intake (T1). At T1, glutathione peroxidase, superoxide dismutase and glutathione reductase increased compared to T0 levels, both considering all subjects as joint and after subdivision in non-smokers and smokers. Differently, a reduction in total antioxidant status was observed at T1. Anyway, total antioxidant status resulted higher than the reference values in both groups at each time point. A decrease in lipid peroxidation, a major indicator of oxidative stress assessed by monitoring thiobarbituric acid reactive substances, was also observed in all subjects at T1. Our results suggested that chabazite/phillipsite/analcime may help to counteract oxidative stress in apparently healthy subjects exposed to different oxidative stress risk factors, such as smoking, thus representing a particular kind of food with potential antioxidant properties.

Key Words: glutathione reductase, glutathione peroxidase, superoxide dismutase, total antioxidant status, zeolites

Natural zeolites are volcanic hydrated micro porous crystals with defined structures based on AlO4 and SiO4 tetrahedral building blocks connected through oxygen atoms and with an “open” structure which can accommodate a wide variety of positive ions. Due to their structure, zeolites exhibit versatile adsorptive, cation exchanger, dehydrating-rehydrating and catalytic properties. Some zeolites are already used in medicine as anti-diarrheal, antibacterial, antifungal drugs and glucose absorbent. Moreover, previous studies also indicated that the zeolite clinoptilolite exerts anticancerogenic and antioxidant effects.

Reactive oxygen species (ROS) are highly reactive molecules which may be both important mediators of some physiological functions and also potential prooxidants. Imbalance between ROS generation and antioxidant capacity induces a condition known as oxidative stress which may play a major role in the initiation and progression of numerous pathologies including cardiovascular dysfunction associated with vascular disease, hyperlipidemia, diabetes mellitus, hypertension and ischemia/reperfusion injury. The potential damage caused by an excess of ROS is controlled by a series of antioxidant defence mechanisms and, among them, a key protective role is played by the antioxidant enzymes glutathione (GSH) peroxidase, superoxide dismutase (SOD) and GSH reductase.

Presently, the antioxidant role attributed to zeolites, and specifically to clinoptilolite, is based on the ability to reduce lipid peroxidation, free radicals levels and also to increase total antioxidant status (TAS) in serum.

Due to their key protective role in preventing or slowing down oxidative damage, antioxidant enzymes could be potential target of zeolites action too. To this aim, in the present study we evaluated whether chabazite/phillipsite/analcime, typical zeolites of the central-souther of Italy, may affect the levels of GSH peroxidase, SOD, GSH reductase and TAS in a group of clinically healthy men, both non-smokers and smokers. Furthermore, since lipid peroxidation is a major indicator of oxidative stress, thiobarbituric acid reactive substances (TBARS) were measured for screening and monitoring lipid peroxidation in our subjects.

Materials, Subjects and Methods

Materials. The fine powder of natural zeolites from central Italy (near Bolsena lake) was obtained by tribomechanical micro-nization and sterilization at 100°C for 24 h (Geomedical srl, Viterbo, Italy). The zeolitic-tuff used contained approximately 33.7% chabazite [CaAl2Si2O8·6(H2O)], 17.0% phillipsite [(K,Na,Ca)2(Si2Al)O10·8·6(H2O)] and 6.1% analcime [NaAl2Si2O8·H2O]. Other components were: sandine (18%), plagioclase (14.6%) and muscovite mica (10.6%). No traces of clinoptilolite were found. The total zeolitic content of the tuff was about 56.8%. The cation-exchange capacity and specific mass were 400 meq/100 g and 1.26 N/m3, respectively. Specific surface area was 1.77 m²/g. Particle size analysis showed a granulometry less then 1 μm for 15% of the particles, 2 μm for 20% of the particles, 5 μm for 40% of the particles, 15 μm for 80% of the particles and 100 μm for 100% of the particles.

Subjects. In this study, 25 clinically healthy male (40 ± 3 years; age mean ± standard deviation (SD)) were enrolled. Subjects gave informed consent to the examination protocol, conducted in accordance with the Declaration of Helsinki and approved by the

*To whom correspondence should be addressed.
E-mail: elena.dozio@unimi.it
institutional committee. These subjects were following a typical Mediterranean diet and leading a non-sedentary life style. Of these, 12 were non-smokers and 13 were smokers (more than 20 cigarettes/day). Subjects received for 4 weeks, twice a day, 1/2 h before breakfast and dinner, 3 g of chabazite/philippsite/analcime as powder suspension dissolved in water. EDTA-anti-coagulated blood samples were taken before (T0) and at the end of the 4 weeks (T1). Plasma samples were obtained after blood centrifugation at 1800 g for 15 min at room temperature and stored at −20°C until assay.

**Biochemical assays.** GSH peroxidase, SOD, GSH reductase and TAS were measured or on whole blood or on plasma samples by specific kits from Randox Laboratories Ltd. (Crumlin, Belfast, UK) on the semi-automated analyzer for colorimetric and turbidometric assays RX Monza™ (Randox Laboratories Ltd.) following manufacture’s instructions. The reference values for the parameters in healthy subjects were: 4171–10881 U/l of whole blood for GSH peroxidase, 164–240 U/ml of whole blood for SOD, 33–77 U/l of plasma for GSH reductase, 1.3–1.7 mmol/l of plasma for TAS. Reported maximum intra- and inter-assay coefficients of variations were 3.79% and 4.90% for GSH peroxidase, 3.58% and 7.07% for SOD, 2.58% and 4.32% for GSH reductase, 3.08% and 3.75% for TAS, respectively. TBARS test was performed using the method described by Richard et al. Briefly, after the reaction of thiobarbituric acid with malondialdehyde, the product was extracted in butanol and was measured spectrophotometrically (excitation: 532 nm, emission 553 nm, slit 10 mm). TBARS levels on plasma were expressed as nmol/ml.

**Statistical analysis.** Box-and-whisker diagrams were used to represent data. The smallest observation (sample minimum), lower quartile (25%), median (50%), upper quartile (75%), and largest observation (sample maximum) are shown. The normality of data distribution was assessed by the Kolmogorov-Smirnoff test. Comparisons between groups were performed using Student’s two-tailed t-test and a p value<0.05 was considered significant. Data were analyzed by GraphPad Software Inc. (San Diego, CA).

**Results**

GSH peroxidase, SOD, GSH reductase and TAS levels were measured before (T0) and after 4-weeks zeolites intake (T1). At T0, no statistically significant differences were observed for GSH peroxidase, SOD, GSH reductase and TAS levels between non-smokers and smokers subjects, although GSH peroxidase and GSH reductase mean values appeared more elevated in the non-smoking group. Among the analyzed parameters, TAS levels resulted higher than the normal values in both groups, whereas all the other parameters were within the normal reference ranges. At T1, GSH peroxidase, GSH reductase and SOD resulted increased compared to basal levels, both considering all subjects as joint and after subdivision in the two sub-groups, non-smokers (1.5 ± 0.3 nmol/ml; p<0.05) and smokers (1.6 ± 0.3 nmol/ml; p<0.01). No statistically significant difference was found between the two groups.

**Discussion**

In the present study we observed that 4-weeks chabazite/philippsite/analcime intake in clinically healthy subjects, both non-smokers and smokers, increased the levels of GSH peroxidase, SOD and GSH reductase, three antioxidant enzymes able to remove ROS, and decreased TBARS, a major indicator of lipid peroxidation utilized for monitoring oxidative stress, thus preventing or slowing down oxidative damage. Anthropologists have always maintained that our ancestor ingested, to cure themselves, different varieties of rocks taken from the surrounding environment. This diet behavior has profound biological bases, bonded to organic need to take the mineral elements necessary for life. Since today developed societies are exposed to a wide variety of exogenous and endogenous stress factors that may influence the generation of ROS and antioxidant activity, the use of integrators and particular kind of food, like zeolites, may help to eliminate free radicals and toxic substances from the body. In addition to the previously suggested antioxidant properties of some zeolites, in the present study we observed that the intake of chabazite/philippsite/analcime, three zeolites widespread in many volcaniclastic deposits in the central-southern of Italy, gave a significant protection against oxidative stress by increasing the levels of important antioxidant enzymes and reducing oxidative stress. These zeolites have been used in our study for the following reasons: they are the most abundant zeolites of Italian soil, they have an overall cation-exchange capacity of 400 meq/100 g and, finally, they have not been previously studied as medicinal food relative to their antioxidant potential. The exchange capacity, which indicates the ability to release beneficial elements while capturing and binding others, has been indicated as an important requirement for the therapeutic application of zeolites. For this

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**Table 1.** Levels of glutathione peroxidase (GLUT PER), superoxide dismutase (SOD), glutathione reductase (GLUT RED), total antioxidant status (TAS) and thiobarbituric acid reactive substance (TBARS) in subjects before (T0) and after 4-weeks zeolites consume (T1). Data are mean ± standard deviation (SD) values

|                  | Whole Subjects (n = 25) | Non-Smoking (n = 12) | Smoking (n = 13) |
|------------------|-------------------------|----------------------|-----------------|
|                  | T0                      | T1                   | T0              | T1              |
| GLUT PER (U/l)   | 6118.44 ± 2227.30       | 8924.64 ± 2479.2**   | 6489.80 ± 2526.4| 9024.00 ± 3187.10** |
| GLUT RED (U/l)   | 34.69 ± 18.68           | 82.20 ± 24.60**      | 40.38 ± 17.86   | 94.98 ± 20.17** |
| SOD (U/ml)       | 141.14 ± 33.11          | 214.14 ± 39.54**     | 135.64 ± 21.87  | 214.62 ± 32.87** |
| TAS (mmol/l)     | 2.49 ± 0.22             | 1.82 ± 0.18**        | 2.44 ± 0.22     | 1.82 ± 0.12**   |
| TBARS (nmol/ml)  | 2.70 ± 0.70             | 1.60 ± 0.30**        | 2.10 ± 0.30     | 1.50 ± 0.30**   |

*p<0.05 vs T0; **p<0.001 vs T0. GLUT PER: glutathione peroxidase; GLUT RED: glutathione reductase; SOD: superoxide dismutase; TAS: total antioxidant status; TBARS: thiobarbituric acid reactive substance.
reason, due to a cation-exchange capacity of 400 meq/100 g, which is greater than that of other zeolites (about 200 meq for the most used and studied clinoptilolite), chabazite/phillipsite/analcime may have the requirements to be more effective than other zeolites.

The subjects included in the study were both non-smoking and smoking. Previous studies which evaluated the effect of smoking on the levels of antioxidant enzymes reported different conclusions, since both significant and not significant differences in the activities of these antioxidant enzymatic systems have been described.(7–10) Although at T0 we observed that GSH peroxidase and GSH reductase mean values were lower in the smoking group, however we did not observe any statistically significant difference. After chabazite/phillipsite/analcime consume, increased levels of all the three antioxidant enzymes were observed in both groups. These data thus may indicate that chabazite/phillipsite/analcime are able to increase physiological mechanisms against oxidative stress in healthy subjects exposed to different risk factors of oxidative stress, such as smoking, and may be considered as protective agents against oxidative stress.

The decrement of TAS levels at T1 seems to be in contrast with previous studies which indicated that zeolites intake may help to increase TAS.(3,4) One possible explanation is that our subjects are healthy and daily following a typical Mediterranean diet rich in natural antioxidants which contribute to increase TAS to levels higher than the normal range. Thus, it is possible that after ingestion, chabazite/phillipsite/analcime remove not only toxins and biological waste but also absorb other molecules. Although chabazite/phillipsite/analcime reduced TAS, however, it is to be noted that it remained higher than normal values. In addition, the observation that TBARS were reduced after zeolites intake suggests that zeolites do not aggravate oxidative stress. On the contrary a general improvement was observed in both groups (non-smoking and smoking). Future studies considering other categories of subjects following different diets and also patients affected by pathologies characterized by increased oxidative stress, will be necessary to better clarify the effect of chabazite/phillipsite/analcime on TAS.

At now, the mechanisms mediating zeolites antioxidant effects are not fully understood and since orally-administered zeolites are not absorbed into the blood it is possible that their in vivo effect may be due to an indirect interaction with biochemical systems, such as toxin and biological waste removal from the gut, activation of the immune system via the mucosal associated intestinal lymphoid tissue and also by increasing the bioavailability of mineral elements, which are important co-factors for the enzymes.(2,11–13)
In conclusion, our results strongly indicated that chabazite/phillipsite/analcime may help to counteract the effects of oxidative stress by increasing the levels of antioxidant enzymes and reducing oxidative stress in apparently healthy subjects exposed to different risk factors of oxidative stress. Future studies will be necessary to better clarify which mechanisms mediate the observed zeolites effects and will be helpful to better identify some other potential applications of such compounds also in fields like cardiovascular prevention and atherosclerosis.

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Abbreviations

GSH glutathione
ROS reactive oxygen species
SOD superoxide dismutase
TAS total antioxidant status
TBARS thiobarbituric acid reactive substances

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