Free Radical Research

ISSN: 1071-5762 (Print) 1029-2470 (Online) Journal homepage: http://www.tandfonline.com/loi/ifra20

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To cite this article: Boris V. Nemzer, Christoph Centner, Denise Zdzieblik, Bruno Fink, John M. Hunter & Daniel König (2017): Oxidative stress or redox signalling – new insights into the effects of a proprietary multifunctional botanical dietary supplement, Free Radical Research, DOI: 10.1080/10715762.2017.1390228

To link to this article: http://dx.doi.org/10.1080/10715762.2017.1390228

Published online: 07 Nov 2017.

FREE RADICAL RESEARCH, 2017
https://doi.org/10.1080/10715762.2017.1390228

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ORIGINAL ARTICLE

Oxidative stress or redox signalling – new insights into the effects of a proprietary multifunctional botanical dietary supplement

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ABSTRACT

Recent interest has focused on maintenance of healthy levels of redox signalling and the related oxidants; these parameters are crucial for providing us with concrete nutritional targets that may help us to better understand and maintain "optimal health". Following the above hypothesis, we performed a pilot double-blind, crossover, placebo-controlled, single dose study to measure the dose-dependent effects of a proprietary plant-based dietary supplement labelled here as S7 (SPECTRA7), related to how it affected the cellular metabolic index (CMI) in healthy human participants ($n = 8$). We demonstrated using the electron spin resonance/electron paramagnetic resonance spectrometer NOXYSCAN that the administration S7 resulted in statistically significant, long-term, dose-dependent inhibition of mitochondrial and cellular reactive oxygen species generation by as much as 9.2 or 17.7% as well as 12.0 or 14.8% inhibition in extracellular nicotinamide-dinucleotide-phosphate oxidase system-dependent generation of $O_2^{\bullet-}$, and 9.5 or 44.5% inhibition of extracellular H_2O_2 formation. This was reflected with dose-dependent 13.4 or 17.6% inhibition of tumour necrosis factor alpha induced cellular inflammatory resistance and also 1.7 or 2.3-times increases of bioavailable NO concentration. In this pilot study, we demonstrated the ability of a natural supplement to affect cellular redox signalling, which is considered by many researchers as oxidative stress. The design and activity of this proprietary plant-based material, in combination with the newly developed "CMI" test, demonstrates the potential of using dietary supplements to modulate redox signalling. This opens the door to future research into the use of S7 for modulation of inflammatory markers, for sports endurance or recovery applications.

Abbreviations: AA: Antimycin A; CGA: chlorogenic acids; CMH: 1-hydroxy-3-methoxycarbonyl-2.2.5.5-tetramethyl pyrrolidine; CMI: cellular metabolic index; ESR/EPR: electron spin resonance/electron paramagnetic resonance; H_2O_2: hydrogen peroxide; hsCRP: high sensitivity C-reactive protein; HDL: high-density lipoproteins; LDL: low-density lipoproteins; NADPH oxidase: nicotinamide-dinucleotide-phosphate oxidase; NOX-15.1: oxygen label (trityl); NO: nitric oxide; $O_2^{\bullet-}$: superoxide anion radical; $O_2(^1\Delta_g)$: singlet oxygen; OH^-: hydroxyl anion radical; ROS: reactive oxygen species; RNS: reactive nitrogen and oxygen species; SOD: superoxide dismutase; SEM: standard error of mean; TNFα: Ntumour necrosis factor alpha; VLDL: very low density lipoproteins

ARTICLE HISTORY
Received 24 August 2017
Revised 5 October 2017
Accepted 5 October 2017

KEYWORDS
Oxidative stress; redox signalling; cellular metabolic index; multifunctional dietary supplement; inflammatory resistance; nitric oxide

Introduction

Oxidation–reduction reactions permeate all fundamental processes, from genome to transcriptome, redox proteome, redox metabolome, bioenergetic, metabolism and other life functions [1]. There has been a significant explosion in the interest in redox signalling and in oxidative stress in many research fields, and an associated excitement within the general public as well. However, these molecular reactions and their often-complicated interrelations have been variously misinterpreted, even by respected health institutions, and often with a complete disconnection from basic redox biology.

A variety of metabolically controlled nicotinamide-dinucleotide-phosphate oxidase oxidases (NOX1-5), metabolically controlled mitochondrial sources of reactive oxygen species (ROS) (including $O_2^{\bullet-}$ and H_2O_2) as well as glutathione- and thioredoxin-related pathways, with powerful enzymatic backup systems, are responsible for fine-tuning of physiological redox signalling. Knowledge about these processes can empower advances in research fields of biochemistry, cell biology, nutritional science, environmental medicine, and molecular knowledge-based redox medicine, and can ultimately lead to new discoveries of preventive and even therapeutic procedures.

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It is extremely important to understand the exact mechanisms and complexity of metabolic syndrome pathways, especially in industrial countries where over 60% of the population is suffering from metabolic syndrome. Metabolic syndrome is defined as a cluster of at least three of the five following medical conditions: abdominal obesity, elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides and low high-density lipoprotein (HDL) levels. Combinations of these conditions result in 16 possible variations of the overall syndrome. The pathophysiology is very complex and has been only partially elucidated. The most important risk factors for the development of metabolic syndrome are diet, genetics, ageing, sedentary behaviour or low physical activity, stress, disrupted chronobiology, mood disorders and excessive alcohol use.

It is generally accepted that the current "Western Diet" especially contributes to the development of metabolic syndrome due to the fact that this diet is mismatched with our biochemistry. The continuous provision of energy via highly processed dietary carbohydrate, lipid and protein fuels, absent the balance provided by physical activity and associated energy demand, creates a build-up of the mitochondrial oxidation products associated with progressive mitochondrial dysfunction and insulin resistance. Additionally, a moderate elevation of a number of markers of systemic inflammation including C-reactive protein, fibrinogen, interleukin-6, tumour necrosis factor alpha (TNF-α) has been closely associated with metabolic syndrome [2,3]. Additionally, these and a number of other related markers have been suggested to be implicated in the eventual development of age-related cognitive declines associated with Alzheimer's, Parkinson's and amyotrophic lateral sclerosis (ALS) [4,5].

Electron paramagnetic resonance (EPR) spectroscopy is a technique that is recognised in the scientific community [6,7] for direct observation of *ex vivo* or *in vivo* formation of ROS. Use of cyclic hydroxylamine spin probe 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl pyrrolidine (CMH) in combination with oxygen label opens the possibility for simultaneous measurement of formation of ROS and oxygen consumption under physiological oxygen concentrations [8]. The advances in instrumentation (bench-top EPR spectrometer NOXYSCAN) provided us with an opportunity to perform a pilot study to investigate the bioactivity of a nutritional supplement SPECTRA7 (S7), a formulation of botanical materials high in preferable phytochemicals such as catechins, including epigallocatechin gallate (EGCG), chlorogenic acids (CGA) and curcumin (see Table 1). The supplement S7 is designed, manufactured and commercially marketed by FutureCeuticals, Inc. (Momence, IL) for the promotion of circulating NO concentrations, support of efficient O_2 utilisation, and healthy oxidative status and metabolism.

Table 1. The composition of the multifunctional nutritional supplement S7 and TAC for S7 per serving.

Phytochemical components	Units	25 mg	50 mg
Chlorogenic acids	mg	6.6	13.2
Catechins	mg	9.2	18.4
EGCG	mg	4.6	9.2
Caffeine	mg	0.85	1.7
Anthocyanins	µg	26.25	52.5
Trigonelline	µg	27.55	55.1
Curcumin	mg	1.05	2.1
Total polyphenols	mg	17.2	34.4

Materials and methods

Study design

Eight healthy participants (age: 43.3 ± 10.7 years, body weight: 59.9 ± 7.4 kg) were enrolled in the investigation. The study was carried out according to the Helsinki declaration for clinical trials. All participating subjects were in good health as confirmed by physical examinations and clinical routine laboratory tests.

Exclusion criteria

Subjects were excluded if they had been diagnosed with type 1 and type 2 diabetes, other acute or chronic disorders (gastrointestinal, pulmonary, renal, cardiac, neurological or psychiatric disorders), known allergies to foods or their ingredients. In addition, subjects were excluded if they were active smokers, using weight-reducing preparations/appetite suppressants, β-blockers, ACE inhibitors or participated in a clinical study within the last 90 d prior to the beginning of this study.

General procedure

The study was a double-blinded, placebo-controlled cross-over study. On different days, after a 7–10 d washout period, subjects received either: i) placebo or ii) 25 mg S7 or iii) 50 mg S7. Examinations began in the morning at 9 am after at least 10 h fasting period.

In order to distinguish between the different pathways involved in the generation of ROS and oxygen consumption the following parameters were measured prior and 60, 120, 180 min after consumption of standard breakfast (bread roll with a glass of water):

- Total cellular ROS/O_2 consumption.
- Extracellular $O_2^{\bullet -}$ formation/O_2 consumption.
- Extracellular H_2O_2 formation/O_2 consumption.

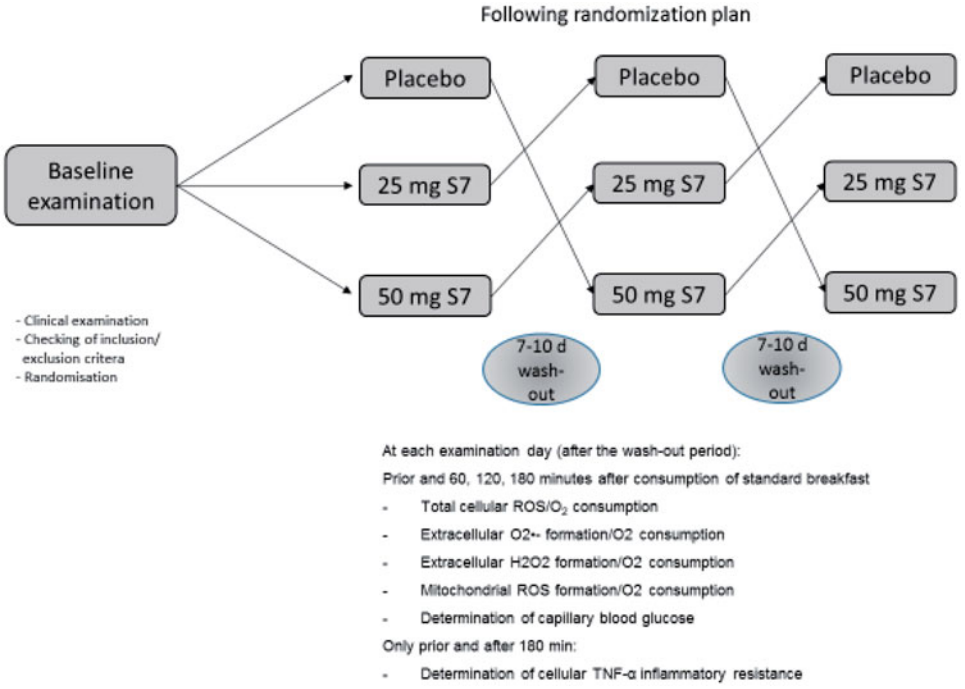

Diagram 1. Design of the study.

- Mitochondrial ROS formation/O_2 consumption.

Performed only prior and after 180 min:

- Determination of cellular TNF-α inflammatory resistance.

In order to investigate the effect on blood sugar prior and 60, 120, 180 min after consumption of standard breakfast (bread roll with glass of water)

- Determination of capillary blood glucose

For details, see Diagram 1.

Cellular metabolic index assay

The principle of the extended "Cellular Metabolic Index" (CMI) assay, which was developed by Noxygen Science Transfer & Diagnostics GmbH (Elzach, Germany), is based upon the monitoring of cellular and mitochondrial generation of ROS by tracing of an EPR signal using benchtop EPR spectrometer "NOXYSCAN" equipped with a Temperature and Gas Controller System (TGC-BIOIII, Noxygen Science Transfer & Diagnostics GmbH). Spin probe CMH, 200 μm) and/or compounds such as superoxide dismutase (SOD) (50 U/ml), catalase (50 U/ml), antimycin A (AA) (10 μm) were mixed into the freshly drawn capillary blood in order to perform various types of observations related to ROS generation under controlled temperature and oxygen concentration ($t = 37\,^{\circ}C$, $pO_2 = 110$ mm/Hg) [9–12]. Addition of oxygen label (NOX-15.1 – 5 μm) to the blood sample allowed us to monitor oxygen concentrations and cellular, as well as mitochondrial, oxygen consumption [13,14]. In order to distinguish pathways involved in generation of ROS and oxygen consumption we measured:

a. Total cellular ROS/O_2 consumption – without the addition of inhibitors.
b. Extracellular $O_2^{\bullet -}$ formation/O_2 consumption – after addition of SOD.
c. Extracellular H_2O_2 formation/O_2 consumption – after addition of catalase.
d. Mitochondrial ROS formation/O_2 consumption – after addition of AA.

We used the following electron spin resonance/electron paramagnetic resonance (ESR) settings for signal detection: centre field: $g = 2.011$; sweep width: 60 G; frequency: 9.76 GHz; power: 20 mW; gain: 1×10^3; modulation amplitude: 1.0 G; sweep time: 5.24 s; number of scans: 10; number of points: 512; total experimental time: 5 min. Calibration of EPR signal was performed using calibration solution that had a standard concentration of CM° (10 μm) or oxygen label NOX-15.1 (5 μm) introduced into oxygen permeable 50 μl Pentoxifylline (PTX) capillary by running of deoxygenation of oxygen label solution using perfusion of pure nitrogen (99.99%).

Cellular TNF-α inflammatory resistance assay

Our cellular inflammatory resistance assay was used to provide information about the ability of S7, in presence of TNF-α, a key factor of inflammation [15], to inhibit TNF-α stimulation of generation of ROS in blood cells. Twenty μm of capillary blood was mixed with a 20-μl solution of human TNF-α (40 ng/ml) and spin probe 1-hydroxy-4-phosphonooxy-2,2,6,6-tetramethylpiperidine (PPH, 500 μm) dissolved in Krebs HEPES buffer (KHB) (20 mm, pH 7.4). Samples filled in a PTX, oxygen permeable capillary tube were placed in the Capillary Treatment Chamber (CTC BIO-IV, Noxygen Science Transfer & Diagnostics GmbH) connected to TGC. The oxygen concentration used for perfusion of CTC was set to 4% oxygen concentration in order to keep the value of pO_2 in the range of 40 mmHg and additional pressure of 10 mmHg. The EPR settings were as follow: centre field: g = 2.011; sweep width: 60 G; frequency: 9.76 GHz; microwave power: 20 mW; gain: 1×10^3; modulation amplitude: 2.2 G; time constant: 40.96 ms; conversion time: 10.24 ms; sweep time: 5.24 s; number of scans: 10; number of points: 46; experimental time: 60 min.

Bioavailable NO concentration assay

After 5 min of centrifugation at $1600 \times g$, heparinised venous blood sample at room temperature was then frozen in liquid nitrogen and kept at −80 °C. Measurement of NOHb content was performed at 77 K with liquid nitrogen-filled quartz finger dewar. EPR spectrometer NOXYSCAN equipped with new designed cavity, operating at 86 kHz field modulation was utilised to collect ESR spectra at the 9.7 GHz X band using the following settings: microwave power, 50 mW; modulation amplitude, 8 G; centre field, 2.01 g; sweep width, 60 G; conversion time, 20 ms; time constant, 80 ms; number of scans 60; total detection time, 600 s. The amount of detected $NO^{\cdot}$, a second key signalling molecule of vascular physiology [16,17], was determined from the calibration curve for the intensity of the EPR signal of erythrocytes treated with known concentrations of nitrite (1–25 μm) and $Na_2S_2O_4$ (20 mm).

Chemicals

The spin probes CMH, 1-hydroxy-4-phosphonooxy-2,2,6,6-tetramethylpiperidine, the metal chelators deferoxamine and diethyldithiocarbamate. KHB, and the oxygen label NOX-15.1 were obtained from Noxygen Science Transfer & Diagnostics. All other chemicals and reagents used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

Statistical analysis

Data are presented as the mean ± SEM. Comparisons were made using Student's *t*-test or ANOVA. Regression analysis was performed with the best fitting for a linear or exponential relationship. Differences are considered significant at $p < .05$.

Results

According to the study protocol, we recruited general healthy participants between the ages of 24 and 55 years with body weight of 59.9 ± 7.4 kg and BMI of 22.1 ± 1.9. Cardiovascular parameters such as heart rate 78.3 ± 6.2 b/min, and systolic 121.5 ± 7.9 mmHg as well as diastolic 76.0 ± 6.8-mmHg blood pressures did not exceed the values of healthy persons. Also, haemogram, metabolic-, inflammatory and lipid-profile values represented in Table 2 do not exceed the range of healthy persons. As Figure 1 immediately below demonstrates, 50 mg of S7 produced significant inhibition of cellular formation of ROS at 60 min. This effect reached maximal value after 2 h and persisted another hour after administration. In comparison to total generation of ROS, cellular oxygen consumption exhibited first significant

Table 2. Laboratory parameters – haemogram, metabolic-, inflammatory- and lipid-profile.

Haemogram		Inflammatory parameters	
Haemoglobin (g/dl)	14 ± 0.6	Leucocytes (tsd/μl)	7.2 ± 1.7
Erythrocytes (mio/μl)	4.5 ± 0.2	CRP (mg/l)	1.5 ± 0.4
Haematocrit (%)	40.0 ± 1.3		
MCH (pg)	31.1 ± 1.7	Aggregability	
MCV (fl)	88.9 ± 3.3	Thrombocytes (tsd/μl)	257.7 ± 24.1
MCHC (g/dl)	35.0 ± 0.9		
Metabolic-profile		**Lipid-profile**	
Insulin (mU/l)	4.5 ± 1.6	Triglyceride/neutral fat (mg/dl)	95.3 ± 12.3
Glucose/whole blood (mg/dl)	106 ± 9.0	Cholesterol	203.3 ± 9.6
HbA1c (mm)	34.8 ± 1.1	HDL-Cholesterol (mg/dl)	62.8 ± 15.4
		LDL-Cholesterol (mg/dl)	12.8 ± 8.3

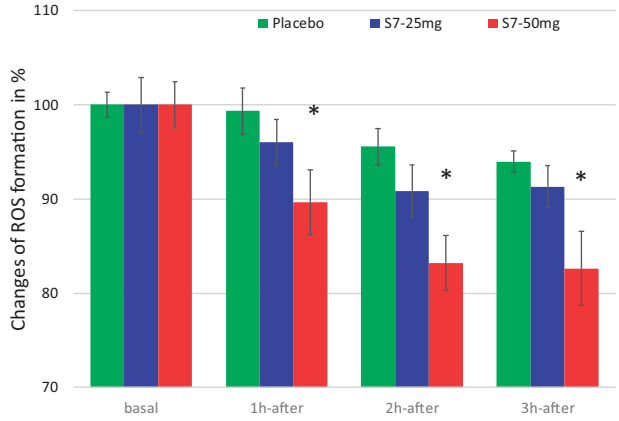

Figure 1. Effect of single dose of S7 on cellular metabolic index "total ROS generation" in human participants. CMI value for a healthy person is age and gender dependent and equal the value 220–240 nm/s. Detection of reactive oxygen species was performed using spin probe CMH (200 μm) and bench-top EPR spectrometer *NOXYSCAN* in eight generally healthy, fasted (minimum 12 h) participants. Green columns (placebo): prior to and 60, 120, 180 min after consumption of standard breakfast (bread roll with glass of water); Blue columns (25 mg S7): after consumption of standard breakfast and capsule with 25 mg of S7; and Red columns (50 mg S7): after consumption of standard breakfast and capsule with 50 mg of S7. For EPR settings please refer to "Material and methods" section. Data are mean ($n = 8$) ± SEM, $^*p < .05$ vs. placebo.

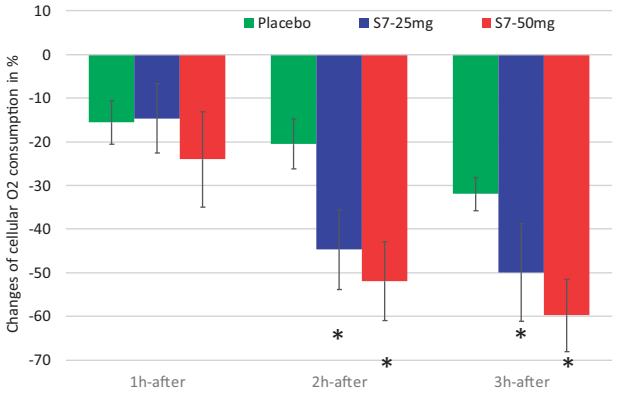

Figure 2. Effect of S7 on cellular oxygen consumption of blood cells. Oxygen consumption analysis was performed simultaneously with detection of ROS using spin-label NOX-15.1 (5 μm) and bench-top EPR spectrometer NOXYSCAN in eight generally healthy, fasted (minimum 12 h) participants. Green columns (placebo): 60, 120, 180 min after consumption of standard breakfast (bread roll with glass of water) and placebo capsule; Blue columns: after consumption of standard breakfast and capsule with 25 mg of S7; and Red columns: after consumption of standard breakfast and capsule with 50 mg of S7. Data are mean ± SEM ($n = 8$), $^*p < .05$ vs. placebo.

changes after 2 h of administration of both 25 and 50 mg S7 dosages indicating long-term changes of redox signalling in human blood cells (see Figure 2). Observation of oxygen concentration changes was possible due to the optimisation of modulation amplitude 1 G, which made it possible to follow EPR amplitude of separately appearing CM-radical and oxygen label EPR lines [9]. We observed that mitochondrial-dependent ROS generation after inhibition of mitochondrial respiratory chain complex III using 10 μm of AA, delivered comparable behaviour as "total" cellular formation of ROS. Only administration of 50 mg S7 induced significant inhibition after 2 h; this inhibition persisted after 3 h. It is important to note here that generation of mitochondrial ROS in healthy persons represented approximately 15% from baseline cellular values in healthy subjects and approximately 25% in metabolic syndrome subjects (data not shown). Surprisingly, mitochondrial oxygen consumption data, one of the important aspects of redox signalling, revealed that there were significant changes after only 60 min of administration of S7 dosages of both 25 and 50 mg. These changes reached the maximal low value after 2 h of administration and stayed low for yet another 60 min of administration. Mitochondrial oxygen consumption represents over 85% of cellular oxygen consumption. In these healthy subjects, we observed a non-significant trend in time-dependent reduction of extracellular superoxide generation. The fact that this trend did not change after administration of S7 or placebo indicated low, healthy expression of enzymes as NOX_2. Because S7 contains botanical materials rich in phytochemicals known to be potent for neutralisation of H_2O_2 (such as catechins, CGAs and curcumin), we evaluated ongoing inhibition of peroxidases and enzymes (such as xanthine oxidase) that generates H_2O_2 [18,19]. This effect was observed only after administration of 50 mg S7 (see Figures 3 and 4). Such effects underpin possibility of inhibition of H_2O_2 (ROS) formation in mitochondria (see Figure 5), based upon reports of H_2O_2 diffusion formed by SOD_2. These effects were confirmed by application of a highly sensitive assay based on detection of extracellular generation of H_2O_2 induced by addition of exogenous TNF-alpha to the blood samples. Three hours after administration of both 25 and 50 mg S7 we noted significant depletion of TNF-alpha induced H_2O_2 formation (see Figure 6). Both inhibition of extracellular peroxidase dependent H_2O_2 formation and TNF-alpha induced generation of H_2O_2 also demonstrated changes in cellular resistance to inflammation as demonstrated in our previous study [9]. In this pilot study, we investigated the effect of S7 on endothelial function based on analysis of circulating, bioavailable NO concentrations [17,20]. The mean value of the examined subjects was in the range 26.4 ± 6.7 nm. These values are not typical for healthy

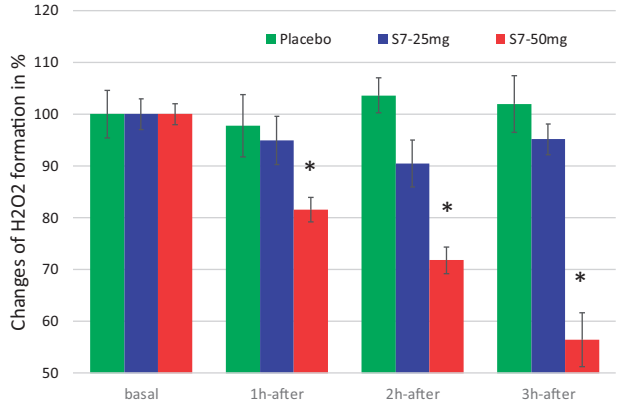

Figure 3. Influence of S7 on "extracellular" H_2O_2 formation in blood cells. The H_2O_2 formation was analysed in human blood using EPR spectrometer NOXYSCAN, spin probe CMH (200 μm) after addition of catalase (50 U/ml) in the blood samples taken prior to, and at 60, 120, 180 min after consumption of standard breakfast (bread roll with a glass of water). Green columns (placebo): after consumption of standard breakfast and placebo capsule; Blue columns: after consumption of standard breakfast and capsule with 25 mg of S7; and Red columns: after consumption of standard breakfast and capsule with 50 mg S7. The values of H_2O_2 generation were calculated as delta value between "total" and "Catalase" sample. Data are mean ± SEM ($n = 8$), $^*p < .05$ vs. placebo.

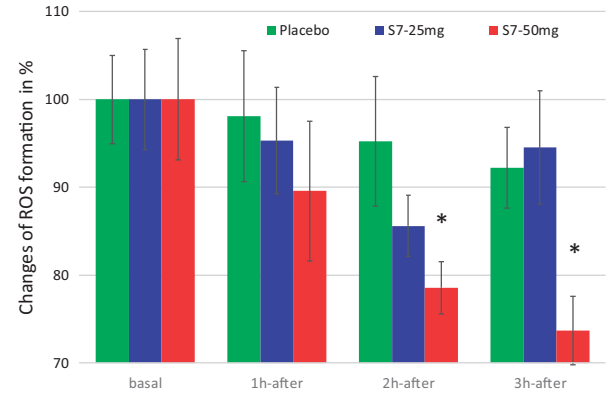

Figure 4. Influence of S7 on "mitochondrial" oxygen consumption of blood cells. Mitochondrial oxygen consumption was performed simultaneously with detection of ROS using spin-label NOX-15.1 (5 μm) and bench-top EPR spectrometer NOXYSCAN after addition of Antimycin A (10 μm) prior to and at 60, 120, 180 min after eating standard breakfast (bread roll with a glass of water). Green columns (placebo): after consumption of standard breakfast and placebo capsule; Blue columns: after consumption of standard breakfast and capsule with 25 mg of S7; and Red columns: after consumption of standard breakfast and capsule with 50 mg of S7. The values of oxygen consumption were calculated as delta value between "Cellular" and "Antimycin A" sample. Data are mean ± SEM ($n = 8$), $p < .01$ vs. placebo.

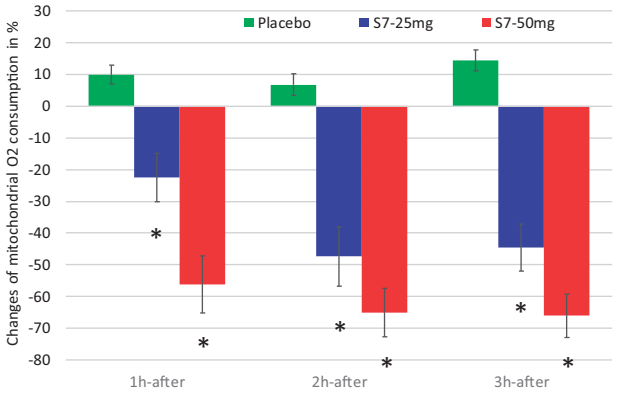

Figure 5. Effect of S7 on "mitochondrial" ROS generation in blood cells collected from human volunteers. Detection of mitochondrial ROS generation was performed using spin probe CMH (200 μm) and bench-top EPR system NOXYSCAN and measured as delta between the value for "total generation" and value after addition of Antimycin A (10 μm) in the blood samples taken prior to, and at 60, 120, 180 min after, consumption of standard breakfast (bread roll with glass of water). Green columns represent placebo values after consumption of standard breakfast; Blue columns (placebo): after consumption of standard breakfast and capsule with 25 mg of S7; and Red columns after administration 50 mg of the S7 capsule with standard breakfast. Data are mean ± SEM ($n = 8$), $^*p < .05$ vs. placebo.

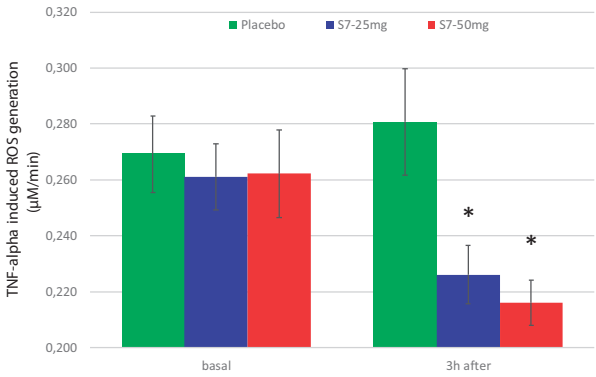

Figure 6. Dose-dependent effects of S7 on TNFα induced a cellular inflammatory response. Induction of extracellular H_2O_2 formation was measured in blood samples from the study subjects after stimulation with 40 ng/ml of exogenous human TNFα and was analysed using EPR system NOXYSCAN and non-membrane permeable spin probe PPH (500 μm). Green columns (placebo): before and 180 min after consumption of standard breakfast (bread roll with glass of water) with placebo capsule; Blue columns: before and 180 min after consumption of standard breakfast and capsule with 25 mg of S7; and Red columns: before and 180 min after consumption of standard breakfast and capsule with 50 mg of S7. Data are mean ± SEM ($n = 8$), $p < .05$ vs. placebo.

persons but are in fact typical for the aged persons. It is interesting that the observed two-fold elevation of circulating NOHb following administration of both 25 and 50 mg S7 was actually much greater (up to 4–5 times) in persons with initially lower baseline values of NOHb. In addition to the observed biological effects on cellular and mitochondrial metabolic activity reflecting changes of ROS generation, oxygen consumption, inflammatory

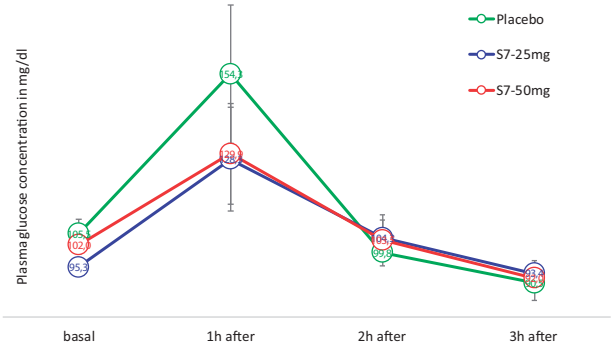

Figure 7. Changes in blood glucose concentration after supplementation of standard breakfast with or without placebo/S7. Green line (placebo): before and 60, 120, 180 min after consumption of standard breakfast (bread roll with glass of water) and placebo capsule; Blue line: after consumption of standard breakfast and capsule with 25 mg of S7; and Red line: after consumption of standard breakfast and capsule with 50 mg of S7. Data are mean ± SEM ($n = 8$), $^{*}p < .05$ vs. placebo.

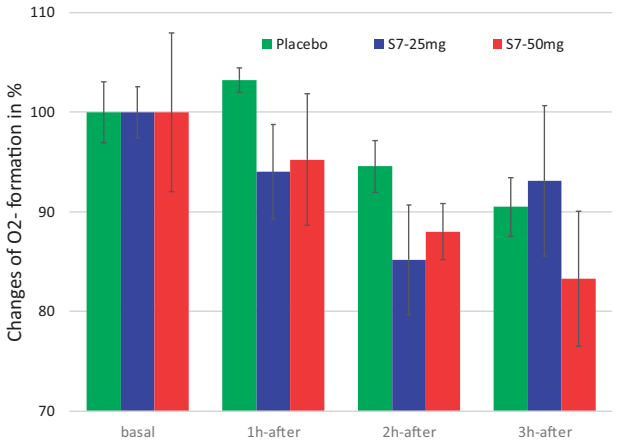

Figure 8. Influence of S7 on "extracellular" superoxide ($O_2^{\bullet -}$) generation in blood cells. Superoxide formation was analysed in human blood using EPR spectrometer NOXYSCAN, spin probe CMH (200 μm) after addition of SOD (50 U/ml) in the blood samples taken prior to and at 60, 120, 180 min. Green columns (placebo): after consumption of standard breakfast (bread roll with a glass of water) and placebo capsule; Blue columns: after consumption of standard breakfast and capsule with 25 mg of S7; and Red columns: after consumption of standard breakfast and capsule with 50 mg of S7. The values of superoxide generation were calculated as delta value between "total" and "SOD" sample. Data are mean ± SEM ($n = 8$).

resistance and endothelial function, we also examined the potential of S7 to limit plasma glucose concentrations induced after consumption of bread roll (see Figure 7).

Discussion

In this pilot study, we delivered evidence of ability to modify the redox signalling of human cells by *in clinico* administration of a botanical nutritional supplement S7 that, at a 50-mg dosage, contains 36.8% catechins (18.4 mg), 26.2% of CGAs (26.2 mg), 18.4% EGCG (9.2 mg), 4.2% of curcumin (2.1 mg), and 3.4% caffeine (1.7 mg). The concentration of vitamins, minerals and other micronutrients in the S7 supplement are in the microgram or nanogram range and can be considered as biologically non-relevant for short-term investigation protocol. The observed multifaceted effects of this new formulation were in compliance with the components of healthy redox balance hypothesis [21] by modulation of:

a. Healthy flux of free radicals.
b. Levels of antioxidants coupled with recycling systems.
c. Nutritional support to help maintain optimal levels of antioxidants and cofactors.
d. Enzyme systems that repair or recycle and replace damaged cellular materials.

Tea polyphenols known as catechins are present in different S7 botanical ingredients but are most concentrated in green tea extract. The main types of catechins present in green tea are epicatechin, epigallocatechin, epicatechin gallate and EGCG [22]. Previous studies reported that catechins may increase the antioxidant capacity of human plasma [23,24], enhance the activities of antioxidant enzymes by scavenging excess free radicals and improving the vitality of intracellular catalase and SOD to inhibit oxidative damage, reducing lipid peroxidation to protect the body based on based on participants' blood samples [25]. In addition, EGCG was shown to promote the expression of p53, p21 and NF-κB, thus inducing the apoptosis of vascular smooth muscle cells so as to inhibit the development of atherosclerosis [26]. Also, catechins can reduce the accumulation of cholesterol and its oxidation products in artery walls when combined with free radicals *in vivo*, thus improving blood circulation and potentially contributing to the prevention of atherosclerosis [27]. Other studies [28–32] also suggested that consumption of tea polyphenols may promote cardiovascular health due to activation of CuZn-SOD activity, and by increasing enzyme expression – a parameter also observed by SOD-dependent extracellular $O_2^{\bullet -}$ generation (Figure 8) in this current study.

Earlier science has suggested that dietary CGA – the major group of coffee polyphenols – may reduce oxidative stress and improve nitric oxide (NO) bioavailability by inhibiting excessive production of ROS in the

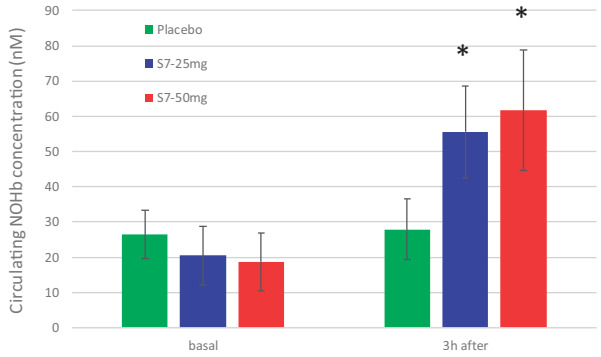

Figure 9. Influence of S7 on circulating NO˙ concentration in blood of human subjects. Bioavailable NO level was analysed in human blood according to material and methods described by the protocol for detection of circulating NOHb concentration in blood samples. Green columns (placebo): before and 180 min after consumption of standard breakfast and placebo capsule; Blue columns: before and 180 min after consumption of standard breakfast and capsule with 25 mg S7; and Red columns: before and 180 min after consumption of standard breakfast and capsule with 50 mg S7. Data are mean ± SEM ($n = 8$), $^*p < .05$ vs. placebo.

vasculature, and lead to the attenuation of endothelial dysfunction [33,34]. It was reported that the initial CGA metabolite, caffeic acid, significantly increased SOD, catalase and glutathione peroxidase (GPx) activity [35]. Consumption of CGA reduced fasting plasma glucose [36–38], increased sensitivity to insulin [39] and slowed the appearance of glucose in circulation after glucose load [40] in animal studies. Recently, it has been reported that CGA also activates adenosine monophosphate-activated protein kinase, a sensor and regulator of cellular energy balance, leading to beneficial metabolic effects, such as suppression of hepatic glucose production and fatty acid synthesis [41] which correlated with our pilot study results (see Figure 7). The most dominant CGA identified in green coffee bean extract are 5-O-caffeoylquinic acid (5-CQA), 4-O-caffeoylquinic acid (4-CQA) and 3-O-caffeoylquinic acid (3-CQA), along with the presence of different isomers [42–44].

Caffeine as another active phytochemical compound in S7 may exert direct and indirect contrasting effects on the vascular tissue by different mechanisms of action and on a wide range of molecular targets. In endothelial cells, caffeine acts directly by increasing intracellular calcium and stimulating the production of NO through the expression of the endothelial NO synthase enzyme causing vasodilatation [45], which may explain the effect of S7 as represented in Figure 9.

Curcumin is considered to be an antioxidant due to the β-diketone group in its structure [46–48]. Joe and Lokesh determined in 1994 that the most important mechanisms by which curcumin is able to promote the majority of its activities are by inhibition of superoxide radicals, hydrogen peroxide and NO radical [49]. Other studies proposed that curcumin also enhances the activity of many antioxidant enzymes such as SOD, catalase, GPx [50] and haeme oxygenase-1 (OH-1) [51]. In accord with these findings, we demonstrated in Figure 8 significant elevations of SOD activity and in Figure 3 increases in catalase activity. These activities reduce lipid peroxidation, thereby decreasing the hepatic damage [52,53]. Another study demonstrated that in human hepatocyte L02 cell line curcumin was able to prevent the ROS formation by increasing SOD activity and reducing glutathione levels after treatment with the antimicrobial feed additive quinocetone as a generator of free radicals [54].

Oxidative and nitrosative stress pathways have shown to be upregulated in subjects with the metabolic syndrome [55]. These pathways contribute significantly to the progression of cardiovascular risk factors commonly associated with the metabolic syndrome such as hypertension, insulin resistance and diabetes leading to atherosclerosis and coronary heart disease or stroke [56].

In particular, oxidative and nitrosative stress have been shown to be associated with endothelial dysfunction. A number of studies have demonstrated that secondary plant compounds such as polyphenols can significantly improve endothelial dysfunction [57].

Another recent meta-analysis reported a 45% reduction of all-cause and cardiovascular mortality in subjects with increased biomarkers of polyphenol intake such as enterolactone [58]. The result of the present study may further improve our understanding how secondary plant compounds, for example, those contained in S7, may help to reduce oxidative stress and improve endothelial function. Another finding of this examination was the reduction in blood sugar by S7 following a standardised test meal. This could possibly be explained by a reduced hepatic glycogenolysis, increased insulin secretion and more important, by a reduced glucose uptake in the small intestine. Polyphenols have shown to reduce hepatic glycogenolysis by inhibiting glycogen phosphorylase and by inhibiting glucose uptake by reducing the activity of the sodium-dependent glucose transporter 1 [59,60]. Therefore, the compounds of S7 may not only reduce oxidative and nitrosative stress but also may help regulate increased blood sugar concentrations in subjects with the metabolic syndrome.

Conclusions

For the first time, we were able to measure the ability of a multifunctional botanical dietary supplement to

influence redox signalling and cellular metabolic activity. Results obtained after ingestion of S7 suggests the potential use of the supplement in correcting dysbalanced redox signalling inherent in metabolic disorders that are accompanied by lowered NO bioavailability, elevated inflammatory response and blood glucose levels. Based upon these early observations, further research is justified into how S7 may ultimately supporting a natural dietary regulation of these in products designed to target sports recovery, sports performance, cardio-health, metabolic health and generally healthier ageing.

Acknowledgements

Herewith, we thank Dr. V. Kagan (Director of the Center for Free Radical and Antioxidant Health, Vice-Chairman of the Environmental and Occupational Health Department at the University of Pittsburgh) for review and helpful discussion during editing of this manuscript.

Disclosure statement

Boris Nemzer, PhD and John M. Hunter are employees of VDF FutureCeuticals. Bruno Fink is an employee of Noxygen Science Transfer & Diagnostics GmbH. No other authors have any potential conflicts of interest.

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