A Double-blind, Placebo-controlled, Randomized, Longitudinal Study on the Effects of a Plant-based Dietary Supplement on Nitric Oxide and Mitochondrial Metabolic Activity

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Abstract
During the past two decades, metabolic dysfunction and concomitant reduction-oxidation reaction (redox) imbalances (i.e., oxidative stress) have been suggested to be associated with numerous age-related chronic diseases. One profound result of metabolic imbalances is endothelial dysfunction, a pathophysiological condition that increases risk of downstream chronic disease consequences. Healthy endothelial function has been positively associated with elevated levels of circulating nitric oxide (NO). Consequently, a natural, plant-based material that may safely increase endogenous NO levels, reduce redox imbalance, and promote improved metabolic response could be of significant interest and benefit. In this first study of its kind, we conducted a longitudinal, randomized, double-blind, placebo-controlled study of S7, a proprietary polyphenol-rich fruit, vegetable, and herb-based material previously reported to reduce reactive oxygen species (ROS) and to increase NO. Specifically, we measured changes in real-time cellular generation of ROS and changes in levels of bioavailable NO (measured as circulating NOHb) in 42 overweight or slightly obese individuals who were recruited into one of three groups: placebo, 25mg of S7, and 50mg of S7. Results suggest that after 90 days of once-daily supplementation, the 25mg and 50mg S7 groups exhibited diminished mitochondrial ROS generation (~54% and ~75%, respectively) compared to placebo, which exhibited a slight increase (>12%) (\( p = 0.049 \)). Furthermore, circulating NOHb levels significantly increased in the 25mg and 50mg S7 groups (33.87% and 53.43%, respectively) compared to placebo (\( p < 0.001 \)). Together, these results suggest that long-term daily supplementation of S7 may provide potential benefits related to healthy endothelial function and reduced mitochondrial dysfunction.

Keywords: endothelial dysfunction, metabolic syndrome, circulating NOHb, cellular metabolic activity, ROS, FMD, Alx 75%, dietary supplement S7

1. Introduction
Obesity is a worldwide epidemic that has significant economic and health consequences. In 2008, an estimated $147 billion U.S. dollars were spent on obesity-related healthcare costs (www.cdc.gov/obesity/data/adult). Additionally, obesity predisposes individuals to many diseases and disorders, including stroke, cardiovascular disease, diabetes, and certain types of cancer costs (www.cdc.gov/obesity/data/adult). In fact, for the first time, some models predict that children in America and Europe could have a shorter life expectancy than their parents (Olshansky et al., 2005). A large part of this risk is because obesity is a central factor in the development of Type 2 diabetes and metabolic syndrome, which have become one of the deadliest cardiovascular risk constellations (Reaven, 2011).

Metabolic syndrome is characterized by endothelial dysfunction, reduction-oxidation reaction (redox) imbalance, hypertension, impaired glucose tolerance, and dyslipidemia, many of which are coupled with the natural aging
process (Reaven, 2011; Rask-Madsen & Kahn, 2012; Yates, et al., 2012; Carrier, 2016; Spahis, Borys & Levy, 2016; McCracken et al., 2018; Kotkowski et al., 2019; Vona et al., 2019). Given that the constellation of symptoms describing metabolic syndrome varies substantially, many researchers have sought physiological biomarkers capable of measuring widespread dysfunction. For example, redox processes pervade practically all fundamental processes, from bioenergetics to metabolism, making them ideal candidates because of their ability to have system-wide effects. Signaling molecules, such as nitric oxide (NO\(^+\)) and superoxide radical (O\(_2\)\(^-\)), have fundamental implications in biology and represent biosignatures related to metabolic syndrome and its role in the development of subsequent pathology, ranging from cardiovascular disease to dementia (Coleman, 2001; Laroux et al., 2001; Korhonen et al., 2005; Stephan et al., 2017; Yuyun, Ng & Ng, 2018). One of the mechanisms through which NO\(^+\) serves as a marker of endothelial dysfunction is through its interaction with superoxide and the subsequent formation of further downstream reactive oxygen and nitrogen species (ROS/RNS) (Laight et al., 1998; Kojda & Harrison, 1999; Zalba et al., 2001; Bauersachs & Widder, 2008; Schulz, Gori & Munzel, 2011). These are relevant for the cardiovascular system since the cardiovascular endothelial homeostasis relies on an equilibrium between these two factors. For example, decreases in NO\(^+\) concomitant with increases in ROS cause endothelial dysfunction and increased risk of cardiovascular disease (Yuyun, Ng & Ng, 2018), one of the most prevalent conditions associated with obesity and metabolic syndrome.

Furthermore, increased ROS production has been posited to be associated with the generation of adipokines. Adipokines are bioactive substances found in adipose tissue that induce generation of ROS (Fernández-Sánchez et al., 2011). In any event, increased ROS production also leads to diminished availability of NO\(^+\) and endothelial dysfunction (Fernández-Sánchez et al., 2011). Thus, NO and ROS, when measured simultaneously, may serve as a robust indicator of underlying processes that significantly contribute to morbid outcomes in obesity and metabolic syndrome.

The role of ROS and NO in oxidative stress is expansive, and pivotal cellular mechanisms have been proposed. For example, mitochondrial dysfunction is associated with increased ROS formation in diabetics (Wu et al., 2018), while also being implicated as a leading factor in heart failure and obesity (Zhou & Tian, 2018; de Mello, Costa, Engel & Rezin, 2018). Interestingly, research has already suggested that combinations of polyphenols (i.e., colorful compounds commonly found in fruits and vegetables that have been shown to have protective biological effects) present in diets rich in fruits and vegetables may be of particular interest in connection with antioxidant properties (Han, Shen & Lou, 2007; Mullen et al., 2011; Yashin et al., 2013; Hussain et al., 2016; Arulselvan et al. 2016; Sarubbo et al., 2018). In vitro, these compounds have demonstrated robust ROS scavenging capabilities while ex vivo studies have demonstrated their effectiveness at reducing oxidative stress (Niu et al., 2015; Serino & Salazar, 2018; Nemzer et al., 2018). Additionally, a recent review suggests that polyphenols may improve mitochondrial function while also improving antioxidant capacity through their disruption of cellular senescence, a process in which cells become dysfunctional and secrete messengers, such as ROS (Serino, & Salazar, 2018). Furthermore, flavonoids, one of the largest categories of polyphenols, have been postulated to increase NO\(^+\), although the exact mechanisms are not yet well understood (Duarte et al., 2014).

In this study, we utilized technological advancements related to the assessment of NO\(^+\) and ROS in order to conduct a double-blind, placebo-controlled, longitudinal study designed to determine the physiological effects of S7, a plant-based, phenolic-rich dietary supplement. Contributing to the novelty of the study, we used scientifically well-recognized electron spin resonance (ESR) spectroscopy (Hawkins & Davies, 2014; Dikalov, Griendling & Harrison, 2007) for direct real-time observation of the physiological levels of cellular, mitochondrial, peroxidase- and NADPH-oxidase dependent ROS formation in whole blood (Nemzer et al., 2018) and for measurement of concentrations of circulating bioavailable NO\(^+\) (NO\(_2\)\(_-\)) that reflects the relative extent of endothelial function (Fink, Dikalov & Fink, 2006). Additionally, we assessed mitochondrial function using a technique previously developed by our laboratory (Nemzer et al., 2018). Advances in instrumentation (a bench-top ESR spectrometer NOXYSCAN System) provided an opportunity to uniquely investigate the bioactivity of S7 on overweight or slightly obese older adults who potentially have an increased susceptibility to metabolic imbalances. S7 is a proprietary formulation of botanical materials high in phytochemicals (Table 1).
Table 1. Phytochemical composition of the nutritional supplement S7 per 25 and 50 mg serving dose

| Phytochemical components | Units | 25 mg   | 50 mg   | Amount (%) |
|--------------------------|-------|---------|---------|------------|
| Catechins                | mg    | 9.2     | 18.4    | 36.8       |
| Chlorogenic acids        | mg    | 6.6     | 13.2    | 26.2       |
| EGCG                     | mg    | 4.6     | 9.2     | 18.4       |
| Curcumin                 | mg    | 1.1     | 2.1     | 4.2        |
| Caffeine                 | mg    | 0.9     | 1.7     | 3.4        |
| Anthocyanins             | µg    | 26.3    | 52.5    | 0.001      |
| Trigonelline             | µg    | 27.6    | 55.1    | 0.001      |
| Vitamin C                | ng    | 2.6     | 5.2     | 1x10⁻⁴     |
| Vitamin E                | ng    | 0.075   | 0.15    | 3x10⁻⁶     |

Given that previous clinical trials have suggested that acute nutraceutical interventions may be effective in attenuating the generation of ROS as well as atherosclerotic events and disease progression (Nemzer, Fink & Fink, 2014; Nemzer et al., 2018) this current longitudinal assessment of NO⁺, ROS levels, and mitochondrial function represents a logical next-step contribution to the literature. We hypothesized that long-term supplementation of S7 would lead to reduced ROS and increased NO⁺ with concurrent improvement in mitochondrial function.

2. Methods

This study was designed to be a 90-day, randomized, longitudinal, double-blind, placebo-controlled investigation of the long-term effects of S7, a proprietary, plant-based dietary supplement that is the hypothesized to new effect of long-term supplementation on NO/ROS.

As such, we sought to recruit individuals who were overweight or slightly obese and therefore at risk for compromised ability to maintain healthy oxidation-reduction levels, but who were not diabetic or otherwise specifically diagnosed with any metabolic condition. The study protocol was registered with and approved by the ethics committee of the Federal Medical Association of Baden-Wuerttemberg (F-2017-030) and was conducted according to provisions of German law, ICH-GCP guidelines, and the general principles of the original World Medical Association’s Declaration of Helsinki.

2.1 Participants

Forty-eight participants were initially recruited and randomly assigned, after matching for age and gender, to one of three groups: placebo (“placebo”), 25mg S7 (“25mg”), or 50mg S7 (“50mg”). Participants were initially screened during a baseline laboratory visit, at which time they were told about the study, provided informed consent, and had basic health parameters collected to determine eligibility for the study. To be eligible for the study, participants 1) had to be between 40 and 70 years of age, 2) had to be overweight BMI > 25 or slightly obese BMI ≤ 34.9, 3) had to be willing to refrain from making any lifestyle changes or altering their diet/exercise regimen for the duration of the study (Fitbit tracker has been used to monitor the exercise level of participants), and 4) had to have an HbA1c < 6.5%. Other exclusion criteria included: smokers, diagnosed Type I or Type II diabetics, any liver/kidney impairments, acute or chronic psychiatric, gastrointestinal, pulmonary, renal, neurological, or cardiovascular disorders, known allergies to foods or other ingredients, used supplements or vitamins, or were taking medications known to affect endothelial function (e.g., ACE-inhibitors or β-blockers). Additionally, participants were excluded if they participated in another clinical trial within 90 days of enrolling. Five participants dropped out of the study, for an attrition rate of 10.4%, and 1 participant was excluded from further analysis because they did not meet inclusion criteria (HbA1c > 6.5%), leaving a total of 42 participants (n = 13, 16, and 13 for the placebo, 25mg, and 50mg groups, respectively). Groups were equivalent on key health measures (Table 2).
Table 2. Demographic and health information for the three study groups, and univariate ANOVA results (with ‘group’ as a fixed factor and each variable as the dependent measure) confirming that the groups did not differ on any parameter at T1. Data represented in the table are mean ± SEM

| Parameter                | Placebo (n=13, 8 women) | 25mg (n=16, 8 women) | 50mg (n=13, 7 women) | Group Differ. (F) | Group Differ. (Sig.) |
|--------------------------|-------------------------|----------------------|----------------------|------------------|---------------------|
| Age                      | 57.8 ± 1.4              | 58.4 ± 1.4           | 57.7 ± 1.7           | 0.14             | 0.88                |
| Waist (cm)               | 109.5 ± 2.8             | 110.3 ± 1.8          | 106.2 ± 2.6          | 0.39             | 0.68                |
| Weight (kg)              | 89.5 ± 3.2              | 92.9 ± 3.1           | 92.7 ± 3.5           | 0.72             | 0.50                |
| BMI                      | 31.5 ± 1.0              | 31.8 ± 1.0           | 31.4 ± 0.6           | 0.26             | 0.78                |
| HDL (mg/dl)              | 53.6 ± 3.1              | 59.7 ± 4.4           | 55.9 ± 3.5           | 0.89             | 0.42                |
| Triglyceride (mg/dl)     | 136.7 ± 14.0            | 179.9 ± 47.6         | 184.7 ± 19.3         | 0.48             | 0.62                |
| Fasting glucose (mg/dl)  | 88.9 ± 2.2              | 90.8 ± 1.8           | 92.0 ± 2.4           | 0.12             | 0.89                |
| sBP (mmHg)               | 138.5 ± 4.1             | 137.6 ± 4.7          | 142.4 ± 4.9          | 0.15             | 0.87                |
| dBP (mmHg)               | 89.3 ± 2.4              | 87.5 ± 2.5           | 88.8 ± 2.1           | 0.71             | 0.50                |
| HR (bit/min)             | 66.1 ± 2.1              | 66.8 ± 2.8           | 63.8 ± 2.7           | 0.34             | 0.72                |
| CMA (nmol/sec)           | 282.1 ± 8.4             | 277.6 ± 9.5          | 275.8 ± 8.1          | 0.07             | 0.93                |
| NOHb (nmol)              | 77.4 ± 6.6              | 86.2 ± 6.4           | 97.8 ± 6.4           | 0.33             | 0.72                |

2.2 Study Design

After the initial consenting visit, participants were sent home with instructions to return in a fasted condition for Time 1 (T1) assessments, which were performed within two days from the initial contact. T1 assessments included a general health evaluation performed by a study physician, cognitive assessments, a venous capillary blood draw, and cardiovascular parameters, which were recorded using the AngioDefender (Everist Health, USA) and the SphygmoCor XCEL (AtCor Medical Inc., USA). At the end of the T1 visit, study participants received encapsulated materials corresponding to their group assignment (placebo, 25mg S7, or 50mg S7), and given explicit instructions regarding ingestion. Specifically, participants were instructed to take 1 capsule every morning with food at approximately the same time every day. After the 90-day study period, participants were scheduled for Time 2 (T2) assessments, which were identical to T1.

2.3 Assays

2.3.1 Cellular Metabolic Activity (CMA)

The extended CMA assay, developed by Noxygen Science Transfer & Diagnostics GmbH (Elzach, Germany), is based upon the monitoring of cellular ROS generation, as well as mitochondrial, peroxidase (H2O2), and NADPH-oxidase (O2) dependent generation of ROS (patent pending). The CMA assay is performed using a bench-top ESR spectrometer equipped with a Temperature and Gas Controller (Noxygen Science Transfer & Diagnostics GmbH, Germany) and a permeable cell membrane spin probe (CMH, 200 µM). For the measurement of extended CMA we added superoxide dismutase SOD (50 U/ml) or catalase (50 U/ml) or Antimycin A (10 µM) to the blood sample allowed for assessment of oxygen concentrations, as well as cellular and mitochondrial oxygen consumption (Komarov et al., 2012).

2.3.2 Bioavailable Nitric Oxide Concentration (NOHb)

Venous blood was taken from a cubital vein using vacutainer containing L-Heparin without upper arm compression and was transferred into a 1ml insulin syringe and spun down (1600 x g) for 5 minutes at room temperature. Afterward, the sample was frozen in liquid nitrogen and kept at –80°C. Measurement of NOHb content was performed at 77°C with liquid nitrogen-filled quartz finger dewar. ESR spectrometer (NOXYSCAN equipped with a newly designed cavity and operating at 86 kHz field modulation) was used to collect ESR spectra at the 9.7 GHz X band using the settings described in Fink et al., 2006 (Fink, Dikalov, & Fink, 2006). The amount of detected NO*, a second crucial signaling molecule of vascular physiology (Dikalov, & Fink, 2005; Pisaneschi et al. 2012) was determined by comparison to the calibration curve for the intensity of the EPR signal of erythrocytes that were treated with known concentrations of nitrite (1–25 µM).
2.4 Chemicals

The spin probes 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH), 1-hydroxy-4-phosphono-oxy-2,2,6, the metal chelators deferoxamine (DF) and diethyldithiocarbamate (DETC), Krebs–Hepes buffer (KHB), and the oxygen label NOX-15.1 were obtained from Noxygen Science Transfer & Diagnostics (Elzach, Germany). All other chemicals and reagents used were of analytical grade and were purchased from Sigma–Aldrich (St. Louis, MO. USA) unless otherwise specified.

2.5 Analytic Plan

Prior to performing statistical analyses, descriptive statistics were determined for each dependent variable and normality was assessed. Outliers were determined using a Tukey fences approach ((Q₁ – 1.5*(IQR)) and (Q₃+1.5*(IQR)), where Q₁ is the first quartile, and Q₃ is the third quartile). Outliers exceeding these boundaries were excluded from analysis. Repeated measures ANOVAs were performed with group as the between-subjects factor and dependent measurements at T1 and T2 as within-subjects factors. Post-hoc comparisons were evaluated for significant effects. In the case of interactions, univariate ANOVAs were performed to determine the nature of the interaction to establish appropriate inferences. Statistical significance was determined at the p < 0.05 level.

3. Results

3.1 CMA

3.1.1 Mitochondrial CMA

Descriptive statistics were determined for each group and each time point. Two participants were excluded from the analysis because of missing data, and 1 outlier was determined from the 50mg group, leaving group sizes of n = 13, 15, and 11 (placebo, 25mg, 50mg, respectively). A repeated-measures ANOVA was conducted with the two-time measurements (T1, T2) as within-subjects factors, and group as a between-subjects factor. Results indicated a significant main effect of time (F(1,36) = 7.252, p = 0.011, partial η² = 0.168) and a significant time by group interaction (F(2,36) = 3.313, p = 0.048, partial η² = 0.155) (Figure 1). A main effect of group was not found (F(2, 36) = 1.311, p = 0.282). To better understand the interaction, we conducted univariate ANOVAs on the T1 and T2, separately. There were no significant differences at T1 (F(2, 36) = 0.236, p = 0.791). T2 differences were significant (F(2, 36) = 3.285, p = 0.049), with least significant difference (LSD) posthoc comparisons suggesting that the placebo group was significantly different from both the 25mg group (p = 0.046) and the 50mg group (p = 0.025). The two S7 groups were not significantly different from each other (p = 0.663). Given these results and that the direction of mitochondrial ROS generation differed between placebo and S7 groups (i.e., placebo increased over 12%, while the 25mg and 50mg S7 groups decreased almost 54% and over 75%, respectively), it is likely that the interaction was driven by S7 supplementation.

![Figure 1](http://jfr.ccsenet.org/JournalofFoodResearch/Vol.10,No.2;2021)
3.1.2 H$_2$O$_2$ CMA

Descriptive statistics were determined for each group and each time point. Two participants were excluded for missing data, and two outliers were determined, leaving group sizes of $n = 13$, $14$, and $11$ (placebo, 25mg, 50mg, respectively). A repeated-measures ANOVA indicated no effect of time ($F(1,35) = 0.824, p = 0.370$), nor was there a time by group interaction ($F(2, 35) = 0.092, p = 0.912$). There was, however, a significant main effect of group ($F(2, 35) = 3.393, p = 0.045$, partial $\eta^2 = 0.162$). LSD post-hoc comparisons indicated that the placebo group was significantly different from both the 25mg ($p = 0.026$) and the 50mg ($p = 0.038$) groups. The 25mg and 50mg groups were not different from each other ($p = 0.969$). These effects are likely driven by qualitative differences in baseline measures, as all three groups demonstrated similar decreases over time (16.38%, 7.92%, and 17.36% for placebo, 25mg, and 50mg, respectively).

3.1.3 NADPH-oxidase CMA

Descriptive statistics were determined for each group and each timepoint. Two participants were excluded for missing data, and one outlier was determined, leaving group sizes of $n = 13$, $15$, and $11$ (placebo, 25mg, 50mg, respectively). A repeated-measures ANOVA indicated no effect of time on NADPH-oxidase ROS generation ($F(1, 36) = 0.918, p = 0.344$), no effect of group ($F(2, 36) = 0.781, p = 0.465$), and no group by time interaction ($F(2, 36) = 2.396, p = 0.105$).

3.2 NOHb

Descriptive statistics were determined for each of the two timepoints and data were checked for normality. One participant’s data was incomplete, and 1 outlier was determined from the 50mg group, leaving group sizes of $n = 13$, $15$, and $12$ (placebo, 25mg, 50mg, respectively). A repeated-measures ANOVA was conducted with the 2 NOHb measurements (T1 and T2) as within-subjects factors and group as a between-subjects factor. Results indicated a significant effect of time ($F(1, 37) = 36.629, p < 0.001$, partial $\eta^2 = 0.497$) (Figure 2). Additionally, there was a time by group interaction ($F(2, 37) = 7.520, p = 0.002$, partial $\eta^2 = 0.289$), as well as a main effect of group ($F(2, 37) = 10.559, p < 0.001$, partial $\eta^2 = 0.365$). LSD post-hoc comparisons revealed significant differences between all three groups (placebo < 25mg, $p = 0.024$; placebo < 50mg, $p < 0.001$; 25mg < 50mg, $p = 0.019$). To determine the nature of the interaction, we conducted univariate ANOVAs at T1 and T2. Results indicated that there were no differences at T1 ($F(2, 37) = 1.595, p = 0.216$), but, and as expected, there were differences at T2 ($F(2,37) = 15.792, p < 0.001$, partial $\eta^2 = 0.461$). LSD post-hoc tests revealed that placebo was significantly different from the 25mg ($p = 0.005$) and 50mg ($p < 0.001$) groups. Additionally, the 25mg group was significantly different from the 50mg group ($p = 0.006$). Specifically, the placebo group exhibited the least NOHb concentration (M±SEM: 84.354±7.961) compared to the 25mg group (M±SEM: 116.453±7.411) and the 50mg group (M±SEM: 148.917±8.286). Because the groups were not significant at T1 but exhibited differences at T2, these data provide strong support that the effects observed on NOHb concentration are being driven by S7. Specifically, over the 90 days, NOHb concentration increased 6.67% for placebo, 33.87% for the 25mg group, and 53.43% for the 50mg group.

![Figure 2](http://jfr.ccsenet.org)

**A.** Bar graph representing the differences between T1 (Day 1) and T2 (Day 90) (M±SEM). **B.** Violin plots demonstrating the raw NOHb data. Violin plots were created with open-source software ggplot2 to visualize the
distribution and kernel probability density of the data within each group at each timepoint. Black dots with connected lines represent the Mt±2*SEM

4. Discussion

Here, we provide evidence supporting our hypotheses that long-term administration of S7, a dietary supplement rich in phytochemicals, reduces ROS and increases NO\(^*\) in overweight or slightly obese participants susceptible to metabolic dysbalance. The observed multifaceted effects of S7 are in agreement with the healthy redox balance hypothesis (Dröge, 2002), which consequently affects NO\(^*\) bioavailability. Given the composition of S7, there are several potential ingredients that may contribute to the underlying mechanisms driving these effects. Specifically, catechins, which represent 36.8% of the material, are polyphenols that have been shown to have protective health benefits through reductions in ROS (Ruidavets et al., 2000; Xiangxin et al., 2002; Hofmann & Sonenshein, 2003; Ohga et al., 2009; Hazafa et al., 2019; Wang et al., 2019). Additionally, S7 has a high percentage of chlorogenic acids (26.2%), that have been shown to decrease ROS and increase ATP production in cells (Youn et al., 2019). These results converge with previous accounts from our lab and others demonstrating that catechins ((-)epigallocatechin, “EGC”, (-)epigallocatechin-3-gallate, “EGCG”) and chlorogenic acids may modify ROS generation (Manach et al., 2005; Nemzer et al., 2018). impact the activity of antioxidative enzymes, reduce lipid peroxidation, promote the expression of p53, p21 and NF-κB (Hofmann & Sonenshein, 2003), and reduce the accumulation of cholesterol in the vessel wall, thus inhibiting development of atherosclerosis (Xiangxin et al., 2002). Furthermore, other lesser ingredients of S7, such as curcumin, for example, have been reported to modulate redox status over the (NRF2) pathway (Shen et al., 2015), with subsequent increases of intracellular Superoxide Dismutase, (SOD) expression, thereby dismutating superoxide radicals into hydrogen peroxide. This is a signaling pathway that further/also leads to expression of Endothelial Nitric Oxide Synthase (eNOS). Such effects consequently serve to increase the bioavailability of NO\(^*\) (Umemura, 2006; Joe & Lokesh, 1994). The observed multifaceted effects of S7’s various compounds seem to be potentially in accord with the components of a healthy redox balance hypothesis (Dröge, 2002). Further research is necessary to confirm this theoretical possibility.

This study confirmed the long-term antioxidative properties of S7 via the reduction of mitochondrial ROS generation concomitant with NOHb increases. Specifically, we observed a greater than 33% increase in NOHb in the 25mg S7 group, and a greater than 53% increase in the 50mg S7 group, as compared to placebo, that had a modest 6.67% increase. The mitochondrial ROS results complemented this pattern wherein placebo increased mitochondrial ROS by approximately 12% while the 25mg and 50mg groups demonstrated substantial decreases of over 54% and 75%, respectively. Thus, we conclude that S7, when administered over a 90-day period, increases levels of bioavailable NO\(^*\), potentially through normalization of mitochondrial ROS generation. Given that the regulation of vascular tone, endothelial function, and cardiovascular function depend heavily on the bioavailability of NO (Stephan et al., 2017; Yuyun, Ng & Ng, 2018), these results may have important implications for various clinical health outcomes including athletic performance. Although we cannot yet confirm the causal relationship between NOHb and mitochondrial ROS generation in this current study, future studies will investigate other potential mediators and mechanisms through which NO\(^*\) increases.

The current study has some limitations. First, the sample size is small, which reduces the power to detect effects. Future studies should be more highly powered in order to detect more subtle effects, which may shed light on other potential mechanisms related to the observed antioxidant properties of S7. Second, we used a participant sample that was overweight or slightly obese and therefore potentially on the cusp of metabolic dysbalance. Thus, we cannot conclude that these results would generalize to other populations of interest, including different age groups. As such, additional studies investigating S7 in other populations would provide intriguing lines of inquiry.

5. Conclusion

In conclusion, for the first time we were able to measure the ability of S7, a polyphenol-rich botanical dietary supplement, to positively increase levels of bioavailable NO\(^*\) and to reduce levels of mitochondrial ROS in a sample of older, overweight or slightly obese adults. These results suggest that S7 has significant potential to improve endothelial function, to increase circulating NO\(^*\) concentrations, to support healthier redox status, and to promote other positive health outcomes through antioxidant effects that are not system specific. Future studies should examine the effects of S7 on other conditions for which dysregulated redox signalling is a hallmark feature (i.e., sports performance and recovery, or specific metabolic or cardiovascular conditions).

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