USANA CellSentials® Supplementation Significantly Increased Circulating Serum Nutrient Levels.

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Summary

We previously demonstrated that one month of CellSentials® supplementation significantly improved self-reported mental and physical health metrics in a study of 70 individuals[1]. In the present study we gain further understanding of the efficacy and impact of CellSentials® on human health in a randomized double-blind placebo controlled clinical trial in which we assess circulating nutrient levels before and after one month of supplementation or placebo in 20 individuals. Seventy-eight percent of circulating nutrients assessed were significantly (Vitamins K, B12, and α-tocopherol (vitamin E)) or modestly (Vitamins A, D, B_6, and folate) improved after supplementation. Additionally, supplementation increased the circulating levels of individuals with low B_{12} levels and folate to within the normal/healthy range. Notably affected nutrients were associated with a myriad of health benefits ranging from increased bone health to neurological improvement, many of which are consistent with the reported health benefits from our previous study and may in part underlie the observed health effects. Finally, the majority of nutrients were within the normal range for healthy individuals after supplementation further supporting previous evaluations assessing the safety of CellSentials® supplementation. These data demonstrate the efficacy of CellSentials® supplementation at increasing the circulating levels of nutrients that are beneficial to human health and further our understanding of the mechanisms by which our product may contribute to previously observed increases in reported quality of life metrics.

Results

1) One month of CellSentials® supplementation was associated with significantly increased circulating nutrient levels.

To assess if CellSentials® supplementation measurably altered circulating nutrient levels, we assayed the levels of vitamins K, B_{12}, A, C, D, and B_6, as well as folate, α-tocopherol (vitamin E), and β/γ-tocopherol in the blood of study subjects before and after one month of supplementation or placebo. As expected, the average change for the placebo group was not significantly different for any nutrient tested (data not shown). The amount of change among the CellSentials® supplemented group was significantly increased relative to placebo ($P < 0.0056$) for three of the 9 nutrients tested (Figure 1), vitamins K ($P = 7.55 \times 10^{-4}$), B_{12} ($P = 4.47 \times 10^{-4}$), and α-tocopherol (vitamin E; $P = 1.81 \times 10^{-3}$). Additionally, four nutrients were modestly increased ($P < 0.01$), vitamins A ($P = 0.018$), D ($P = 0.014$), and B_6 ($P = 5.07 \times 10^{-3}$) as well as serum folate ($P = 9.72 \times 10^{-3}$). The average difference between placebo and supplementation is quite large for all trending nutrients (increases of 6.21, 2.67, 11.50, 74.62 units for folate, vitamin A, D and B6, respectively), nominally significant P-values may reflect the relatively small sample size of the study and the large amount of variation known to be associated with circulating nutrient levels [2-4]. β-γ tocopherol and vitamin C showed no changes after supplementation. **Overall, our data indicated that one month of CellSentials® supplementation was effective at increasing circulating nutrient levels.**

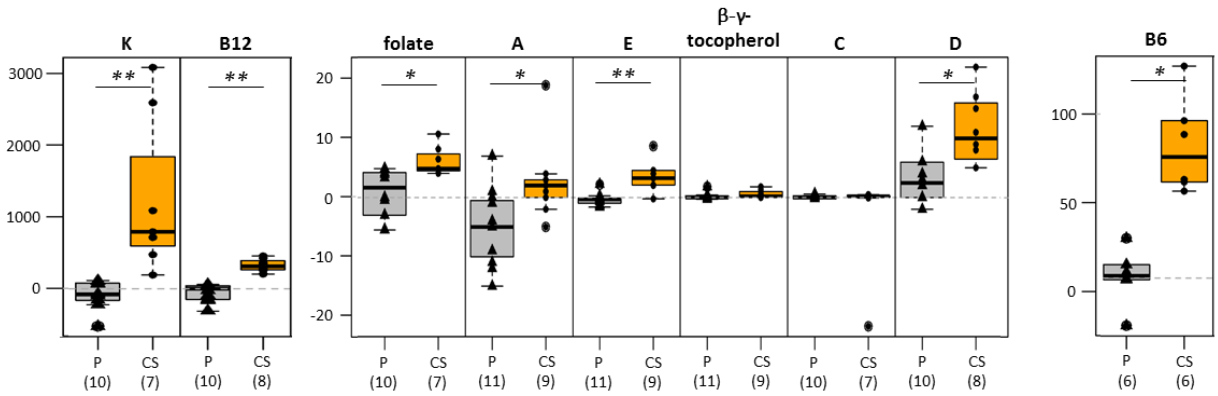

Figure 1. Boxplots showing differences in the circulating levels of nine nutrients after 1 month of placebo (P; grey) or CellSentials® supplementation (CS; orange). Significantly different nutrients are marked with a double asterisk (P< 0.0056) and moderately different (P< 0.01) with a single asterisk. Individual level differences are shown as filled circles (supplementation) or triangles (placebo) within each boxplot. A difference of 0 is marked with a grey dashed line. Difference was calculated for each individual by subtracting the baseline level from the level after supplementation or placebo. Nutrient boxplots are split by magnitude difference for display purposes.

2) CellSentials® supplementation increased circulating nutrient levels of individuals with low or deficient nutrient concentrations.

We identified individuals with deficient circulating nutrient levels at baseline and assessed if levels were increased after supplementation. Forty-four percent of individuals assayed had low levels of B_{12} at baseline and supplementation increased circulating levels to within the normal/healthy range in all instances (P=0.014). Importantly, levels in the placebo group remained relatively unchanged. (P=0.476; Figure 2). Although B12 deficiency is commonly defined as circulating levels ≤ 200 pg/mL, 5-10 % of individuals with levels ≤ 400 pg/mL may develop symptoms of deficiency, specifically neuropsychiatric and hematological abnormalities (Quest diagnostics, Madison NJ), thus our cutoff for low B12 in circulation was placed at 400 instead of 200 pg/mL. Additionally, one individual had deficient levels of folate before supplementation (4.5 ng/mL). This individual was within the normal range after one month of supplementation (9.0 ng/mL). **This data demonstrated that just one month of CellSentials® supplementation was able to increase circulating nutrient levels of individuals with low or deficient circulating nutrient levels.**

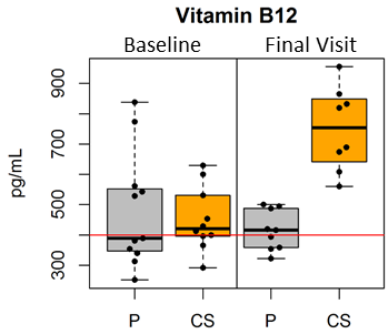

Figure 2. Boxplot showing circulating B_{12} levels at baseline (left panel) and after one month of supplementation (right panel) for placebo (P; grey) and CellSentials® (CS; orange). Individual data points are shown as filled circles within each boxplot.

3) Supplementation induced increases in circulating nutrients were within the normal reference range for healthy individuals.

As CellSentials® supplements have increased dosages of nutrients, relative to the recommended daily allowance of many countries, we verified that assayed nutrient levels were within the normal reference range for healthy individuals in the Salt Lake area. CellSentials® associated increases in circulating levels of vitamins B_{12}, A, and E were within the normal reference range for healthy individuals (Table 1). For vitamins K and B_6, 57 and 100 percent of individuals were above the normal reference range. We note that there is no upper limit for circulating levels of vitamin K. Moreover, harmful levels of B_6 (200mg/day) are well above the 35mg dosage in CellSentials® and increased circulating levels may benefit a myriad of activities in the body as B6 plays a vital role in the function of over 100 enzymes that catalyze essential chemical reactions, including nervous system function[5]. **We showed that one month of CellSentials® supplementation did not increase assayed nutrient levels above normal/healthy levels.**

Table 1. Circulating nutrient level ranges for placebo and CellSentials® supplementation.

	Placebo Follow-up		CellSentials Follow-up		Population Reference Range
	Mean	Range (Min-Max)	Mean	Range (Min-Max)	
Vitamin K (pg/mL)	384.0	231.0-697.0	1579.1	505.0-3283.0	80-1160
Vitamin B12 (pg/mL)	450.7	292.0-629.0	750.2	560.0-955.0	200-1100
Serum Folate* (ng/mL)	19.0	12.2-24.0	20.9	9-24.0	>5.4 normal 3.4-5.4 borderline low
Vitamin A (mcg/dL)	56.9	43.0-78.0	64.3	48.0-93.0	38-98
Vitamin B6 (ng/mL)	18.7	3.8-35.9	84.3	56.6-126.2	2.1-21.7
α-tocopherol (mg/L)	9.8	6.6-11.9	13.3	9.7-23.3	5.7-19.9
β/γ-tocopherol (mg/L)	1.6	1.0-3.4	1.8	1.0-3	< 4.3
Vitamin C (mg/dL)	1.1	0.7-1.7	1.1	0.1-1.4	0.2-1.5
Vitamin D (ng/mL)	35.0	25.0-46.0	37.4	30.0-47.0	30-100 normal 20-29 insufficient <20 deficient

** > 24 off scale*

Discussion

Our study indicates that CellSentials® supplementation effectively increased circulating nutrient levels both of individuals that were deficient as well as those within the normal/healthy range. The advantages of increasing circulating levels for deficient individuals is clear. For example, reduced vitamin D levels have been associated with a myriad of chronic health issues, such as increased bone fracture incidence and cardiovascular health [6]. Although vitamins E, A, K and most B vitamins were already within the healthy range, elevation of circulating levels may still be advantageous. Elevated levels of vitamin K were associated with bone and cardiovascular health as well as increased clotting capabilities in many studies (reviewed in[7]). Additionally, B vitamins in general are required for red blood cell production and tissue repair, and necessary to convert the food we eat into energy. By these mechanisms and others B vitamins are frequently associated with increased vitality (energy) and brain health[8,9], this is consistent with our previous study in which individuals reported increased energy and overall mental health after one month of CellSentials® supplementation[1]. Finally, vitamin E is an antioxidant that protects against reactive oxygen species (ROS) that lead to cellular damage [10,11], increases in vitamin E may augment ROS protection. We note that while certain publications raise concern regarding elevated levels of circulating vitamin E and its

association with health issues; the daily dosages in these studies are 60% higher (≥400 IU) than the CellSentials® daily allotment [12,13].

As outlined above, each nutrient can be associated with individual health benefits; therefore it is important to note that overall health is the result of the global effect of nutrient consumption. Observational and cell culture studies have shown promising effects of supplementation on various aspects of health [14-16], however intervention studies or randomized controlled clinical trials are in many instances conflicting [17] (reviewed in[18]) . Supplementation effects are influenced by duration of supplementation, various genetic, epigenetic, phenotypic, nutritional and other environmental factors that need to be taken into account before relevant effects can be observed [4,15,19-21] (reviewed in[22]). For example, our previous whitepaper demonstrated age and gender effects of supplementation. Specifically, after one month of CellSentials® supplementation older men (but not older women) reported increased vitality relative to younger men and older women (but not older men) reported reduced incidence of bodily pain relative to younger women[1]. Moreover, although vitamin D has been shown to have little effect on the blood pressure of healthy individuals, a recent study demonstrated that one year of vitamin D supplementation lowered central blood pressure among adults with vitamin D deficiency [23]. Finally, there is conflicting evidence on the effect of vitamin E to improve immune response, however it has been shown that baseline immune health can affect immune response and may mitigate the effects of vitamin E supplementation[24,25]. These studies and others highlight the need for an in depth understanding of study populations and clinical characteristics that may influence outcomes of interest. The present study has taken the first step toward this, by demonstrating the efficacy of CellSentials® supplementation at increasing circulating nutrient levels in deficient and non-deficient individuals. Future studies may expand on this work to identify cell-signaling pathways associated with these improvements or identify further avenues of personalization by identifying subsets of a population where these responses are augmented or reduced.

Materials and Methods

Study participants. To assess the effects of multivitamin and multimineral supplementation on circulating nutrient levels, a randomized, double-blind, placebo controlled clinical trial was conducted in 23 "healthy" participants at USANA Health Sciences. Inclusion criterion were: (i) informed consent prior to entry, (ii) within the age range of 20 to 65 years, (iii) no reported medical conditions that would interfere with daily supplementation, (iv) comprehensive metabolic panel results within normal reference ranges, (v) BMI between 17.5 - ≤ 30, (vi) systolic blood pressure between 90-130 mmHg, (vii) diastolic blood pressure between 55-90 mmHg, (viii) fasting blood glucose between 65-110 mg/dL, (ix) total cholesterol < 140 g/dL, (x) LDL cholesterol < 130 mg/dL, (xi) triglyceride level < 150 mg/dL. Major exclusion criterion included: (i) pregnant or lactating females, (ii) chronic or acute use of prescription or over-the-counter medications that interfere with absorption of test supplement, (iii) gastrointestinal conditions that may affect consumption of the treatment or placebo tablets, (iv) acute or chronic illness that prevents informed consent or study participation; including insulin-dependent and orally controlled diabetics, (v) history of alcohol or drug abuse. Non-Caucasian individuals were omitted resulting in 20 healthy participants, 11 male and 9 female. Eleven and nine individuals were assigned to the placebo and supplementation group, respectively. Subjects were instructed to take placebo or supplement twice daily for 28 days. This trial was registered at clinical trials.gov (NCT03146312).

Dietary supplementation. This study was a randomized, double-blind placebo controlled clinical trial consisting of a 28-day washout period (for those currently taking supplements), followed by 28 days of supplementation, USANA CellSentials®, or placebo. Participants were instructed to take supplement or placebo twice a day for the full 28 days. Compliance was measured as: (number of dosage units taken/number of dosage units expected to have been taken) X 100. All individuals had a compliance greater than 70%.

Sample collection and circulating nutrient level measurements. Venous blood was drawn from 12-hour fasted individuals immediately before (baseline) and after 28 days of supplementation (follow-up). Samples were shipped

to Quest Diagnostics laboratory for assessment via the nutritional status panel. This panel assesses vitamin K, vitamin B12, serum folate, vitamin A, vitamin B6, alpha-tocopherol, beta-gamma tocopherol, vitamin C, and vitamin D levels.

Statistical Analyses. Non-Caucasian donors (N=3) were excluded from analyses. Statistical analyses were performed using Rv3.4.1. Clinical characteristics between placebo and supplement groups were assessed using a Wilcoxon rank-sum test. The difference between follow-up and baseline circulating nutrient profiles were also assessed using a Wilcoxon rank-sum test. A multiple-testing threshold of 0.0056 was required for statistical significance.

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Author contributions

ML, TM, and JC designed and implemented the clinical trial and data collection. JN-J designed and implemented further downstream statistical analyses and wrote the manuscript. RS contributed to manuscript preparations.

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