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The Effect of Short-Term NAD3® Supplementation on Circulating Adult Stem Cells in Healthy Individuals Aged 40-70 Years

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Abstract

Objective: This study aimed to assess the impact of acute and short-term supplementation with NAD3 ®, a theacrine-containing supplement, on circulating adult stem cell numbers in a healthy male and female population aged 40-70 years of age.

Methods: A double-blind, placebo-controlled crossover study with 12 participants randomized to receive either NAD3® or a placebo for seven days. Blood samples were collected after an overnight fast, before and after the seven-day supplementation period, as well as one and two hours after the final supplement dose. Using flow cytometry, circulating stem cells, including lymphocytoid $CD34^+$ stem cells ($CD45^{dim}CD34^+$), stem cells associated with vascular maintenance and repair ($CD45^{dim}CD34^+CD309^+$), $CD34^+$ stem cells linked to a progenitor phenotype ($CD45^{dim}CD34^+CD309^{neg}$), circulating endothelial stem cells ($CD45^{neg}CD31^+CD309^+$), and mesenchymal stem cells ($CD45^{neg}CD90^+$) were quantified.

Results: Acute NAD3® supplementation did not result in the mobilization of stem cells from the bone marrow. However, seven days of daily NAD3® supplementation resulted in selective changes in circulating stem cell numbers. A significant time*treatment interaction was observed for $CD4^{5dim}CD34^+$ cells (p=0.04) and CD45dim$CD34^+CD309^{neg}$ cells (p=0.04), indicating a decrease in cell numbers with supplementation. There was also a trend towards an increase in circulating endothelial cells (p=0.08) with seven days of NAD3®supplementation.

Conclusion: Short-term NAD3® supplementation demonstrated an effect on the quantity of bone marrow-derived stem cells in circulation. The study suggests that this theacrine-containing supplement may play a role in modulating adult stem cell populations, emphasizing the potential impact of NAD3® on regenerative processes. Further research with extended supplementation periods and larger sample sizes is warranted to elucidate the functional consequences of these changes and explore the therapeutic implications for age-related declines in stem cell function.

Categories: Nutrition, Integrative/Complementary Medicine
Keywords: supplement, circulating stem cells, nicotinamide adenine dinucleotide (nad+), aging, theacrine

Introduction

NAD3® is a commercially available supplement that contains theacrine (1,3,7,9-tetramethyluric acid), cuprous niacin and Wasabi japonica (a plant similar to horseradish). Theacrine is a major purine alkaloid found in leaves of wild tea plant species such as Camellia kucha. It is structurally similar to caffeine and has been shown to have various health benefits [1] e.g., antioxidative [2] and anti-inflammatory properties [3], fatigue reduction [4], and suppressive effects on breast cancer cell metastasis [5]. It also affects cognitive performance and psychometric parameters; the theacrine supplement (TeaCrine®) at a dose of 200 mg/day led to a significant increase in energy, and a trend towards improved concentration and did not affect heart rate or blood pressure. It is also suggested to improve measures of subjective feelings [6] . Theacrine-containing products have mostly been tested in younger adults [7], however, in the study by Roberts et. al. where the theacrine-containing NAD3® supplement was used, safety was assessed in a population 40-60 years of age [8].

Clinical studies have demonstrated that NAD3® supplementation can improve biomarkers of lipid metabolism [8]. In vitro NAD3® treatment of C2C12 muscle cells also increased cellular nicotinamide adenine dinucleotide (NAD+) levels concomitantly with the upregulation of the enzyme nicotinamide phosphoribosyl transferase (Nampt) [9]. NAD+ is a coenzyme derived from the vitamin niacin, and Nampt is a rate- limiting NAD+ biosynthetic enzyme that plays a critical role in energy metabolism, cellular senescence and aging [10]. NAD3® administration has also been shown to increase the NAD+:NADH ratio, which serves as a proxy of mitochondrial electron transport chain activity and overall cellular oxidative

metabolic capacity, in human peripheral blood mononuclear cells (PBMCs) [8]. The pathway of NAD+ synthesis is highly regulated and NAD+ levels are known to decrease with age [11][12].

Hematopoiesis and immunity are maintained by self-renewable hematopoietic stem cells (HSCs) that are located within the bone marrow [13][14]. Adult HSCs are in a quiescent state to prevent DNA damage and depletion of the stem cell pool. Expansion, maintenance, and differentiation of these stem cells are tightly regulated to assure longevity. Adult stem and progenitor cells, including HSCs, mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs), also contribute to peripheral tissue repair and rejuvenation. With age, the number and frequency of HSCs in the bone marrow increase, however, there is a reduction in their regenerative capacity and an increase in cellular senescence [15]. Aged HSCs also show a skewed differentiation potential to the myeloid lineage and decreased differentiation into the lymphoid lineage, resulting in decreased adaptive immunity and an inability of the body to raise an appropriate immune response [16]. Consequently, elderly populations have reduced adaptive immune function, vaccine failure, increased systemic inflammation and susceptibility to infectious diseases [17]. Even though the number of myeloid cells is increased, their quality is also compromised [18]. Due to the low metabolic rate of HSCs, the mitochondria have not typically been considered critical in restoring function in aged hematopoietic stem cells. However, recent studies suggest an association between NAD+ levels, mitochondrial activities, and HSC longevity in sustaining health and age-related diseases [19]. Various factors contribute to the reduction in HSC functionality. However, the NAD+ precursor, nicotinamide riboside, has been shown to improve the cellular health of aged bone marrow and improve the reconstitution potential of aged HSCs, demonstrating its unique role in aging-induced loss of stem cell function [20]. Supplementation with NAD+ precursors can also restore NAD+ levels in a variety of tissues and organ systems and reverse stem cell dysfunction in aged mice [11][21] [22]. The restoration of regenerative capabilities appears to occur through the amelioration of [23] mitochondrial dysfunction and suppression of cellular senescence [22].

Hematopoietic stem and progenitor cells can be mobilized from the bone marrow. Although this process is crucial for the maintenance and rejuvenation of peripheral tissues with aging [24][25], the prognostic value of circulating stem cells is not well defined. It has been shown that levels of circulating stem inversely correlate with the risk of negative health outcomes, e.g., cardiovascular disease, suggesting that a higher number of circulating stem and progenitor cells might improve tissue damage repair [26].

Drapeau et. al. had previously demonstrated that acute consumption of the polyphenol-rich extracts from sea buckhorn berries resulted in the rapid and selective mobilization of the HSCs, MSCs and EPCs in a population between 28-70 years of age [27]. The effect of NAD3® supplementation on the mobilization of various stem cell types in an aged population has not yet been investigated. In the current study, we measured levels of circulating stem cells after short-term supplementation and acute consumption of the theacrine-containing supplement, NAD3®, in a non-diseased population of male and female adults aged 40-70 years.

Materials And Methods

Experimental design

This study followed a randomized, double-blinded, placebo-controlled, crossover design. Study was registered with ClinicalTrial.gov (NCT02512107). Healthy males and females were recruited via social media. Initial screening was performed using an online screening questionnaire followed by an on-site screening visit. Twenty-two people provided written consent (IRB approved protocol #PRO-FY2023-21) and underwent an in-house screening procedure after which nine people were excluded due to ineligibility. One participant dropped out after the first laboratory visit and their data was excluded in the final analysis. Twelve subjects completed the study. See Figure *1* for the consort flow diagram of the study design. Participants were healthy men and women between 40-70 years of age with vital signs within normal range and no known chronic diseases. Participants had a sedentary to lightly active lifestyle and did not smoke. Participants were asked to refrain from alcohol consumption and strenuous exercise 48 hours prior to each laboratory visit.

Study participants completed four laboratory visits. Participants came to the lab after a 10-hour fast for a baseline visit (Day 1) and again after 7 days (Day 8) of supplementation (NAD3® or placebo) for each of the study arms. To reduce circadian influences on study outcomes, all study visits occurred between 7-10 am and each participant was scheduled to come to the laboratory at the same time for each laboratory visit. Participants were instructed to avoid stressful situations prior to the lab visits, and if there was a stressful occurrence, to reschedule the visit. No participants had to reschedule. During the baseline visits of each arm, blood was collected in K2-EDTA and lithium heparin vacutainers, and test materials (capsules) were distributed. The first capsule was taken with water during the lab visit immediately after blood collection. One capsule was then taken daily for the following 6 days. After the 7th day (Day 8), participants came to the lab after an overnight fast without consumption of the test material. Similar procedures were followed as mentioned for the baseline visit. After the first blood collection, the test material was consumed, and two additional blood collections then occurred one and two hours after test material consumption.

Study Supplement

NAD3®supplement contained the following (per capsule): 344 mg microcrystalline cellulose,156 mg of a proprietary blend of Wasabia japonica (freeze-dried rhizome) cultivar, 97.0% theacrine, copper nicotinic acid chelated complex. Each placebo capsule contained 500 mg of color-matched microcrystalline cellulose.

Measurement of Health parameters

The following metabolic parameters were measured using a Piccolo Xpress chemistry analyzer (Abbott Diagnostics) at the initial laboratory visit: low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TRIG), very low-density lipoproteins (VLDL), aspartate transaminase (AST), alanine transaminase (ALT), cholesterol (CHOL) and glucose (GLU). Insulin was measured using an ELISA assay (Eagle Biosciences).

Diet analysis

All participants completed a 3-day food log prior to each laboratory visit. Participants were encouraged to consume a similar diet prior to each visit. Nutrient analysis was performed using The Food Processor™ software.

Stem cell evaluation by flow cytometry

White blood cell counts were determined at each visit using an Abaxis Vetscan HM5 hematological analyzer (Zoetis). Within one hour of blood collection, stem cells were stained with fluorescently labeled antibodies and immediately analyzed by flow cytometry. Replicate samples of 50 µl of heparinized whole blood were stained with the following antibodies to phenotype various stem cells population: CD45-PacBlue (clone H130, Biolegend), CD34-PeCy7 (clone 561, Biolegend), CD309-PE (clone A16085H, Biolegend), CD31-FITC (clone WM59, Biolegend) and CD90-PE (clone 5E10, Biolegend). Staining was performed on whole blood using Cal-Lyse® fixation as per the manufacturer's instructions. Briefly, whole blood was incubated with a specific antibody combination for 15 minutes at room temperature in the dark. 50 ul of Cal-Lyse® lysing solution was added and incubated at room temperature for 10 minutes. 0.5 mL of deionized H20 was added, vortexed and incubated at room temperature for an additional 10 minutes. Samples were then immediately analyzed using an Attune™ flow cytometer (Life Tech). 500 000 events were collected for each replicate sample. Data were analyzed by Attune ™ software and converted to cells/µl of whole blood.

Statistical analysis

All data are mean ± SD unless otherwise indicated. Statistical analyses were performed using GraphPad Prism 9.5.1. Repeated-measures two-way ANOVA or a fixed-effect model was used to determine potential differences in stem cell populations between treatments. Sidak's post hoc analysis was used to detect differences between specific parameters. Statistical significance was accepted at $p<0.05$.

Results

This was a randomized, double-blind, placebo-controlled, crossover study. Twelve subjects completed the study. See Figure *1* for the consort flow diagram of the study design.

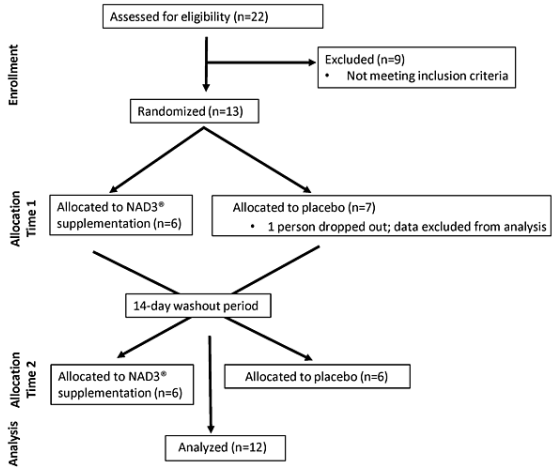


FIGURE 1: Consort Flow diagram

Participants were healthy men and women between 40-70 years of age with vital signs within normal

range and no known chronic diseases. Participants had a sedentary to lightly active lifestyle and did not smoke. Participants were asked to refrain from alcohol consumption and strenuous exercise 48 hours prior to each laboratory visit.Twelve participants finished the study and 100% were compliant in consuming test material and adhering to the study protocol. The study population included 2 males and 10 females. The average age and BMI of the study cohort was 50.58±8.45 years and 27.5±4.0 kg/m2, respectively. Additional anthropometric data for individual participants are provided in Table *1*.

Parameter	Baseline (n=12)
Age, years	50.6±8.5
Male	2/12 (16.7%)
Female	10/12 (83.3%)
Height, cm	165.8±10.4
Weight, kg	76.4±16.8
Body mass index, kg/m2	27.5±3.9
Resting HR, bpm	74.2±8.9
Resting SBP, mmHg	116.3±8.2
Resting DBP, mmHg	74.3±8.3

TABLE 1: Anthropometric data of study participants.

Anthropometric data of 12 participants collected at the initial visit. Values are mean ±SD. (HR, Heart Rate; SBP, systolic blood pressure; DBP, diastolic blood pressure)

Health parameters for the study cohort are provided in Table *2*. Lipid, glucose, and liver parameters were all considered to be normal.

Parameter	Baseline (n=12)
ALT, U/L	24.2±6.7
AST, U/L	21.8±4.8
Cholesterol, mg/dL	189.6±45.3
Triglycerides, mg/dL	100.6±54.0
HDL, mg/dL	64.0±17.8
LDL, mg/dL	101.6±30.9
VLDL, mg/dL	20.1±10.8
Glucose, mg/dL	101.6±9.7
Insulin, mIU/L	5.5±4.0

TABLE 2: Health parameters of study participants.

Health parameters 12 participants collected at the initial visit. Values are mean ±SD. (ALT, Alanine transaminase. AST, Aspartate Aminotransferase, HDL, High density lipoprotein, LDL, Low density lipoprotein, VLDL, Very low density lipoprotein)

Participants were requested to follow their habitual diet during the study period and eat a similar diet in the days prior to each laboratory visit. No significant differences were detected for total daily calories consumed or macronutrient (protein, carbohydrate, and fat) consumption during either the NAD3® supplementation or placebo arms of the study. As theacrine has the potential to interact with adenosine receptors (similar receptors that are blocked by caffeine), caffeine intake was also monitored. There was no significant difference in caffeine consumption detected between lab visits. Nutrient analysis results are shown in Table

3.

	NAD3® (n=12)		Placebo (n=12)				
Parameter	Day 1	Day 8	Day 1	Day 8	P1	P2	P3
Kcal/day	1598±411.1	1578±502.4	1490±434.9	1617±377	0.48	0.83	0.33
Protein (g/day)	69.0±16.0	75.5±26.9	71.1±25.70	65.7±19.0	0.88	0.65	0.12
Carbohydrates (g/day)	171.1±46.1	175.5±67.6	156.6±46.3	186.1±55.6	0.07	0.92	0.17
Fat (g/day)	68.3±25.2	64.2±23.1	63.0±22.6	66.1±21.5	0.91	0.84	0.41
Caffeine (mg/day)	118.4±101.7	152.3±128.3	135.3±109.8	169.2±178.6	0.13	0.74	0.99

TABLE 3: Nutrient consumption prior to each laboratory visit.

Data are mean±SD. Significance determined with repeated measures two-way ANOVA for time (P1), treatment (P2) and time*treatment interaction (P3)

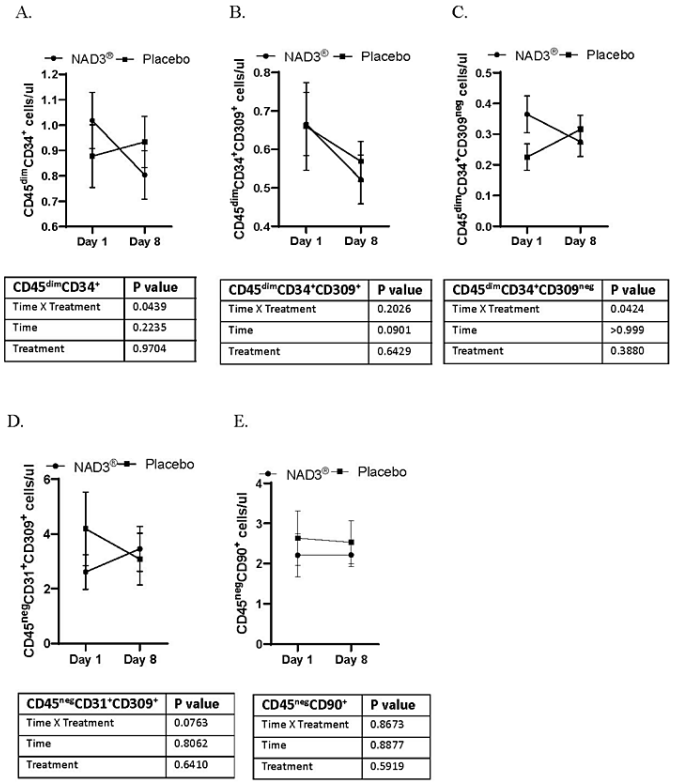
To determine if supplementation affected bone marrow function and immune cell differentiation, white blood cell numbers and composition were determined after one week of supplementation. There was no significant change in the absolute number of white blood cells (time*treatment interaction, p=0.51), lymphocytes (time*treatment interaction, p=0.6), monocytes (time*treatment interaction, p=0.98) or neutrophils (time*treatment interaction, p=0.4) numbers. The composition of the white blood cell population was also not altered (p>0.05) with NAD3® supplementation (Table 4).

	NAD3® (n=12)		Placebo (n=12)				
Parameter	Day 1	Day 8	Day 1	Day 8	P1	P2	P3
WBC (10^9/L)	5.6±1.7	5.2±1.2	5.5±1.7	5.5±1.2	0.42	0.80	0.51
Lymphocytes (10^9/L)	1.8±2.8	1.7±0.2	1.9±0.3	1.8±0.4	0.23	0.36	0.60
Monocytes (10^9/L)	0.07±0.03	0.08±0.05	0.08±0.03	0.1±0.04	0.20	0.22	0.98
Neutrophils (10^9/L)	3.7±1.6	3.4±1.1	3.6±1.5	3.6±1.1	0.27	0.96	0.40
Lymphocytes (%)	34.2±9.4	34.5±7.9	32.9±13.0	33.7±7.2	0.79	0.75	0.90
Monocytes (%)	1.3±0.5	1.7±0.9	1.5±0.4	1.9±1.0	0.11	0.36	0.90
Neutrophils (%)	64.5±9.3	63.8±8.2	62.3±8.5	64.4±7.9	0.56	0.81	0.27

TABLE 4: Leukocyte absolute number and percentage at baseline and after 8 days of supplementation.

Data are mean±SD. Significance determined with repeated measures two-way ANOVA for time (P1), treatment (P2) and time*treatment interaction (P3).

We next determined if one week of supplementation influences the absolute number of circulating stem cell populations; lymphocytoid CD34+ stem cells (CD45dimCD34+), stem cells implicated in vascular maintenance and repair (CD45dimCD34+CD309+), CD34+ stem cells associated with a progenitor phenotype (CD45dimCD34+CD309neg), circulating endothelial stem cells (CD45negCD31+CD309+) and mesenchymal stem cells (CD45negCD90+) were measured before and after supplementation. A significant time*treatment interaction was detected for CD45dimCD34+ cells (Figure 2A, p=0.04), and CD45dimCD34+CD309neg cells (Figure 2C, p=0.04) with no effect on CD45dimCD34+CD309+ cell numbers. Sidak's post hoc analysis revealed a marginal reduction in CD45dimCD34+ cells only with NAD3® (p=0.05), not observed with the placebo. There was also a trend towards a time*treatment interaction in the absolute number of CD45negCD31+CD309+ (Figure 2D, p=0.08) with increased numbers with supplementation. No significant time*treatment interaction was seen for absolute numbers of CD45dimCD34+CD309+ (p=0.20) and CD45negCD90+ cells (p=0.86) (Figure 2B and E).



CD45^{dim}CD34^{+}	P value
Time X Treatment	0.0439
Time	0.2235
Treatment	0.9704

CD45^{dim}CD34^{+}CD309^{+}	P value
Time X Treatment	0.2026
Time	0.0901
Treatment	0.6429

CD45^{dim}CD34^{+}CD309^{neg}	P value
Time X Treatment	0.0424
Time	>0.999
Treatment	0.3880

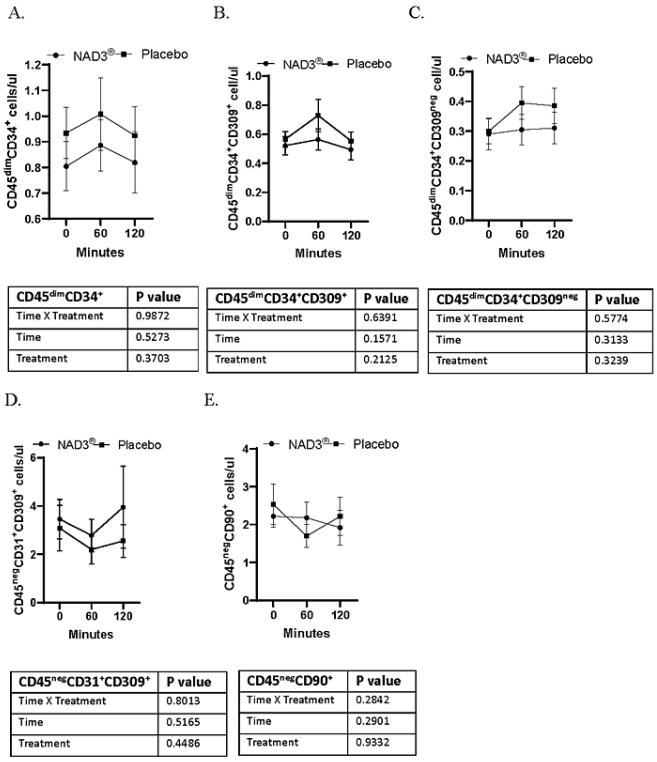
CD45^{neg}CD31^{+}CD309^{+}	P value
Time X Treatment	0.0763
Time	0.8062
Treatment	0.6410

CD45^{neg}CD90^{+}	P value
Time X Treatment	0.8673
Time	0.8877
Treatment	0.5919

FIGURE 2: Absolute number of stem cells (cells/ul of blood) measured in whole blood after one week of supplementation with NAD3® or placebo

Line graphs of concentration of various cell populations; A. CD45dimCD34+, B. CD45dimCD34+CD309+, C. CD45dimCD34+CD309-, D. CD45-CD31+CD309+ and E. CD45-CD90+, measured in blood at baseline and on day 8 of supplementations. Data are mean ± SEM, n=12. Significance determined by repeated measures two-way ANOVA.

Previous studies have shown acute stem mobilization in response consumption of a proanthocyanidin-rich extract of sea buckthorn berries. We therefore determined the acute effect of NAD3® supplementation on the absolute number of circulating stem cells measured one and two hours after the consumption of the supplement. No time*treatment effect was observed at 1 and 2 hours after supplement consumption for CD45dimCD34+ (Figure *3A*, p=1.0), CD45dimCD34+CD309+ (Figure *3B*, p=0.64), CD45dimCD34+CD309neg (Figure *3C*, p=0.58), CD45negCD31+CD309+ (Figure *3D*, p=0.80) and CD45negCD90+ cells (Figure *3E*, p=0.28).



CD45dimCD34+	P value
Time X Treatment	0.9872
Time	0.5273
Treatment	0.3703

CD45dimCD34+CD309+	P value
Time X Treatment	0.6391
Time	0.1571
Treatment	0.2125

CD45dimCD34+CD309neg	P value
Time X Treatment	0.5774
Time	0.3133
Treatment	0.3239

CD45negCD31+CD309+	P value
Time X Treatment	0.8013
Time	0.5165
Treatment	0.4486

CD45negCD90+	P value
Time X Treatment	0.2842
Time	0.2901
Treatment	0.9332

FIGURE 3: Absolute number of stem cells (cells/ul of blood) measured in whole blood immediately prior to supplement or placebo consumption and 1 and 2 hours after consumption

Line graphs of concentration of various cell populations; A. CD45dimCD34+, B. CD45dimCD34+CD309+, C. CD45dimCD34+CD309-, D. CD45negCD31+CD309+and E. CD45negCD90+, measured in blood at baseline and after 1 and 2 hours of supplement intake. Data are mean ± SEM, n=12. Significance is determined by repeated measures two-way ANOVA.

Discussion

A central physiological feature of aging is a diminished ability to maintain tissue homeostasis. Hematopoietic stem have a critical function in this process and a decline in the capacity of these cells to replenish tissues result in the ineffective repair of damaged tissues [28].

NAD+ has gained considerable attention in the context of aging and particularly its potential role in stem cell longevity. It is known that NAD+ levels decrease with age, and compounds that increase NAD+ levels can improve the pool and pluripotency of stem cells [22]. NAD+ precursor compounds e.g., nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) have also been shown to restore aged stem cells through the NAD+/SIRT pathway and suppress senescence [19] [22]. In vitro studies by Mumford et. al. demonstrated that the commercially available supplement NAD3®, can increase intracellular NAD+ levels and sirtuin activity [9]. Sirtuin proteins are believed to be responsible for the improvement in bone marrow cells resulting from increased NAD. Studies by Roberts et al. demonstrated the effect of supplementation

with NAD3® on the blood lipid levels and NAD levels in peripheral blood mononuclear cells (PBMCs). Although no change in NAD+ and NADH levels were observed with supplementation in PBMCs, there was an increase in the NAD+:NADH ratio compared to placebo, indicating that NAD3® can impact the NAD+ metabolome [8].

Stem cell mobilization, a process by which hematopoietic stem cells (HSCs) are released from the bone marrow into the bloodstream, is a natural response to various physiological signals and is important for peripheral tissue repair. Potential strategies have been studied to increase stem cell mobilization associated with aging, e.g., dietary interventions (such as caloric restriction, omega-3 fatty acids and antioxidants), exercise, and hormonal therapies. Drapeau et.al. also demonstrated that a polyphenol extract from sea buckthorn berries causes rapid and selective mobilization of specific stem cell types [27].

In the current study, we investigated the effect of NAD3® supplement on mobilization of various stem cells population (CD34+ stem cells (CD45dimCD34+), stem cells implicated in vascular maintenance and repair (CD45dimCD34+CD309+), CD34+ stem cells associated with a progenitor phenotype (CD45dimCD34+CD309neg), and circulating endothelial stem cells (CD45negCD31+CD309+) and mesenchymal stem cells (CD45negCD90+)) in an aging, but otherwise healthy population. No metabolic dysfunction was detected in the study population, and diet and caffeine consumption were consistent throughout the study period.

White blood cell numbers and composition were measured before and after supplementation, but supplementation did not alter total white blood cell, monocyte, lymphocyte or neutrophil numbers. Changes in circulating stem cell numbers were also monitored before and after 1 week of supplementation. NAD3® supplementation resulted in a significant decrease in the CD45dimCD34+ population and specifically the CD45dimCD34+CD309neg cells. This population is associated with a progenitor phenotype, while the CD45dimCD34+CD309+ cells contain the transmembrane tyrosine kinase, also known as the vascular endothelial growth factor receptor-2 (VEGFR-2), and is implicated in vascular maintenance and repair [27]. CD45dimCD34+CD309+ cells were not significantly different from placebo. The optimal level of circulating progenitor cells is not known, although it has been suggested that higher baseline numbers are beneficial for peripheral tissue repair. However, during aging the number and frequency of HSC in the bone marrow of mice and humans increase, but these cells exhibit a concomitant decrease in regenerative capacity [15]. In a mouse model of G-CSF-induced stem cell mobilization it was found that the ability to mobilize HSCs was 5-fold higher in aged mice due to changes in cell adhesion to bone marrow niche [29]. It is therefore possible to speculate that improving stem cell health and reducing senescence in an aged population can reduce progenitor number in circulation. A limitation of the current study is that we did not determine the regeneration capability of the circulating stem and progenitor cells to determine if there was a change in the functionality of the cells after supplementation.

Interestingly, there was a trend towards an increase in circulating endothelial progenitor cells (CD45negCD31+CD309+) with NAD3® supplementation. Endothelial stem cells are thought to be involved in blood vessel formation and vascular homeostasis. Depletion of NAD has previously been suggested to contribute to the impairment of EPCs mobilization in diabetic conditions, suggesting a potential therapeutic value of NAD in the prevention or treatment for cardiovascular complications of diabetes [30]. The duration of supplementation in this study was limited to one week; therefore, an extended period of supplementation may be necessary to observe a significant change. Nonetheless, these data are intriguing given our relatively small sample size and short period of supplementation.

Acute changes were also determined by monitoring stem cell numbers at 1 and 2 hours after supplement ingestion. No effect of NAD3® supplementation was detected on the mobilization of previously described HSCs and progenitor cells. This result suggests that that there is no acute effect of NAD3® supplementation on the adhesion of stem cells to the microenvironment within the bone marrow.

Conclusions

In conclusion, one week of NAD3® supplementation did not cause an acute mobilization of bone marrow derived stem cells. However, supplementation did result in selective changes in the circulating stem cells numbers; there was a decrease in CD34+ progenitor cells and a trend toward an increase in endothelial stem cells. Although it is not clear what the functional consequence of these changes are, it does suggest that oral NAD3® supplementation affects the physiology of bone marrow derived stem cells. Future studies should utilize a longer supplementation period and larger sample sizes to ascertain if NAD3® affects various populations of stem and progenitor cells. Finally, it is essential to assess the regenerative potential of these circulating cells post-supplementation to determine if there are any changes in the functionality of the cells attributable to the intervention.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. University of Memphis

issued approval PRO-FY2023-21. This study was approved by the University of Memphis Institutional Review Board. . **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** Compound Solutions provided funding for the project. . **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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