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The Effect of Vitamin D and E and Coenzyme Q-10 on Endothelial Function in a Young Population

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Abstract

Background: Oral antioxidants have been shown to improve vascular function in older individuals. It is usually assessed by the blood flow response to occlusion. In the present investigation, 2 tests of vascular function with different mechanisms were assessed after vitamin administration in young individuals.

Methods: Thirty six physical therapy students participated in a series of experiments to see if the administration of vitamins (300 mg of coenzyme Q-10, 1000 mg of vitamin C and 1000 IU of vitamin E), taken daily, would impact resting blood flow in the forearm skin and the blood flow response to 4 minutes of vascular occlusion or the response to local heat (42°C) for 6 minutes. Vitamins were administered for 2 weeks. Half of the subjects took the vitamins and half did not.

Results: The results of the experiments showed that the resting blood flow in the skin of the forearm was nearly double in the vitamin group compared to the control group. Whereas the blood flow response to occlusion was not different between the two groups of subjects ($p>0.05$). The blood flow response to heat for the vitamin group peaked at 60.8 +/- 28.5 flux, and for the control group peaked at 43.6 +/- 22.6 flux, a significant difference ($p <0.05$).

Conclusion: The response to heat, unlike the response to occlusion, can be altered by administration of large doses of vitamins in young, fit subjects. This may be due to the fact that the response to occlusion is believed to be mediated by a different mechanism than the response of the skin blood flow to heat.

Keywords: Free radicals; Vitamins; Local heat; Blood flow; Circulation

Introduction

Vascular endothelial function is critical for the health of the organs in the body [1]. It was once believed that endothelial dysfunction was only seen with age and diseases such as diabetes [2]. This reduction in endothelial function causes an impaired blood flow response to stressors on the skin such as heat and pressure and reduced blood flow to vital organs such as the heart and kidney causing senescence in the cardiovascular system [3]. With what the World Health Organization calls an epidemic of obesity and diabetes, endothelial impairment is even being seen in young people [1].

One factor believed to be a principal cause of endothelial dysfunction is high concentrations of free radicals in the body. Free radicals are commonly produced and neutralized in the body [4]. Some free radicals are produced and used for cellular communication, and others are produced as a natural product of cellular metabolism [5-8]. For example, nitric oxide is released from mitochondria and vascular endothelial cells to increase circulation in the tissue [7]. Two to five percent of oxygen used by mitochondria forms free radicals [9]. With exercise, oxidative phosphorylation increases dramatically, increasing the production of free radicals [10]. For example, using electron spin resonance spectroscopy [11] there was a 70% increase in free radical production from electrically stimulated rat muscle compared to controls. It is of no surprise, then, that exercise is considered an inflammatory process [10].

When free radical levels reach a critical level, rather than increasing blood flow, they biodegrade nitric oxide and prostacyclin into inactive forms. In the presence of free radicals such as hydrogen peroxide, nitric oxide is reduced to peroxynitrite (ONOO) a free radical with no influence on circulation [1]. Bioconversion of nitric oxide to peroxynitrite is believed to be one of the mechanisms associated with the reduction in circulation at rest and during stress in older people and people with diabetes [1].

A common measure of endothelial function is flow mediated vasodilation (FMD). FMD is mediated by shear stress on large arteries through a prostaglandin mechanism [1] and is a measure of macrovascular function. High free radical levels in young men has been shown to be negatively correlated with FMD [12]. Another measure of endothelial function is the skin response to local heat [1]. When heat is applied to the skin, there is an increase in skin blood flow mediated by two different mechanisms. Initially, tactile neurons in the skin release Substance P and Calcitonin Gene Related Peptide when the skin is exposed to local heat [3,13]. This causes an increase in potassium permeability in vascular smooth muscle surrounding the endothelial cell [13-15]. Relaxation of vascular smooth muscle then increases blood flow. But this response only lasts a few minutes. The sustained response to increasing temperature in the skin is mediated by TRPV-4

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voltage gated calcium channels in the vascular endothelial cells [16-19].Above a temperature of 35°C, these cells cause an exponential increase in calcium influx into the endothelial cell from the interstitial space.Calcium activates the enzyme nitric oxide synthetase producing endothelial nitric oxide [20]. Nitric oxide, a potent vasodilator, diffuses into the surrounding smooth muscle activating cyclic GMP which in turn increases potassium permeability and relaxes vascular smooth muscle [13,15,21-23]. High concentrations of free radicals neutralize nitric oxide and prostacyclin, the 2 principal vasodilators, and reduce blood flow at rest and in response to heat.

Numerous studies have examined the administration of vitamin A, C and E, known potent antioxidants, on free radicals and performance. For example, since free radicals are associated with muscle soreness and the inflammatory response to exercise, various studies have shown that oral doses of vitamin E or C increase the antioxidant capacity and a reduction in muscle soreness [24,25]. In a study of chronic smokers, FMD was reduced in smokers but increased in the same population with administration of vitamin C (1000 mg) and E (500 units) for 25 days. Another study of smokers also showed a low response to FMD which was reversed with 4 weeks administration of 600 IU of vitamin E for 4 weeks [26]. Gross measures of vascular function such as protection from damage from myocardial infarction have been shown with as little as 14 days of vitamin E supplementation by scavenging free radicals, improving antioxidants and maintaining Ca(2+) levels [27,28].

Considering the fact that numerous studies have examined the benefit of vitamins in reducing free radicals in the body [29-31], it is surprising very little has been done to see if vitamin supplementation can alter direct measures of endothelial function, such as the response to local heat or occlusion mediated vasodilatation. The hypothesis to be tested in this study, then, was that even in young individuals, coenzyme Q-10, Vitamin E and C would have an effect on resting blood flow, and the blood flow response to occlusion and local heat.

Subjects

Thirty six subjects participated in the randomized, double blind experiments. The subjects were randomized in 2 groups. Sixteen subjects were control subjects (8 male, 8 female), not taking any vitamin supplements, and 19 subjects took vitamin supplements (9 female and 10 male). The non-vitamin group (control group) had an average age of 24.9 +/- 2.6 years, an average height of 171.9 +/- 10.5 cm, and average weight of 70.3 +/- 13 kg. Their average BMI was 23.8 +/- 2.6. The resting blood pressure was averaged 105.8 +/- 9.2 over 67.8 +/- 6.1 mm Hg, and their average heart rate was 72.6 +/- 14.8 beats per minute. For the vitamin group, the average age was 23.9 +/- 1.4 years, the average height 169.6 +/- 8.1 cm, and the average weight was 68.2 +/- 12.9 kg. The BMI average was 23.6 +/- 3.7. The average resting blood pressure was 103.9 +/- 6.1 over 66.9 +/- 7.7 mm Hg. The average resting heart rate was 76.2 +/- 14.7. Statistical analysis (unrelated t-test) showed no significant difference between any of the demographic parameters measured here.

Thus, all subjects were of a similar age, were not taking alpha blockers, beta blockers, alpha agonists or antagonists, or any other medication that would affect peripheral blood flow. They were not taking calcium channel blockers or any pain medications. All subjects were vitamin naïve for at least a month prior to the beginning of this study. No subjects were smokers. All subjects were physical therapy students at Azusa Pacific University with similar levels of activity. The control group took no supplements since the measures here, the blood flow at rest and in response to heat or occlusion are quantitative and not influenced by what the subject thinks they should do. All subjects were told that the vitamins may or may not have an effect to avoid any bias. All methods and procedures were approved by the institutional review board of Azusa Pacific University. All subjects signed a statement of informed consent.

Methods

Measurement of skin temperature

Skin temperature was measured with a Thermistor (SKTRX202A) manufactured by BioPac systems Inc. (Goleta, CA). The Thermistor output was sensed by an SKT100 Thermistor amplifier (BioPac Systems Inc., Goleta, CA). The output, which was a voltage between 0 and 10 volts, was then sampled with an analog to digital converter at a frequency of 1000 samples per second with a resolution of 24 bits. The sampling system was a BioPac MP-150 analog to digital converter. The converter data was stored and later analyzed with Acknowledge 4.1 software. Data was analyzed over a 10 second period. The Thermistor's were calibrated at the beginning of each experimental day by placing the Thermistor used in the study in a controlled temperature of water bath which was calibrated against a standard thermometer.

Measurement of blood pressure

Blood pressure was measured by auscultation of the right arm with a sphygmomanometer. Systolic and diastolic pressures were determined as per the standards of the American heart association. The systolic being determined as the first tapping heard through the stethoscope and the diastolic as the changing quality from a tap to a muffle. The blood pressure cuff was applied to the arm and inflated to 200 mm Hg, and the pressure was released at 3 mm Hg per second as per the standards of the American heart association.

Measurement of heart rate

Heart rate was measured with a fingertip pulse oximeter on the other arm at the same time blood pressure was measured (Santa Medical Model SM-110, Arcadia, California).

Measurement of skin blood flow

To measure skin blood flow, a BioPac systems laser Doppler flow probe was used (LDL202A) (BioPac Inc., Goleta, California). The laser Doppler flow probe was a fiber optic probe which provided an infrared beam to measure blood flow on the skin. It was plugged into a module which contained a laser and a standard temperature compensation unit. The device was connected to an MP-150 BioPac system and was used to measure blood flow (in flux) continually throughout the experiment.

Control of skin temperature

Skin temperature was controlled by a BioPac (BioPac Inc., Goleta, California) Isotemp water bath. The water bath, with a flow rate of 1

1. NAS Report "Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters," Feb. 15, 2011, Summary of Committee Findings.
2. Ibid.
3. NAS Report, Chapter

liter per minute, had its output connected through a water jacket that could be placed around the arm. The water temperature was 42°C, and when the jacket was placed on the arm, it would warm it rapidly. The water bath was calibrated with a standard thermometer prior to the experiments.

Vitamins

The vitamins used in the study was a multivitamin (Kirkland multi), 300 mg of coenzyme Q-10, 1000 mg of vitamin C and 1000 IU of vitamin E taken daily.

Procedures

After subjects either didn't take vitamins at all (control group), or took vitamins for 2 weeks as described under methods, two series of experiments were conducted. In one, subjects sat in a seated position and a thermistor and a flow probe were placed on their arm above the brachioradialis muscle. They sat in a controlled temperature room (23°C) for 10 minutes, and then baseline flow measurements and skin temperature was recorded. The water jacket was then placed on the arm, and for 6 minutes, skin temperature and blood flow were recorded. In a second series of experiments on the opposite arm, the blood flow probe was placed also above the brachioradialis muscle, and an occlusion cuff was placed under the axilla and inflated to 200 mm Hg for 4 minutes. The pressure was then released and the blood flow was measured for an additional 2 minutes.

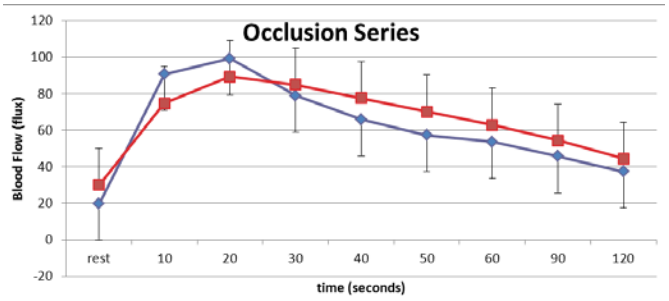

Figure 1: Illustrated here is the mean (+/- SD) blood flow measured in the control subjects (diamonds), and the group that took vitamins (squares). Each data point shows the mean +/- the standard deviation for blood flows measured in flux, measured at rest and at 10, 20, 30, 40, 50, 60, 90, and 120 seconds post occlusion.

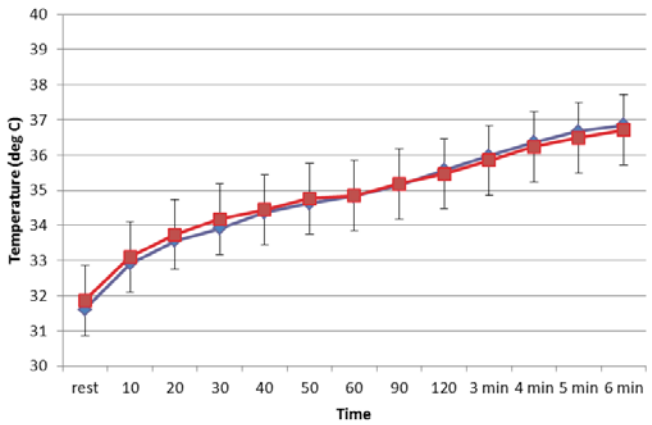

Figure 2: Illustrated here is the average skin temperature of the control group (diamonds), and vitamin group (squares) measured at rest and at 10, 20, 30, 40, 50, 60, 90, and 120 seconds post application of heat, and at 3, 4, 5 , and 6 minutes post application of heat. Each point shows the mean response +/- the respected standard deviation for the group.

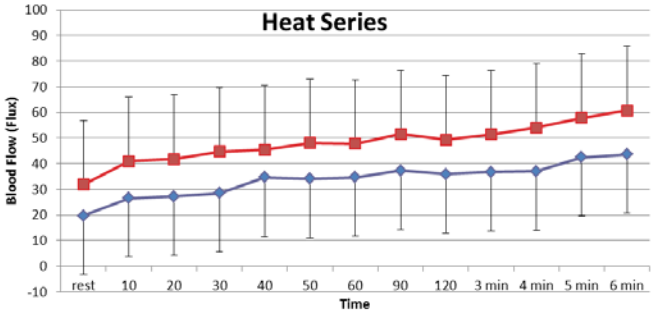

Figure 3: Illustrated here is the blood flow measured on the forearm (in flux) measured at rest and at 10, 20, 30, 40, 50, 60, 90, and 120 seconds post application of heat as well as 3, 4, 5, and 6 minutes post application of heat. Each data point represents the mean for the control subjects (diamonds), and the vitamin group (squares) +/- the respective standard deviation.

Results

Results of the experiment are shown in Figures 1, 2 and 3. For both series of the experiments, the resting blood flow was significantly

4. The FBI issued a subpoena in February 2002 requiring submission of samples from "each distinct B. anthracis Ames strain stock in your possession, which differs in source or in other parameters prescribed by the requesting agency" (no other parameters are mentioned in the documents). Both institutional and personal inventories were covered (NAS Report, p. 105; FBI "Supplemental Documents:" "Preparing and Shipping TSA Slants for B. anthracis Ames").
5. NAS Report pp. 104-107 and 119. The Report notes that the repository was unlikely to have been comprehensive because of uncertainty in whether all Ames-strain possessors had been identified, the lack of specificity in the subpoena protocol, uncertainties in compliance with the protocol, incomplete information on Ames-strain transfers between laboratories, the possibility that some stocks had already been destroyed in the months before the FBI sent its subpoena, and because repository collection was based on the integrity of those asked to provide samples rather than on standards of custody or evidence (which would have required that FBI agents collect the samples).
6. Eleven repository samples were not viable, and 112 samples gave inconclusive assay results, which were discarded (NAS Report, pp. 109-110). Thus, 123 samples, submitted by an unreported number of laboratories, have not been considered.
7. NAS Report (2009) Also, note that an FBI Press Release of March 6, 2009, discussing a presentation at the 2009 American Society for Microbiology meeting in Baltimore, said that eight samples matched the genetic profile of the letter material 110-112.
8. FBI documents B2M10 p. 25 and B3D16.
9. FBI document B3D16.
10. See, for example B. anthracis assay results on 30 repeat samplings of one liquid sample (NAS Report 117).
11. See NAS Report eg 114-117 and p 107.
12. NAS Report FBI document B2M10 pp. 25 and 104-112 (Appendix V of Report on Statistical Analysis, where the names of some laboratories are handwritten next to data on their samples).
13. A hand-drawn diagram in FBI document B3D16 indicates that Ivins sent a sample to DRES on June 21, 1998 and that an FBI repository sample from DRES tested positive for A1, negative for A3 markers; the same diagram indicates the two shipments to BMI in 2001.

higher in the vitamin group than the control group. The average resting blood flow in the vitamin group was 30.1 +/- 16.5 flux, and for the control group the average resting blood flow was 19.7 +/- 9.8 flux. This difference was significant ($p = 0.03$). From the resting blood flow, when occlusion was applied to the arm, and then released after 4 minutes, as has been reported widely in the literature, there is a rapid increase in blood flow peaking at about 20 seconds after the occlusion was released and returning close to normal by 2 minutes. As shown in Figure 1, the peak blood flow for the control group averaged at its highest, 99.2 +/- 27.5 flux and for the vitamin group, at its highest it averaged 89.2 +/- 34.1 flux (Figure 1). There was no significant difference between the 2 groups for peak blood flow ($p = 0.35$), or at any point throughout the entire 2 minute post occlusion (ANOVA, $p > 0.05$). However, results were different in the second series of experiments. As shown in (Figure 2), when heat was applied to the arm, skin temperature rose continually to a maximum skin temperature for both groups of subjects averaging 36.7 +/- 0.9°C. At rest or throughout the heat exposure (6 minutes), there was no statistical difference in the skin temperature from either of the 2 groups (ANOVA, $p > 0.05$). However, as shown in (Figure 3), blood flow at rest and throughout the heat exposure was higher in the vitamin group than the control group. Analysis of variance (one way ANOVA) showed the blood flow to be significantly higher at each measuring point throughout the entire 6 minute period in the subjects taking vitamins compared to the control group ($p < 0.05$). Thus, blood flow, for the vitamin group peaked at 60.8 +/- 28.5 flux, and for the control group peaked at 43.6 +/- 22.6 flux.

Discussion

In the present investigation, the effect of coenzyme Q-10, Vitamin E, a multivitamin supplement and a dose of vitamin C on vascular endothelial function in younger individuals was examined. Endothelial function was assessed in 2 ways. The first was the response to heat. The sustained skin blood flow response to local heat is believed to be largely mediated by nitric oxide, although in younger individuals prostacyclin is also involved [13,32]. The second means of assessing endothelial function is by the response to occlusion. Classically, if the occlusion to the circulation is accomplished for 4 minutes, the sudden rise in skin blood flow above basal levels after the release of arterial occlusion, called reactive hyperemia, is characterized by 2 phases. A maximal peak that occurs within a few seconds, and then finally a more prolonged hyperemic period that is believed to represent blood flow debt repayment [33-35]. As such, reactive hyperemia has been used as a common clinical tool to assess micro-vascular and macro-vascular function in patients with diabetes, cardiovascular disease, and other stressors that affect the blood flow in the body [34,35].

The resting blood flow before the reactive hyperemia and the resting blood flow prior to heat was altered by a 2 week course of vitamins. Since resting blood flow, even in younger individuals, in a mildly warm room is mediated by nitric oxide [8], damage to these vasodilators by free radicals produced from digestion of food (especially high fat meals) [36] and environmental factors such as secondary cigarette smoke [37,38], can bioconvert nitric oxide into peroxynitrite [1]. Free radicals also have the ability of impairing production of prostacyclin, the second vasodilator found in younger individuals, into a bio inactive form [1]. While the effect of free radicals on blood flow is well known, it is still surprising that in this young, healthy population, resting blood flow significantly increased with the use of antioxidants and coenzyme Q-10. Other studies on obese young men have showed elevated free radicals and impaired circulation [12]. But this same study showed that if vitamin C was impaired, even lean men had reduced vascular response to occlusion. These data then agree with the present investigation in that lean healthy men here benefitted in increasing resting blood flow with vitamin C and E intake.

The reactive hyperemia (either the initial phase or the sustained plateau) were not altered in younger individuals who were taking the doses of vitamins used in the present investigation. It is possible that a longer dose (months), or in older individuals or individuals with diabetes there may have been an effect. However, this was not seen in this group of individuals. There is some small dependencies of occlusive reactive hyperemia on nitric oxide [34]. But in large arteries and in the microcirculation, recent studies have shown that other factors largely determine to blood flow response to occlusion [34,35]. When nitric oxide synthetase was blocked by L-NAME in the skin, the blood flow response after either 5 or 15 minutes was not altered [34]. Some studies have reported a nitric oxide mediated reaction to occlusion [39-41]. But these studies differ in that they measured blood flow on the entire forearm by venous occlusion plethysmography and therefore measured the circulation in skin and muscle as well. Here only skin was measured. If the blood flow response is not mediated by free radicals, this may explain the lack of response to vitamins.

The response to local heat, which is also mediated by these 2 free radical sensitive compounds, that is nitric oxide and prostacyclin was also increased in people who took the course of vitamins and coenzyme Q-10.

Since multivitamins were used along with higher doses of coenzyme Q-10, it is hard to determine which had the effect on resting blood flow and blood flow response to heat. Potentially, the answer could be all of the above, or a single vitamin such as vitamin C or even coenzyme Q-10. Further investigation is warranted. However, at least in the present investigation it was surprising with only a 2 week dose a large increase in resting blood flow was seen in these individuals. In older individuals, and people with diabetes where resting free radicals are higher, it is possible that even a more dramatic effect would be seen. However, this was beyond the scope of the present investigation. Further, it is possible that a longer duration of vitamins and antioxidants may have an effect on vascular occlusion in this population. Further studies need to be conducted for examining the role of individual vitamins, the intensity of the vitamin regime, and how this interacts in younger and older people compared to levels of free radicals in the blood.

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