# THE EFFECT OF CAROTENOID BETA-CAROTENE ON IMMUNE AND REPRODUCTIVE HEALTH

JISHNU JETWANI[1], MAIDAH ARIF[1], ZIYA BAHAYANI[1], DIYA KUNISETTY[1], PARTH SHETH[1], AND RAHMA ARIF[1]

[1] *Study conducted through the Youreka Canada Program at University of Calgary*

*Compiled April 20, 2024*

**This study was done through the Youreka Canada program held in conjunction with the University of Calgary and the Foothills Medical Centre. All researchers extend their gratitude for the opportunity and mentorship provided by the above listed organizations.**

**This study investigates the impact of beta-carotene supplementation on human immune and reproductive health. Utilizing the paired T-test, Wilcoxon signed rank test, and correlation strength tests, we analyzed a sample of 48 male participants, comparing changes in their semen principle components (PC), bacteria killing, bacteria suppression, and immune PC's, before and after supplementation with beta-carotene. Our studies found a novel correlation between supplementation and immune health, with an $\alpha$= 0.05, we found evidence that the carotenoid beta-carotene increases immune function; specifically, the immune PC's and bacteria killing. This is further supported by the no correlation findings in before/ after tests on the placebo group and an analysis of the biological uses of beta-carotene in the human body. Further research is required.**

http://dx.doi.org/10.1364/ao.XX.XXXXXX

## 1. INTRODUCTION

The carotenoid beta-carotene has been shown to be important in animal mating due to its properties in enhancing attractiveness. The carotenoid trade-off hypothesis states that animals must balance the use for carotenoids for coloration and for antioxidants which are used to neutralize reactive oxygen species (ROS). Thus, animals with strong coloration must also have high antioxidant levels due to the trade-off, making them more likely to find a mate. Since, ROS damage DNA and semen health, animals supplemented with excess beta-carotene, a carotenoid, should have healthier semen.

The chemical nature of beta-carotene further suggests an overlooked connection between beta-carotene and reproductive health. The carotenoid beta-carotene is converted into vitamin A in the human body through cleavage. Studies have shown that vitamin A is a key component in facilitating spermatogenesis and increased fertility. Furthermore, beta-carotene acts as an antioxidant, boosting viability and motility of sperm which in turn regulate reproductive hormone levels, and protects sperm from oxidative damage to DNA and lipid membranes. This further supports the potential correlation between beta-carotene and a lower level of DNA fragmentation in male semen as chemically, beta-carotene plausibly 'shields' DNA.

In regards to immune function, beta-carotene supports immune function by enhancing activity of immune cells, such as T cells, and by promoting the production of antibodies. Furthermore, carotenoids contribute to strong mucosal surfaces in the respiratory and gastrointestinal tracts, serving as a barriers against pathogens. Vitamin A also aids in regulating the balance between pro/anti inflammatory signals.

There has been historically conflicting evidence regarding the impact of beta-carotene on human immune/reproductive health, with studies finding positive, null, or negative correlations between the two.

For these reasons, our group choose to study the connection between carotenoid supplementation and immune/reproductive health. Our study looks into the relationship between supplementation, immune health, and reproductive health through analysis of data gathered in past studies. The methods of analysis are described in the next section.

## 2. MATERIALS AND METHODS

Supplementation data was collected from a study led by Yong Zhi Foo. It consisted of various measurements taken before and after supplementation, for a placebo group and a beta-carotene supplemented group. Immune PC1 was weighted with bacterial killing and suppression, immune PC2 with lysozome activity and bacterial immunity. The following is quoted from Yong's study:

"Semen PC1 was weighted most strongly by variables related to rapid progressive motility, PC2 was weighted most strongly by variables related to the linearity of the sperm movement, PC3 was weighted most strongly by high sperm concentration and percentage motile sperm with low levels of left-right head

movement."

"Forty-three Caucasian men with a mean age of 21 years 11 months (M = 21.93, SD = 4.23) were recruited for the supplementation study from the University of Western Australia community. Each of them received either course credit or transport remuneration. All of them identified themselves as heterosexual and reported that they did not suffer from any immunological, endocrine, or metabolic disorders...Participants first attended a 1.5-h laboratory session, which was held between 12pm and 6pm to reduce any potential changes in the physiological variables to be measured that might arise due to circadian rhythm. They were asked to refrain from consuming any food or flavored drinks 1 h before the session, not wear any make-up or tanning agents, and be clean-shaven. Urine (10 mL) was first collected in a sterile bottle for oxidative stress measures. Saliva (5 mL) was then collected for immune function measures. Participants collected the saliva in a sterile bottle using the passive drool method after rinsing their mouth with water and waiting approximately 15 min. The urine and saliva samples were stored immediately in a 4°C fridge and transferred to a 80°C freezer within 4 h of collection."

The format of the data analyzed is shown in figure 1.

| PARTICIPANT | TREATMENT | CHANGE LYTIC ACTIVITY | CHANGE IMMUNE PC1 | CHANGE IMMUNE PC2 | CHANGE SEMEN PC2 |
|---|---|---|---|---|---|
| M0001 | Beta-carotene | 0.0950 | -1.63 | 1.32 | -0.99 |
| ... | .. | ... | ... | ... | ... |
| M9305 | Placebo | -0.03695 | -0.49 | -3.12 | -0.23 |
| ... | ... | ... | ... | ... | ... |

**Fig. 1.** Visual aid for format of the data. Different variables were anaylzed than shown.

The testing was done using R Studio using the ggplot and standard packages. We began each test with a Shapiro-Wilk and Levene test to test for normality and for equal variance. If both were satisfied, we proceeded with a paired T-test. Otherwise, we proceeded with a Wilcoxon Signed Rank Test.

If the result for some parameter came with a p-value less than $\alpha$=0.05, we proceeded with the same tests on the placebo group for that parameter. The results of all tests are summarized in the table below (Figure 2).

As shown above, we found significant P-values($<\alpha$) for the following parameters: bacteria killing, bacteria suppression, immune PC1, and immune PC2. For Immune PC2, the Shapiro-Wilk before had a p-value$>\alpha$, meaning the data was not normally distributed. Instead, we continued with the Wilcoxon Signed Rank Test which showed a significant p-value. For all the significant groups, we then ran the same tests on the placebo groups, resulting in the p-values in the rightmost column. As depicted, all of these p-values were not significant, leading us to believe that the results of significance were due to the manipulated variable: supplementation of beta-carotene.

We found non-significant P-values($>\alpha$) for the following parameters: Semen PC1, Semen PC2, Semen PC3. For the Semen PC1/2, we found that the Shapiro-Wilk for either before or after had a p-value$>\alpha$, meaning the data was not normally distributed. We then proceeded with the Wilcoxon Signed Rank Test, which all yielded p-values$>\alpha$, not significant.

| | Shapiro wilk before | Shapiro wilk After | Levene's test | Weighted T-Test | Wilcoxon Signed Rank Test | Interpretation | Placebo |
|---|---|---|---|---|---|---|---|
| Bacteria Killing | 0.5314 | 0.5457 | 0.2934 | 0.04467 | N/A | **Significant** | 0.9709 |
| Bacteria Suppression | 0.04946 | 0.4032 | 0.7239 | N/A | 0.02137 | **Significant** | 0.07567 |
| Semen PC1 | 0.9986 | 0.04928 | 0.7975 | N/A | 0.2226 | Not Significant | N/A |
| Semen PC2 | 0.04792 | 0.697 | 0.961 | N/A | 0.3229 | Not Significant | N/A |
| Semen PC3 | 0.2169 | 0.9992 | 0.77 | 0.5937 | N/A | Not Significant | N/A |
| Immune PC1 | 0.796 | 0.8971 | 0.5599 | 0.03284 | N/A | **Significant** | 0.7282 |
| Immune PC2 | 0.3809 | 0.0179 | 0.6641 | N/A | 0.01956 | **Significant** | 0.1937 |

**Fig. 2.** Summary of testing results.

Figures 3, 4, 5, and 6 depict boxplot graphs for all significant parameters. In the following, we refer to group 1/2 as the beta-carotene supplemented group before/after respectively, and group 3/4 as the placebo group before/after respectively.

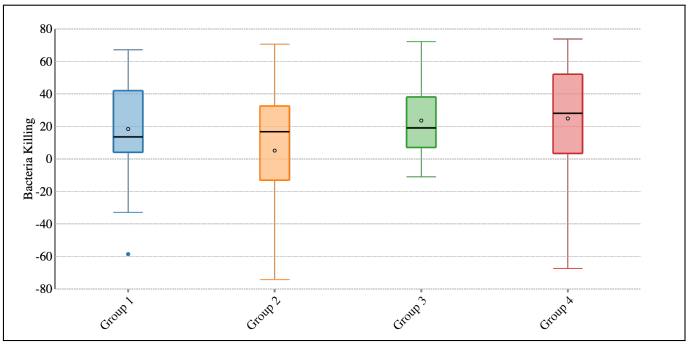


**Fig. 3.** Boxplot for Bacteria Killing

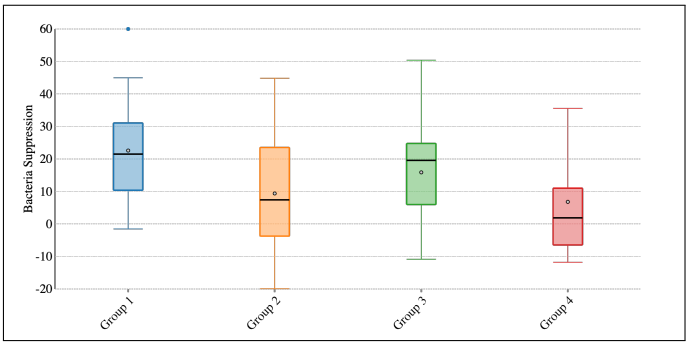


**Fig. 4.** Boxplot for Bacteria Suppression

Note that these boxplots are not accurate depictions of the effect of beta-carotene. Figures 4,5,6 depict a negative correla-

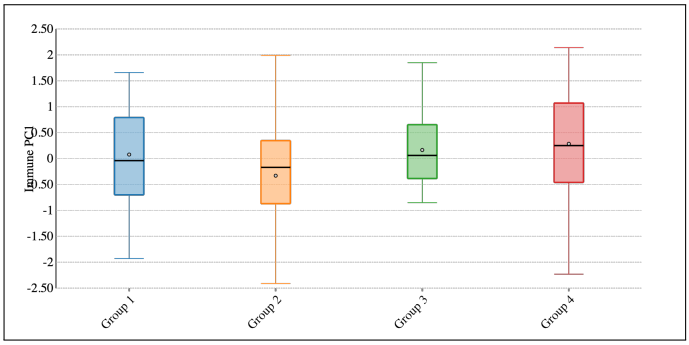


**Fig. 5.** Boxplot for Immune PC1

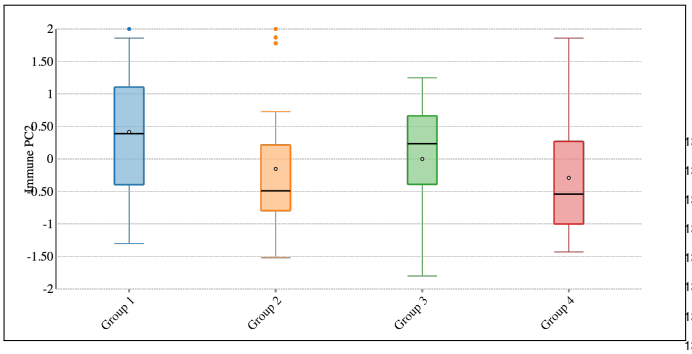


**Fig. 6.** Boxplot for Immune PC2

tion and Figure 3 depicts a positive correlation, but this is not accurate because the starting values of these parameters varies greatly between the subjects (as shown by the error lines). For a more accurate interpretation, we turn to correlation strength tests, in Figure 7. If the data was normally distributed, we used a Pearson's test, otherwise we used Spearman's test.

| | Pearson's Test | Spearman's Test | Significance? |
|---|---|---|---|
| Bacteria Killing | 0.6430978 | N/A | **Significant** |
| Bacteria Suppression | N/A | 0.0009881423 | Not Significant |
| Immune PC1 | 0.6618748 | N/A | **Significant** |
| Immune PC2 | N/A | 0.4605881 | **Significant** |

**Fig. 7.** Results for correlation strength tests

By these tests, we can see that there is a slight positive correlation for Immune PC1, Immune PC2, and for Bacteria Killing.

These results are discussed in the next section.

## 3. DISCUSSION

### A. Semen Health

There was no significant correlation found between beta-carotene and semen health in the weighted T-test and Wilcoxon test.

There was reasonable evidence to suggest that supplementation should aid sperm health, as evident by the carotenoid trade-off hypothesis. According to this theory, the body must carefully balance the distribution of carotenoids between their roles as antioxidants and pigments. Carotenoids as pigments are shown to enhance facial attractiveness and coloration, both

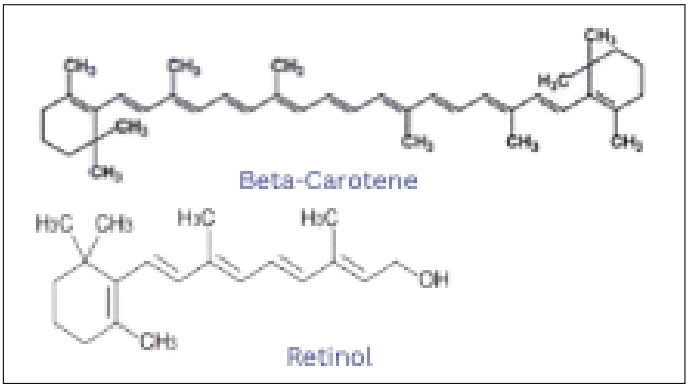


**Fig. 8.** The process of cleavage in which the central double bond in beta-carotene breaks to produce two retinol molecules.

important traits for finding a mating partner. Carotenoids as antioxidants are used in order to neutralize reactive oxygen species which are known to damage immune function and semen health when present in excess. Usually, carotenoids are ingested only in fruits and vegetables which do not compose a majority of the diet of most animals, making them a scarce resource. Thus, when the body is supplemented with an excess of carotenoids, the need to balance their usage should not come into effect as there is 'enough to go around'. Thus, supplementation should increase sperm health as the body no longer has to balance resources. We believe the Carotenoids did not increase sperm health due to the complexity and various factors that go into sperm health beyond oxidative stress. Carotenoids aid in alleviating oxidative stress but do not aid in other sperm health factors like hormonal balance and DNA integrity. Furthermore, all participants in the study were from the same region meaning they would have a similar diet. This could've caused no results to be shown as their diets may have already been strong in fruits/vegetables in which beta-carotene is naturally strong, so no result was seen. A study by Ming-Chieh Li showed that fertilization rate increased after male participants from the USA were supplemented with beta-carotene along with vitamin C. This could mean that the reason results were not seen in our study was due to diet or due to beta-carotene impacting factors of fertilization that were not measured in our study (Semen PC's).

### B. Immune Health

There was a significant positive correlation found between beta-carotene and the immune PC's and bacteria killing, shown in figure 7.

The improvement in immunological markers that was shown after taking beta-carotene supplements show promising results about the role that beta-carotene and its metabolite, retinol (vitamin A), play in immune regulation. Through enzymatic reactions such as cleavage by beta-carotene-15,15'-dioxygenase (BCO1) and beta-carotene-9',10'-dioxygenase (BCO2), beta-carotene is converted to retinol(Vitamin A) in the body. Subsequent metabolic activities result in the synthesis of retinol and its derivatives. This enzymatic cascade is essential for utilizing beta-carotene's immunomodulatory effects. The strong immune function regulator retinol works through a number of pathways, such as promoting the formation of antibodies, controlling inflammatory reactions, and modifying T cell development and proliferation. Furthermore, retinol aids in the preservation of

mucosal integrity, acting as a defense against infections that invade the gastrointestinal and respiratory systems.

This means that, whereas the carotenoid trade-off theory provides information about the dynamics of carotenoid distribution in the body, the complex mechanisms involved in beta-carotene metabolism provide insight into the immunomodulatory effects of the substance. This work adds to a better knowledge of beta-carotene's ability to improve immune function by illuminating the relationships between the nutrient and physiological systems. It also emphasizes the need for more research into the diverse effects of beta-carotene on human health.

### C. Reflection

Further research is needed to explore the role of beta-carotene in immune function. Our study had a relatively small sample size of 48 males; the study should be repeated with a larger sample size consisting of both genders. Furthermore, the study should be repeated using a population from a different location that consists of a people with different diets. Different diets lead to different amounts of beta-carotene and vitamin A present in the body, which can enable us to see the effect of supplementation more clearly. Doing this would eliminate the sampling bias that results from diet.

As previously mentioned, we believe beta-carotene may impact other factors of reproduction not measured in our study, as other studies have shown an increase in fertility rate after supplementation. However, it is not completely known why beta-carotene did not increase the semen PC's.

## 4. CITATIONS

Li, M.-C., Chiu, Y.-H., Gaskins, A. J., Mínguez-Alarcón, L., Nassan, F. L., Williams, P. L., Petrozza, J., Hauser, R., Chavarro, J. E. (2019, July 9). Men's intake of vitamin C and beta-carotene is positively related to fertilization rate but not to live birth rate in couples undergoing infertility treatment. The Journal of nutrition. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6825820/

Foo, Y. Z., Rhodes, G., Simmons, L. W. (2017, February 13). The carotenoid beta-carotene enhances facial color, attractiveness and perceived health, but not actual health, in humans. Academic.oup.com. https://academic.oup.com/beheco/article/28/2/570/2990210?login=false

Keogh, L. M., Byrne, P. G., Silla, A. J. (2018, August). Effect of long-term dietary beta-carotene supplementation on sperm concentration and motility in an endangered amphibian. Animal Reproduction Science. https://www.sciencedirect.com/science/article/abs/pii/S0378432018303737

De Cosmi, V., Parazzini, F., Agostoni, C., Noli, S., Cipriani, S., La Vecchia, I., Ferrari, S., Esposito, G., Bravi, F., Ricci, E. (2021, October 4). Antioxidant vitamins and carotenoids intake and the association with poor semen quality: A cross-sectional analysis of men referring to an Italian fertility clinic. U.S. National Library of Medicine. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8520935/

Hughes, D. A. (1999, August). Effects of carotenoids on human immune function. The Proceedings of the Nutrition Society. https://pubmed.ncbi.nlm.nih.gov/10604207/ Chew, B. P. (1993, September). Role of carotenoids in the immune response. Journal of Dairy Science. https://www.sciencedirect.com/science/article/pii/S0022030293776195