Dietary micronutrient levels in HIV+ smokers and local alveolar immune function

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*Abstract**

Rationale: Human immunodeficiency virus (HIV) incidence has been correlated with increased immune suppression in the alveolar cells of HIV+ smokers. While smokers are urged to increase dietary intake of vitamin C, the effect of other nutrients, specifically antioxidants, on the immune function of alveolar cells in HIV+ smokers with compromised lung health has not been addressed. Some evidence suggests that greater consumption of antioxidant nutrients is linked to improved lung function. Our initial work demonstrated significant correlations between dietary antioxidants and lung function in a population of HIV+ smokers prior to smoking cessation.

Objective: To determine the association between alveolar immune suppression and dietary antioxidant intake in an HIV seropositive population (n=43) before smoking cessation.

Methods: Immune suppression was assessed using regulation of TLR-2, TLR-4 and NOD-1, NOD-2, and LL-37 (cathelicidin). Dietary records, from 24-hour recalls, analyzed with nutrient analysis software (NDS-R V.2011) were employed to establish antioxidant nutrient consumption and were obtained at baseline. Linear regression models were fit for each diet/host defense molecule comparison. The models were controlled for age, gender, and cotinine level.

Results: The population was 81.81% male and 61.4% white with a mean age of 43.1 (s=9.68). No significant correlation between dietary antioxidant intake and innate immune function was found. Antioxidant vitamin consumption within this population on average was consistently lower than national RDA guidelines. Mean intakes of vitamin D, vitamin C, and vitamin E were 33%, 84%, and 70% of RDA levels respectively. Only vitamin A needs were met by the study population. All copper, iron, magnesium, and zinc RDA requirements were exceeded.

Conclusions: Although no relationship was established between dietary antioxidant intake and key host defense molecule regulation in alveolar macrophages, further studies are needed to determine whether changes in diet over time are related to macrophage expression**.**

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Introduction

HIV-infected smokers are at increased risk for a number of pulmonary infections and complications including bacterial pneumonia and tuberculosis. This may be related to smoking associated abnormalities in lung host defense. Notably, dietary intake of micronutrients has been associated with improved innate immune function. Potential markers of innate immunity may be better understood with Toll-like (TLR) and NOD-like (NLR) receptors, key pattern recognition receptors (PRRs), which play essential roles in the innate immune response. Vitamins A, D, zinc, and magnesium, have been linked to PRR signal modulation and expression. Whether dietary micronutrient intake is associated with lung immune function, specifically in PRR regulation, among HIV-infected smokers has not been fully established.

Primary Objective

This project is designed to determine the association between dietary micronutrient intake and alveolar macrophage expression of host defense molecules among HIV-infected smokers prior to smoking cessation.

Background and Significance

With approximately 124,477 deaths every year, chronic obstructive pulmonary disease (COPD) remains the third leading cause of death in the United States and is considered a major health concern (American Lung Association, 2011). Furthermore, smokers are 12 to 13 more times likely to die from COPD as non-smokers. In the U.S. alone, an estimated 13.1 million adults have COPD and 24 million adults showed signs of impaired lung function in 2008. Due to the disease's debilitating effects, COPD was projected to cost the nation approximately $49.9 billion in 2010.

Characterized by chronic bronchitis and emphysema, the damage done to the lungs is oftentimes irreversible. Chronic bronchitis, or the inflammation and scarring of the bronchial epithelial lining, leads to the thickening of bronchial tubes, mucus build-up, and ultimately obstructed air passageways, if left untreated. Starting with the destruction of alveoli, emphysema results in the decreased ability to transfer oxygen to the bloodstream, reduced lung elasticity, and thereby shortness of breath. These two ailments combine to produce chronic obstruction of the lungs and other symptoms experienced by those afflicted with COPD.

Individuals infected with human immunodeficiency virus (HIV) and autoimmune deficiency syndrome (AIDS) are prone to developing opportunistic microbial infections, such as those that accelerate the progression of COPD in the bronchi. Thus, the smoking HIV/AIDS-infected population is at particular risk for acquiring COPD.

Despite treatment with anti-retroviral therapy (ART) and increases in life expectancy of those affected by HIV/AIDS, non-opportunistic lung-diseases, such as COPD, are a growing cause of death in this population. According to Morris and colleagues (2011), approximately 4%

of deaths in the HIV-infected population in 1998 were due to obstructive airway diseases. Furthermore, ART may be contributing to this increased mortality through immune reconstitution inflammatory syndrome, which modifies the inflammatory cascade and potentially produces COPD symptoms. Additionally, lower levels of glutathione, a key moderator of damage done by reactive oxygen species, and increased oxidative stress, caused by HIV infection, combine to negatively affect the oxidant-antioxidant balance in the lungs and cause further damage (MacNee, 2009).

COPD is especially prevalent within this population. Diaz and colleagues (2000) observed emphysema in 23% of an HIV-infected, smoking population. Among HIV sero-positive and –negative veterans, the rate of COPD was significantly higher in the sero-positive population (Crothers et al, 2006).

Toll-like receptors (TLRs) and NOD-like receptors (NLRs), key pattern recognition receptors (PRRs) in the innate immune response that initiate the formation and release of pro-inflammatory chemokines and cytokines, have been shown to play a role in COPD progression. Droemann and colleagues (2005) found decreased surface-expression of TLR-2 in the alveolar macrophages (AMs) of smokers and those with COPD, reflecting weakened pulmonary immune response to bacterial infection. Furthermore, mice with decreased levels of TLR-2 have been shown to be more susceptible to developing *Streptococcus pneumoniae* (Echchannaoui et al, 2002). Soluble PRRs function as key down-regulators of surface-expressing receptors, preventing unnecessary inflammation from occurring. Von Scheele and colleagues (2011) found that there were higher levels of soluble TLR-2, derived from neutrophils, in the sputum of patients with COPD, which indicated increased surface receptor shedding. TLR-4 has also been implicated in immune response in respiratory infection. Schurr and colleagues (2005) found

that TLR-4 signaling, upon gram-negative bacterial infection, controlled the majority of innate immune response gene expression that was crucial for survival of the mice studied.

NLRs perform additionally important functions in maintaining innate immunity. NOD1 and NOD2, intercellular PRR proteins, have been found in bronchial epithelial cells, AMs, and endothelial cells of individuals with COPD. Responsible for activating NFκ-B-dependent pro-inflammatory cytokine and chemokine production as well as reactive oxygen species formation, these receptors are essential to innate immune response (Opitz et al, 2010). Kinose and colleagues (2009) found NOD1 and NOD2 gene expression at significantly higher levels in COPD patients in bronchial epithelial cells, despite similar levels of TLR2 and TLR4 gene expression, when compared to smoking, non-COPD controls. Accordingly, increased levels of pro-inflammatory cytokines, products of PRR activation, have been associated with COPD. Heightened cytokine levels may contribute to COPD pathogenesis through excessive inflammation (Keatings et al, 1996).

Similarly, PRR expression is altered in those afflicted with HIV and AIDS. Infection with HIV/AIDS has been associated with impaired PRR function in the lungs. Nicol and colleagues (2008) showed depressed TLR surface expression in HIV infected monocytic cells and decreased signaling of AM TLRs. TLR gene expression was also significantly down-regulated in AMs. HIV infected AMs appear to have decreased myeloid differentiation factor 88 (MyD88)-dependent TLR-4 signaling, critical to acute inflammatory response, whereas MyD88-independent signaling is conserved (Tachado et al, 2010). This indicates the HIV sero-positive population has altered innate lung immune function that could contribute to an increased susceptibility to acquiring infection, which could potentiate COPD progression.

In addition to acquired illnesses, dietary intake can also affect COPD progression and development. According to Romieu (2006), correlations have been established between dietary consumption of vitamins A, C, E, and some minerals and attenuated COPD development. Furthermore, higher dietary intake of fresh fruit was linked to decreased deterioration of lung function. Omega-3 fatty acids have been associated with slower decline in the lung function as well. Certain micronutrients have been shown to affect PRRs, which may contribute to these enhanced immune effects. Vitamins A and D are key factors in gene regulation and have been indicated to impact TLR signaling. Vitamin A was found in epithelial cells to significantly down-regulate TLR2 and its co-receptor CD14 (Liu et al, 2004). The results of Sadeghi and colleagues (2006) suggest Vitamin D3 (1,25(OH)2D3) reduces the expression of TLR2 and TLR4 and impairs the production of TNF-α in human monocytes. Several studies also suggest that if there are inadequate cellular levels of 1,25(OH)2D3, vitamin D receptor-mediated expression of antimicrobial genes is also down-regulated (Liu et al, 2006; Liu et al, 2007) The production of cathelicidin, an important antimicrobial peptide, is specifically induced by TLR-activated vitamin D receptors. Liu and colleagues (2006) found that levels of cathelicidin were positively correlated with levels of 1,25(OH)2D3.

Several minerals have also been implicated to affect TLR signaling. Sugimoto and colleagues (2012) showed that magnesium sulfate (MgSO4) reduces both constitutive and TLR-stimulated pro-inflammatory cytokine production. However, zinc has been shown to have level-dependent inhibitory and stimulatory effects on TLR-4 signal transduction. According to Haase and Rinke (2009), a moderate rise in concentration of free zinc can have stimulatory effects on a TLR-4-signaling, however higher levels are inhibitory in human macrophages. Zinc chelators cancel the effects of LPS stimulation of TLR-4 and inhibit the activation of mitogen-activated

protein kinases (MAPKs) and the release of the pro-inflammatory modulators that they induce (Hasse et al, 2008).

Omega-3 and other polyunsaturated fatty acids may have inhibitory effects on NLR and TLR signaling. Lee and colleagues (2010) found that omega-3 fatty acids repressed the activation of NFk-B and IL-8 expression induced by NOD ligands in monocytes. The affect is similar in TLR signaling. Omega-3 fatty acids have been found to suppress TLR-4 and TLR-2 monocyte signaling as well, resulting in reduced induction of inflammatory cytokines (Lee et al, 2003).

Given the significance of alterations made to the expression and function of lung host defense molecules by smoking and HIV/AIDS, the prospect of micronutrient attenuation of these effects warrants investigation.

Methods

Subjects

Participants were HIV-infected smokers involved in a prospective study of a specialized smoking cessation intervention, funded by the National Heart, Lung, and Blood Institute (RH10HL090313-01). All participants were HIV sero-positive adults who smoked five or more cigarettes a day on average, and were interested in quitting within a 30-day period (Ferketich et al, 2012).

PRR Quantification

Bronchoalveolar lavage (BAL) was performed in the right middle lobe and alveolar macrophages were isolated. Macrophage mRNA levels were quantified by real time PCR using Syber green and primers designed to allow for multiple gene comparisons within the same run. For this analysis, mRNA levels of antimicrobial peptide LL-37 and immune cell receptors (TLR-2, TLR-4, NOD-1, NOD-2) were examined. BALs performed at baseline and at secondary visits for participants who continued to smoke during the cessation trial were used for this analysis.

Dietary Analysis

Dietary records, from 24-hour recalls, were analyzed with Nutrition Data System for Research software version 2009 (NDS-R V.2009), developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN, excluding vitamin and supplement intake. These records were employed to establish micronutrient consumption. Total vitamin A equivalents,

vitamin D, vitamin E, magnesium, iron, zinc, selenium, and copper were the primary nutrients analyzed.

Statistical Analysis

Linear regression models were fit, using SPSS version 21, for each diet and host defense molecule comparison and adjusted for subject kcal/kg body weight to accommodate for energy availability. DRI levels for the micronutrients of interest were used to evaluate the relationship between "high" and "low" micronutrient consumption and levels of host defense markers. "High" and "low" level statuses for each micronutrient were defined as above or at the USDA's DRI, or below the USDA's DRI, for both male and female participants across age groups. Independent t-tests were used to determine whether a difference exists between participant levels of vitamins A, E, and D, and Mg, Zn, Se, and Cu, and mean levels of pattern recognition receptors TLR-1, TLR-2, NOD-1, NOD-2, and LL-37.

Results

Descriptive Statistics

The population was 84.6% male, 62.5% white, with a mean age of 43 years. Generally, average levels of micronutrients in the diet for this population were lower than the DRI for Vitamins D, E, and C for both men and women, and Fe for women only (*Table I*). Levels of all micronutrients varied significantly within the population. According to the World Health Organization, the daily caloric intake needs for those with symptomatic HIV increase 20-30% to maintain normal body weight (WHO, 2003). Both male and female subjects on average did not meet this recommendation. Subsequently, levels of micronutrients were adjusted for subject kcal/kg body weight, in order to reflect total energy intake.

Table I: Population Statistics

Mean Micronutrient	Men (n=45)	Women (n=10)	Overall
Kcal	2263.5 ± 892.5	1672.0 ± 593.3	2195.9 ± 858.9
Vitamin A (µg)	1191.5 ± 1437.6	938.11 ± 1529.0	1285.7 ± 1434.6
DRI	900	700	
% DRI	**132.4%**	**134.0%**	
Vitamin D (µg)	3.7 ± 4.2	2.3 ± 2.4	3.7 ± 4.0
DRI	15	15	
% DRI	**24.6%**	**15.4%**	
Vitamin E (mg)	6.2 ± 7.2	5.5 ± 3.0	6.6 ± 6.6
DRI	15	15	
% DRI	**41.5%**	**36.7%**	
Vitamin C (mg)	63.0 ± 63.0	46.7 ± 59.5	67.3 ± 60.0
DRI	90	75	
% DRI	**70.0%**	**79.3%**	
Mg (mg)	259.0 ± 126.2	238.3 ± 171.0	264.9 ± 133.0
DRI	420	320	
% DRI	**61.7%**	**74.5%**	
Fe (mg)	13.8 ± 6.7	11.2± 5.5	14.1 ± 6.5
DRI	8	18	
% DRI	**173.0%**	**61.9%**	
Zn (mg)	11.1 ± 5.8	11.6 ± 5.7	11.6 ± 5.8
DRI	11	8	
% DRI	**100.7%**	**144.9%**	
Se (µg)	126.45 ± 49.43	123.3 ± 54.2	121.6 ± 45.1
DRI	55	55	
% DRI	**217.1%**	**216.4%**	
Cu (mg)	1.1 ± 0.4	1.0 ± 0.6	1.1 ± 0.5
DRI	0.9	0.9	
% DRI	**117.3%**	**115.9%**	

Linear Regression Models

No significant linear relationships were found between micronutrient and host receptor cell levels, with and without adjustment for kcal/kg. Linear regression models did not establish that dietary intake of Vitamins A, D, and E, nor Mg, Fe, Zn, Se, or Cu could significantly predict alveolar macrophage levels of TLR-2, TLR-4, NOD-1, NOD-2, and LL-37. Model coefficients, adjusted R^2, and significance levels adjusted for kcal/kg bw are given for all micronutrient models for each host receptor molecule (*Tables II, III, IV, and V*).

Table II: Simple linear regression micronutrient models for TLR-2

Model	Adjusted R^2	Coefficient	Sig.
Vit A	0	0.000042	0.774
Vit D	0	0.051	0.720
Vit E	0	0.001	0.79
Mg	0	-0.001	0.79
Fe	0	-0.032	0.74
Zn	0	-0.006	0.79
Se	0	-0.014	0.47
Cu	0	-0.579	0.67

Table III: Simple linear regression micronutrient models for TLR-4

Model	Adjusted R^2	Coefficient	Sig.
Vit A	0	0	0.76
Vit D	0	-0.138	0.49
Vit E	0	0.061	0.76
Mg	0	-0.004	0.64
Fe	0	-0.032	0.82
Zn	0	0.016	0.89
Se	0	-0.020	0.19
Cu	0	-0.465	0.79

Table IV: Simple linear regression micronutrient models for NOD-1

Model	Adjusted R^2	Coefficient	Sig.
Vit A	0	0	0.83
Vit D	0	-0.028	0.36
Vit E	0	-0.016	0.60
Mg	0	0	0.83
Fe	0	-0.004	0.82
Zn	0	0.001	0.83
Se	0	0	0.83
Cu	0	0.029	0.82

Table V: Simple linear regression micronutrient models for NOD-2

Model	Adjusted R^2	Coefficient	Sig.
Vit A	0	0	0.977
Vit D	0	-0.010	0.68
Vit E	0	-0.003	0.94
Mg	0	0	0.70
Fe	0	0.001	0.98
Zn	0	-0.005	0.87
Se	0	-0.002	0.56
Cu	0	-0.077	0.75

Table VI: Simple linear regression micronutrient models for LL-37

Model	Adjusted R^2	Coefficient	Sig.
Vit A	0	0.000012	0.88
Vit D	0	0.003	0.96
Vit E	0	-0.006	0.89
Mg	0	0	0.99
Fe	0	0.001	0.99
Zn	0	0.008	0.78
Se	0	0.000027	0.99
Cu	0	-0.02	0.99

Within-Group Comparisons

Although mean TLR-4 levels were significantly different between "high" and "low" vitamin D status, no other significant differences were found between host receptor cell levels in the "high" and "low" micronutrient level groups. Mean host cell receptor levels for each group are given in *Table VII*.

Table VII: T-test results for above and below DRI group comparison of mean PRR levels
(*) Significant difference was found between high and low status groups and average PRR levels

Micronutrient	TLR2	TLR4	NOD1	NOD2	LL37
Vitamin A					
< DRI	9.1 ± 3.0	6.4 ± 4.0	1.2 ± 0.50	4.3 ± 16.4	2.8 ± 2.5
> DRI	9.5 ± 3.9	7.1 ± 3.7	1.3 ± 0.74	1.5 ± 0.66	4.6 ± 6.2
Vitamin D					
< DRI	9.5 ± 4.0	6.6 ± 4.1	1.3 ± 0.71	3.1 ± 12.1	3.7 ± 5.1
> DRI	8.7 ± 4.0	4.9 ± 3.0	1.1 ± 0.56	1.3 ± 0.91	3.1 ± 2.7
Vitamin E					
< DRI	9.6 ± 3.7	7.2 ± 4.1	1.4 ± 0.72	3.4 ± 13.2	3.8 ± 5.5
> DRI	8.6 ± 3.1	5.7 ± 3.0	1.1 ± 0.38	1.3 ± 0.52	3.4 ± 2.6
Mg					
< DRI	9.1 ± 3.7	6.7 ± 3.9	1.3 ± 0.67	3.1 ± 12.2	3.8 ± 5.1
> DRI	9.9 ± 2.5	7.1 ± 3.9	0.96 ± 0.36	1.1 ± 0.62	3.1 ± 2.9
Fe					
< DRI	9.2 ± 2.7	4.9 ± 1.8	1.2 ± 0.47	1.0 ± 0.39	4.6 ± 5.3
> DRI	9.23± 3.7	7.2 ± 4.1	1.3 ± 0.68	3.1 ± 12.3	3.5 ± 4.8
Zn					
< DRI	9.0 ± 2.9	6.2 ± 3.8	1.3 ± 0.55	4.2 ± 15.4	3.6 ± 3.8
> DRI	9.6 ± 4.1	7.3 ± 3.9	1.3 ± 0.74	1.2 ± 0.66	3.8 ± 5.8
Se					
< DRI	10.6 ± 4.2	8.3 ± 7.1	1.4 ± 1.1	1.0 ± 0.70	1.3 ± 0.63
> DRI	9.2 ± 3.5	6.7 ± 3.7	1.3 ± 0.62	2.8 ± 11.4	3.9 ± 4.9
Cu					
< DRI	9.4 ± 2.6	6.2 ± 4.3	1.2 ± 0.53	6.4 ± 21.1	3.8 ± 4.4
> DRI	9.2 ± 3.8	7.0 ± 3.7	1.3 ± 0.68	1.3 ± 0.67	3.7 ± 5.0

Discussion

This investigation aimed to determine if a relationship between micronutrient consumption and macrophage levels of certain pattern recognition receptors exists, given the general relationship between health and micronutrient status. Although no significant associations were found between the receptor and micronutrient levels, there are still some findings of interest with implications for future research.

As described previously, the study population was significantly deficient in several vitamins and minerals, and on average, did not meet recommended daily energy intake levels. These deficiencies may be related to lower levels of food security and the nutritional competence of the subjects involved, a majority of whom were of low socioeconomic status (SES). Some studies have observed the positive correlation between decreasing SES, decreasing micronutrient status, and negative immunological effects (Janicki-Deverts et al, 2009). Whether these immune impacts can be observed on the level of specific PRRs remains uncertain, but the potential correlation has implications for future research. Diet quality and patterns associated the food insecure population may have additional effects. For example, increased consumption of processed, high-fat, and high-sugar foods, and decreased consumption of fruits and vegetables have been associated with food insecurity (Nnakwe, 2008). Specific food groups consumed in greater levels and dietary patterns in the food insecure population have been associated with increases in the activity of some innate immune receptors *in vitro* (Erridge, 2010; Nettleson et al, 2011). The potential for added dietary stimulation of inflammatory receptors in food insecure individuals, in addition to the

effects that *whole* foods could have on these receptors, open new avenues for future research and present greater challenges to the present analysis.

The lower than recommended average caloric intake of the population confounds this analysis as well. Decreased energy availability, especially in individuals under chronic physical stress, has been well established to induce immune deficiency. For example, there is a greater incidence of respiratory infections in endurance athletes, who are calorically deficiency (Hagmar et al, 2008). As HIV-seropositive individuals have higher resting metabolic rates and therefore greater energy requirements, maintaining adequate caloric levels is crucial to maintaining immunity (Fenton & Silverman, 2008). Furthermore, the lower than recommended average energy intake of this population could have compromised subject synthesis of some PRRs, as has been demonstrated in animal models (Sun et al, 2001; Fock et al, 2007). Taken together, the population's low average energy availability could limit this investigation's ability to discern the specific effects of micronutrient levels on innate immune function.

There are many other factors that can be attributed to the absence of significant findings. The amount and quality of dietary data is very limited. Although there is no 'gold standard' of nutritional assessment, the use of four to six 24-hour dietary recalls in combination with a Food Frequency Questionnaire (FFQ) has been found to create a complete and accurate picture of subject dietary intake (Carroll et al, 2012). In this study, only one 24-hour recall was taken per subject, without the use of an FFQ. Micronutrient levels from this single dietary record were related to baseline levels of the PRRs, which may poorly portray the effect of subject dietary intake on the whole. Subject dietary patterns over an extended period of time, which are not encompassed in this analysis, should also be considered when determining the

affect of micronutrients on PRR expression and innate host immunity. For example, levels of the fat-soluble vitamins A, D, and E, assessed in this analysis are affected by long-term dietary intake, as they are stored in the body over time. Additionally, the accuracy of the dietary data is subject to participant bias and may not truly depict intake during the 24-hour period recalled. The time period between each subject's BAL and 24-hour recall was also variable and introduces another complication, as subject micronutrient levels on the day BALs were taken could have been altered given probable day-to-day dietary alterations.

In addition to caveats that arise in using this form of dietary data, there are additional significance issues pertaining to the population studied. The group consisted of 44 HIV-seropositive smokers, which was of insufficient size to form a strong correlation between PRR expression and dietary factors, despite the use of second-visit data to increase sample size. The group studied may not be representative of the general population, as all were HIV-seropositive and interested in smoking-cessation. Variance within the population could present other challenges to analysis significance. The population was 81.8% male and 61.4% white with a mean age of 43.1 ± 9.7, which, although controlled for, introduces additional confounding factors particularly regarding sex and age affects on the PRRs being analyzed (Volkova et al, 2011). Additionally, the affect of immune deficiency on micronutrient metabolism and status could also contribute to insignificance of results, as the HIV seropositive population is naturally deficient in many micronutrients (WHO, 2003).

Although there are many limitations to this analysis, future studies can benefit from the methodological insights gained. In order to acquire an accurate understanding of the affect of micronutrient profile on certain immune receptors in the lungs, several aspects of study design should be changed. Micronutrient levels should be assessed in a more vigorous manner.

Subject micronutrient profiles should not only be evaluated using the gold standard of nutritional assessment by incorporating an appropriate number of 24-hour dietary recalls and an FFQ, but also via appropriate biomarker analysis. Body levels of some micronutrients are manipulated over long periods of time and may not be accurately represented with short-term dietary recall data. The use of biomarkers could accommodate for this by adding another layer of micronutrient status data from which to make comparisons to receptor data. Additionally, a randomized, immunocompetent, non-smoking, mid- to high-SES population should be used in future studies assessing the affect of certain micronutrients on innate immune function of the lungs, as the characteristics of this population introduced many potentially confounding factors.

Conclusion

Despite evidence that micronutrients in the diet affect levels of pattern recognition receptors important in innate host immunity, this analysis found no significant relationships between these factors. Given the limitations of the study population and dietary data, further investigation is needed to verify the influence of certain micronutrients on receptor levels. Although this analysis lacks significant results, smokers affected by HIV and AIDS could benefit from future research elucidating the specific dietary requirements necessary for optimal immune function.

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