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Synergistic Influence of Multiple Lifestyle Factors on Cellular and Molecular Immune Markers: A Cross-Sectional Study in Healthy Adults

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Abstract

Although individual lifestyle factors have been recognized to modulate immunity, the combined and synergic effects of diet, physical activity, sleep and lifestyle habits on cellular and molecular immunity profiles are poorly understood. In this cross-sectional study, 150 healthy adults (age range 18-65) were assessed. Dietary quality, physical activity, sleep and negative behaviors were evaluated through validated questionnaires (DQI, IPAQ, PSQI and PSS). The complete blood count, lymphocytic subsets (CD4+, CD8+, NK cells), IgG, IgA, inflammatory cytokines (CRP, IL-6), endocrine markers (cortisol, DHEA), and oxidative stress markers (MDA, SOD, glutathione) were measured in both peripheral blood and saliva samples. High quality diet and physical activity showed significant relationships with increased levels of white blood cell, antibody production (IgG, IgA), and antioxidant defense (SOD, glutathione, MDA). At the same time, good sleep normalized hypothalamic-pituitary-adrenal (HPA) axis, which led to decreased cortisol and systemic inflammatory cytokines (CRP, IL-6). On the other hand, psychological stress, alcohol and smoking impaired immune efficacy. Interestingly, a synergistic and cumulative effect was noticed: those who applied more than one of the healthy lifestyle dimensions had the best immunological balances, with reduced chronic inflammation and oxidative damage. Increased cortisol was found to be negatively associated with CD4 + counts and IgG, while increased DHEA was found to be positively associated with favorable immune responses. The conclusion is that integrated healthy lifestyle factors synergistically optimize the immune function level in the cellular and molecular level, reducing low-grade chronic inflammation and oxidative stress. The results of these studies offer a comprehensive model for evidence-based preventive medicine.

Keywords: Lifestyle Factors; Immunomodulation; Oxidative Stress; Inflammatory Cytokines; Preventive Health

1. Introduction

Human immune system is a complex network comprising of innate and adaptive immune mechanisms capable of protecting the host from invasions of disease-causing organisms and spontaneous malignancies through immunocytes, cytokines and immunoglobulins [1]. Understanding the modulatory determinants of immunological efficiency is of critical importance to the development of public health strategies and preventive medicine. In the last ten years there has been a growing epidemiological and clinical evidence that lifestyle factors (food intake, physical activity, sleep habits, handling of daily stress) represent crucial factors for immune plasticity, either promoting immune resilience or fostering chronic, low-grade inflammation [2].

A healthy diet containing micronutrients such as vitamins A, C and D, trace minerals such as zinc and selenium, and antioxidants, play a fundamental role in promoting the activity of lymphocytes and B cells, which in turn increase antibody production and minimize oxidative damage which may suppress immune function [3]. On the other hand, eating foods rich in sugars and saturated fats activates the inflammatory pathways and leads to more oxidative stress, compromise of immune responses and increased risk of chronic diseases [4].

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Chronic physical activity also has powerful immunomodulatory properties. Moderate-to-vigorous exercise has been shown to increase the expression of anti-inflammatory cytokines, improve the cytotoxic response of natural killer (NK) cells and subsets of T lymphocytes, and optimize bioenergetics of immunocytes, which increases pathogen surveillance and clearance [5]. In contrast, excess sedentary time has a strong association with immunosenescence and increased chronic low-grade inflammation [6].

In addition, sleep architecture is a key regulator of the hypothalamic-pituitary-adrenal (HPA) axis, regulating the daily rhythm of cortisol production that in turn affects leukocyte trafficking and immunoglobulin production [7]. The disruption of sleep quality or the loss of sleep alters the physiological balance towards hyper-inflammation, thus reducing immune memory and increasing infectious diseases susceptibility [8]. In addition, unhealthy habits like smoking, excessive drinking, chronic psychological stress cause dramatic systemic changes, such as chronic inflammatory responses and lipid peroxidation, eventually compromising long-term immune responses [9].

Although numerous papers have been published on the effects of each factor alone, there is a critical need for scientific research on the combined, interactive effects of these factors on cellular and molecular biomarkers in healthy populations [10]. To fill this gap, this cross-sectional study comprehensively investigates the independent and interactive effect of diet, physical activity, sleep and behavioral habits on a multi-dimensional panel of biological markers such as number of leukocytes, percentages of T-cell subsets (CD4 + , CD8 +), the percentage of NK cells, the levels of immunoglobulins (IgG, IgA), inflammatory mediators (hs-CRP, IL-6), endocrine markers, and oxidative stress indices (MDA, SOD, glutathione) in a well-defined population. In this study, this comprehensive, multi-dimensional approach is being used to support this concept with solid empirical evidence for multi-dimensional lifestyle modifications as the primary interventions to reduce inflammation and improve public health.

2. Materials and Methods

2.1. Study Design and Participants Recruitment

This study was designed as a cross-sectional study to assess the link between various aspects of lifestyle, such as diet, physical activity, sleep, and behavioral patterns, with indicators of the immune system in healthy adults. A total of 150 people aged 18-65 years were recruited. People with chronic diseases, those on medications that may influence immune function or have experienced acute infections during the past two weeks were excluded. Demographic information such as age, sex, body mass index (BMI), educational background, income and marital status were obtained to control for confounding factors and ensure the analysis represented the impact of lifestyle factors on immune function only.

2.2. Sample Collection and Laboratory Processing

Venous blood samples (15 mL) were collected from each participant following an 8-hour fast in order to ensure stable immune indicators that were not affected by recent meals [11]. Serum was separated by centrifugation at 3,000 rpm for 10 min and stored in aliquots at -80C^0 to avoid repeated cycles of freeze-thawing that may have an effect on protein and cytokines [12]. Saliva samples were collected in the morning at least before any food or drink intake to analyse the hormonal markers and the immune markers associated with stress [13]. Sterile collection tubes and calibrated equipment were used and 10% of the samples were reanalyzed to check for accuracy and confirm that the biological data provided an accurate representation of the pick-up status of participants [14].

3. Measurement of Independent Variables

3.1. Dietary Evaluation

Dietary intake was measured with a Food Frequency Questionnaire (FFQ), for which the subjects reported the frequency and amount of food intake during the previous month. Data were analyzed to get average daily intake of macro-nutrients (protein, fat, carbohydrates, fiber, and total calories) and the micronutrients linked to immune function (vitamins C and D, zinc, iron, and selenium). As a measure of fat quality, saturated fats or omega-3 fatty acids were categorized, and probiotics, dietary fiber, refined sugars, caffeine, and soft drinks intake were calculated. Participants were categorized based on Diet Quality Index (DQI) in low, medium, and high-quality diet groups which then were correlated with immune markers to determine diet quality and immune response relationship [15].

3.2. Physical Activity Quantification

Physical activity was estimated by one International Physical Activity Questionnaire (IPAQ) measuring the exercise frequency, type, intensity and weekly duration. Data were converted to MET-minutes/week for the classification of participants in low, moderate and high physical activity [16]. Resistance activities, such as weightlifting, daily sedentary time, and type of exercise (aerobic or resistance) were also recorded along with intensity. These classifications were related to disease signs of the immune system, with white blood cell counts, antibodies, and cytokines to assess the detailed influence of physical activity on the immune system [17].

3.3. Sleep Quality Assessment

Sleep was measured with use of the Pittsburgh Sleep Quality Index (PSQI), which uses data on nightly sleep duration, regularity of sleep, and sleep disturbance in addition to data on daytime napping and sleep-wake timing. Researchers also also recorded conditions such as snoring or sleep apnea. Participants were divided into good and poor sleep quality groups based on PSQI scores and the two groups were correlated with immune markers such as antibodies, cytokines and white blood cell counts to analyze the effect of sleep quality on immune function [18].

3.4. Behavioral and Psychosocial Metrics

Daily behaviors were obtained on the basis of standardized questionnaires, and included the number of cigarettes smoked daily, weekly alcohol intake, the levels of stress (measured by the Perceived Stress Scale, PSS), number of hours of social interaction per day, social support levels and dietary supplements (vitamins and herbs) [19]. Participants were divided according to smoking, alcohol and stress levels and these classifications were correlated across measurements like the amount of immune cells, antibodies and inflammatory cytokines to determine the impact of lifestyle behaviours on immune function [20].

4. Biological and Immunological Assays

4.1. Hematological Analyses

Venous blood samples were analyzed using CBC analyzer and used to determine number of white blood cells, neutrophils, lymphocytes, monocytes, red blood cells, hemoglobin and hematocrit [21]. Mean values were compared between groups of participants classified on the basis of diet, physical activity, sleep quality and lifestyle behaviors, giving a fundamental assessment of innate and adaptive immunity [22].

4.2. Inflammatory Cytokines and Biomarkers

High sensitivity C-reactive protein (hs-CRP) was determined by using enzyme immunoassays (ELISA kits) and inflammatory cytokines (IL-2, IL-4, IL-6, IL-10, TNF-a) were detected by using multi-target (ELS) assays [23]. All samples were tested twice to ensure reliability, and the findings were correlated with independent variables to test the correlation between lifestyle variables and systemic inflammation [24].

4.3. Immunoglobulin Quantification

Serum levels of IgG , IgA, and IgM antibodies were analyzed by specific following by specific enzyme immunoassay (ELIAs), with the duplicate measurements for each sample. Results were classified into low, normal and high levels, and correlated with the groups of participants analyzed to determine the effect of lifestyle factors on adaptive immune responses [25].

4.4. Cellular Immune Analysis

Flow cytometry was used to study immune cells such as CD4+, CD8+, B cells, natural killer (NK) cells and regulatory T cells (Treg). Blood samples were prepared, fluorophore labeled antibodies were added, cells were analysed by a flow cytometer. Results were reported as percentage of each cell type and were associated with lifestyle variables to determine the effect on immune cells distribution and function [26].

4.5. Endocrine and Stress Hormones

Salivary cortisol, DHEA, and adrenaline concentrations were analyzed with the kit (ELI) based on the enzyme immunoassay (EIA), and the collected samples were the morning before any activity or food intake. These results were correlated with stress levels, sleep and physical activity due to the influence of biological stress on immune function and immunological balance between pro- and anti-inflammatory immune responses [27].

4.6. Oxidative Stress and Antioxidant Capacity Markers

Malondialdehyde (MDA) levels were measured as an indicator of cytotoxic oxidative damage, and levels of GSH and superoxide dismutase (SOD) were measured as indicators of the antioxidant capacity. Serum samples were analysed by the enzymatic and spectrophotometric methods according to standard protocols. These indicators were correlated with lifestyle factors, diet, physical activity and sleep to look at the impact of oxidative stress on immune function[28].

4.7. Statistical Analysis

The description statistics were summarized by mean ± SD and percentage. Pearson or Spearman correlations tests were used based on the distribution of the data. Multiple regression analysis was performed to study the independent effect of the different variables on the immune indicators, while also controlling for factors such as age, sex and BMI, which may influence the results. Two-tailed $p < 0.05$ was used for the data analysis.

5. Results

5.1. Modulatory Effects of Diet Quality on Immune Indices

The stratification of the 150 healthy participants by the Diet Quality Index (DQI) showed that systemic immune markers were directly correlated with nutritional quality, in a dose dependent manner. The high-quality diet group had significantly more mean number of white blood cells (WBC) per uL (7.8 ± 1.2 × 10 3 /uL) than the medium (6.9 ± 1.1 × 10 3 /uL) and low quality diet groups (6.2 ± 1.0 × 10 3 /uL, p<0.05). In addition, the circulating levels of adaptive immunoglobulins (IgG and IgA) were positively related to high diet quality, while systemic inflammatory measures were inversely related to high diet quality, with considerable decreases in the concentrations of hs-CRP and IL-6 (Table 1).

Table 1 Influence of Diet Quality on Leukocyte Counts, Immunoglobulins, and Inflammatory Biomarkers**.**

Diet Quality	WBC ($\times 10^3/\mu L$)	IgG (mg/dL)	IgA (mg/dL)	CRP (mg/L)	IL-6 (pg/mL)
Low	6.2 ± 1.0	900 ± 85	210 ± 30	4.5 ± 1.2	8.2 ± 1.5
Medium	6.9 ± 1.1	1050 ± 95	250 ± 25	3.2 ± 1.0	6.7 ± 1.3
High	7.8 ± 1.2	1180 ± 100	280 ± 28	2.1 ± 0.8	5.1 ± 1.1

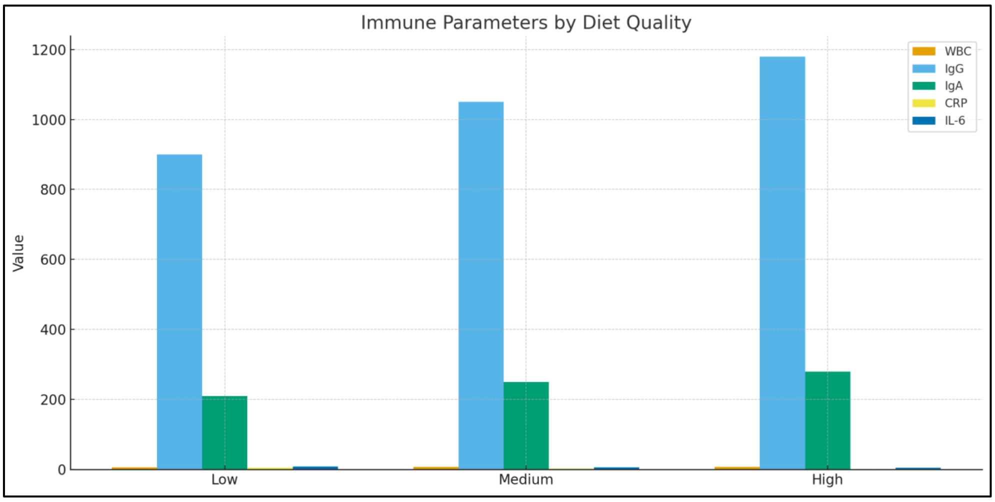

Figure 2 Immune parameters by diet quality

5.2. Effect of Physical Activity on Immune Indicators

The participants were categorized based on the physical activity (low, medium, high). Physical activity was linked to NK cell, CD4+, CD8+, elevated SOD, glutathione, and lower MDA and CRP, which was linked to improved immune defense, and a decrease in oxidative stress and inflammation [30].

Table 2 Association of Physical Activity Levels with Immune Cell Subsets and Oxidative Stress Markers

Activity Level	NK cells (%)	CD4+ (%)	CD8+ (%)	SOD (U/mg)	MDA (µmol/L)	CRP (mg/L)
Low	12 ± 3	35 ± 4	28 ± 3	5.2 ± 0.8	3.8 ± 0.6	4.2 ± 1.1
Medium	18 ± 4	40 ± 4	32 ± 4	6.8 ± 0.9	3.0 ± 0.5	3.0 ± 0.9
High	25 ± 5	45 ± 5	36 ± 5	8.2 ± 1.0	2.2 ± 0.4	2.0 ± 0.7

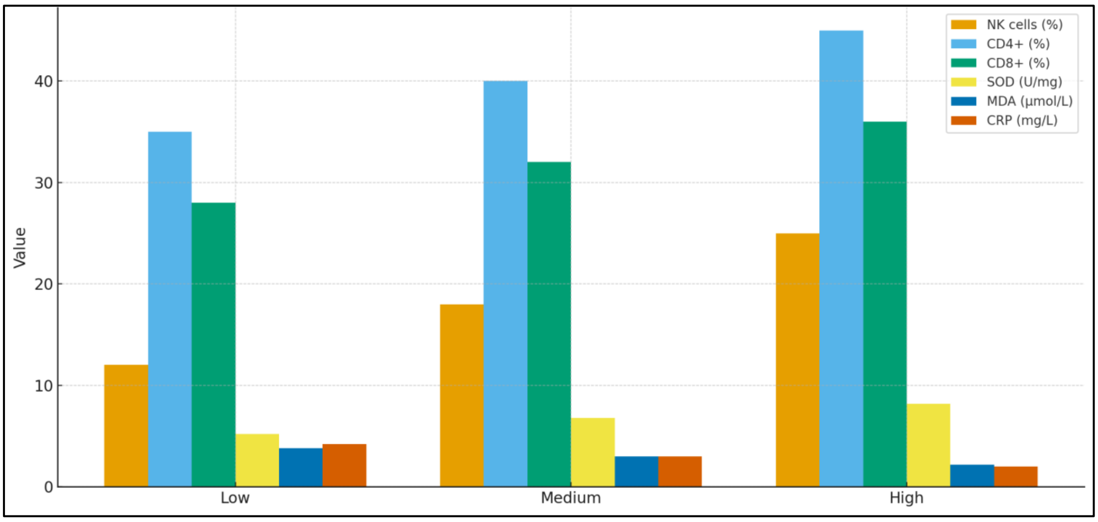

Figure 3 Immune and oxidative stress markers by activity level

According to (Table 2), the irregularly increased physical activity can improve both innate and adaptive immune functions and decrease oxidative stress and inflammation.

5.3. Impact of Sleep and Sleep Quality on Immune Markers.

Sleep was rated as either good or poor. In comparison with poor sleep, good sleep was also linked with higher levels of IgG and IgA, CD4+, lower levels of CRP, IL-6, and lower levels of cortisol, which demonstrated the importance of sleep in the production and maintenance of immune response and hormonal stress [31].

Table 3 Sleep Quality on Antibody Levels, T Cells (CD4 +) Inflammatory Markers, and Cortisol.

Sleep Quality	IgG (mg/dL)	IgA (mg/dL)	CD4+ (%)	CRP (mg/L)	IL-6 (pg/mL)	Cortisol (ng/mL)
Poor	950 ± 90	220 ± 25	36 ± 4	4.3 ± 1.1	7.8 ± 1.4	18 ± 2
Good	1150 ± 95	270 ± 28	44 ± 5	2.2 ± 0.8	5.2 ± 1.0	13 ± 1

The results of (Table 3) confirm that good sleep increases the levels of antibody and immune cells and decreases inflammation and hormonal stress.

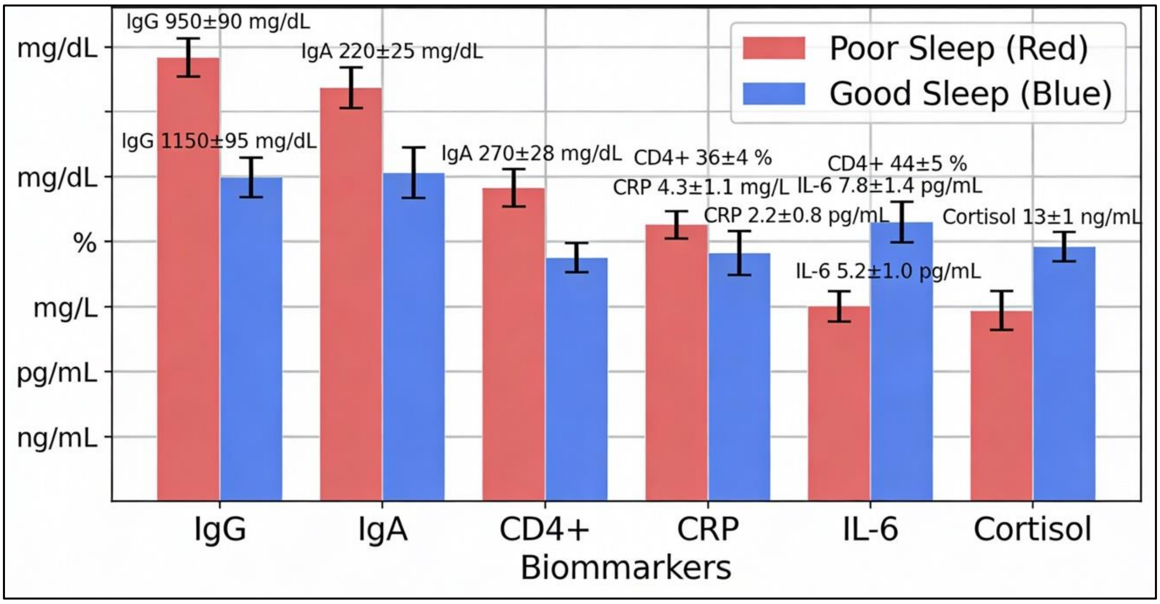

Figure 4 Effect of sleep quality on immune biomarkers

5.4. Effect of Behaviors and Life Style on Immune Indicators

Participants were categorized depending on their smoking (non-smoker, low, high), alcohol intake (low, medium, high) and stress levels (low, medium, high) [32]. High levels of smoking, high consumption of alcohol, and high levels of stress have been linked to increased CRP, IL-6, MDA and decreased NK cells, IgG, and CD4+, which reflects the negative influence of these behaviors on immune function and inflammatory response [33].

Table 4 Effect of Lifestyle Behaviors (Smoking, Alcohol, Stress) on Immune function and Inflammation

Behavior Category	NK cells (%)	IgG (mg/dL)	CD4+ (%)	CRP (mg/L)	IL-6 (pg/mL)	MDA (μmol/L)
Healthy	24 ± 4	1170 ± 95	46 ± 5	2.0 ± 0.7	5.0 ± 1.0	2.1 ± 0.4
Moderate	17 ± 4	1020 ± 90	40 ± 4	3.5 ± 1.0	6.8 ± 1.3	3.0 ± 0.5
Unhealthy	11 ± 3	900 ± 85	34 ± 3	4.7 ± 1.2	8.0 ± 1.5	3.9 ± 0.6

(Table 4) shows that there is an inverse relationship between unhealthy behaviors and immune function, with higher levels of inflammation and oxidative stress and low levels of immune cells and antibodies.

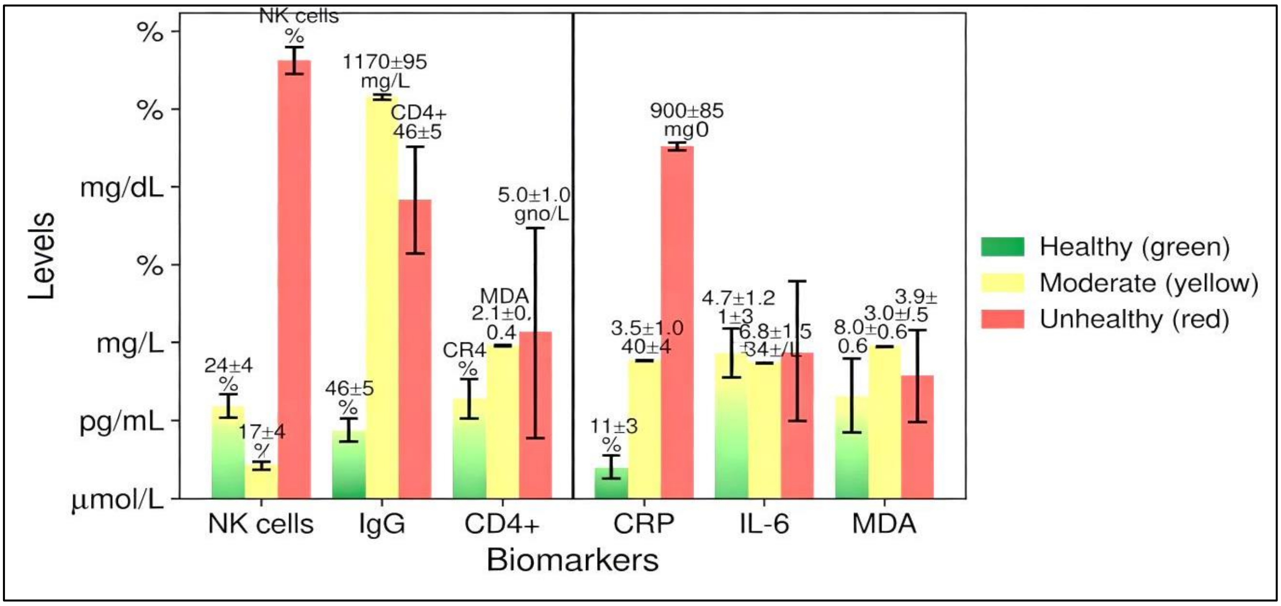

Figure 5 Biomarkers by behavior category

5.5. Interactive Dynamics of Diet and Physical Activity on Inflammatory Profiles

Multivariate clustering showed a significant additive effect between the quality of diet and the level of physical activity. Low-grade systemic inflammation was also found to be best protected by a high-quality dietary regimen combined with a high physical activity level; and the low-grade systemic inflammation was less well protected by a single healthy behavior in isolation (Table 5).

Table 5 Synergistic Effects of Diet Quality and Physical Activity Interactions on Inflammatory Outcomes.

Diet Quality	Physical Activity	CRP (mg/L)	IL-6 (pg/mL)
High	High	1.8 ± 0.6	4.9 ± 0.9
High	Low	2.5 ± 0.7	5.8 ± 1.1
Low	High	2.9 ± 0.8	6.5 ± 1.2
Low	Low	4.5 ± 1.1	8.1 ± 1.4

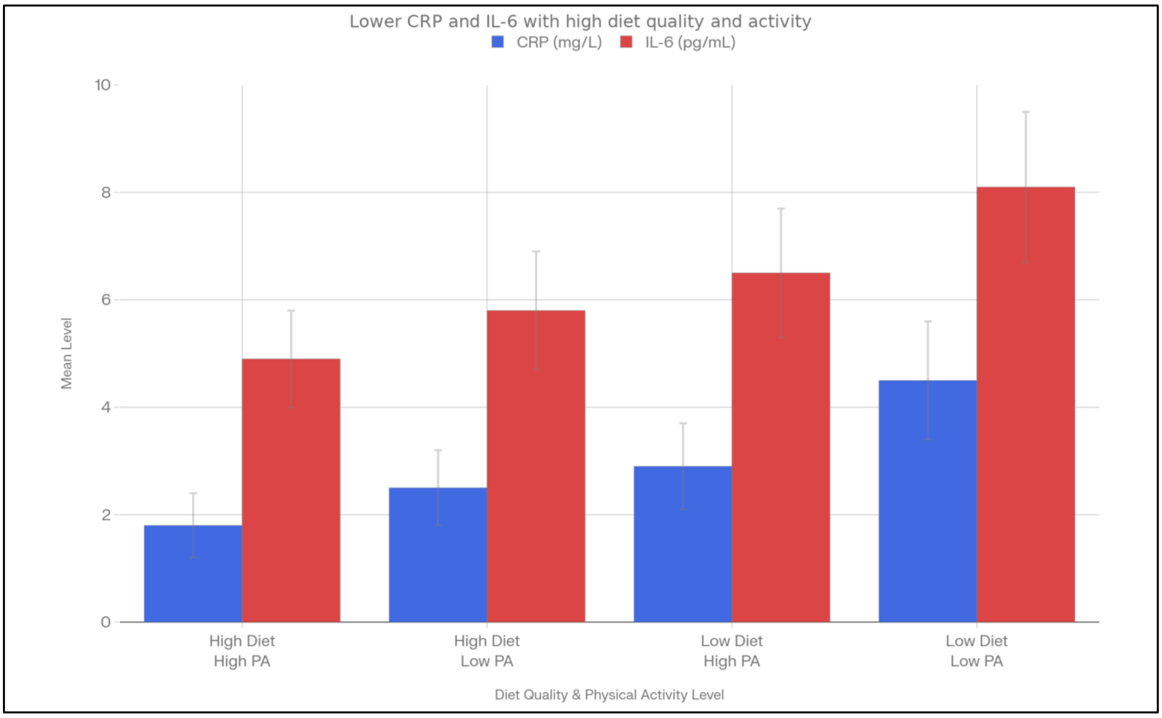

Figure 6 Combined Impact of Diet Quality and Physical Activity on CRP and IL-6 Levels

5.6. Combined Impact of Sleep and Healthy Behaviors on signs of Immune Function

To derive the best immune system outcomes (CD4 +, IgG) and the least systemic inflammation, analyses of the relationships between sleep parameters and the categorization of daily behaviour found that only those participants who had excellent sleep architecture combined with healthy behaviour patterns achieved these outcomes (Table 6).

Table 6 Interactive Impact of Sleep Quality and Lifestyle Tiers on Cellular and Humoral Responses.

Sleep Quality	Healthy Behaviors	CD4+ (%)	IgG (mg/dL)	CRP (mg/L)
Good	Healthy	46 ± 5	1180 ± 95	1.9 ± 0.6
Good	Unhealthy	40 ± 4	1050 ± 90	2.8 ± 0.8
Poor	Healthy	41 ± 4	1100 ± 92	2.9 ± 0.9
Poor	Unhealthy	34 ± 3	900 ± 85	4.5 ± 1.1

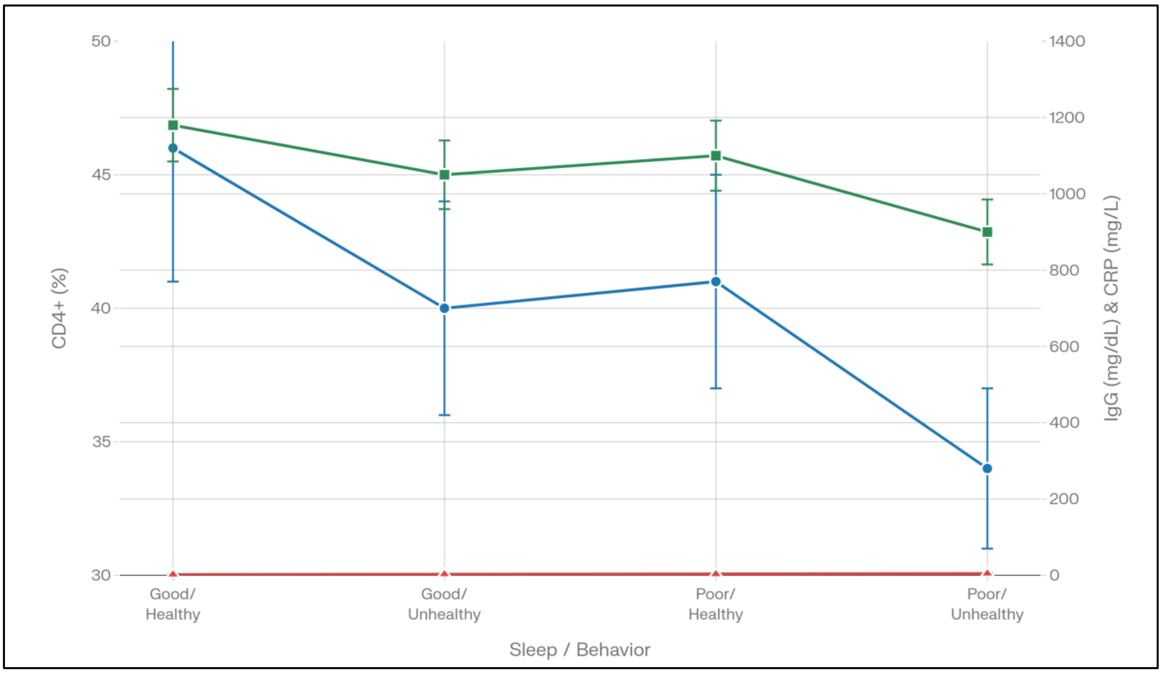

Figure 7 Sleep Quality, Healthy Behaviors vs. CD4+, IgG, CRP Levels

5.7. Endocrine Profiling and Structural Immune Signatures

Chronic activation of the biological stress axis (high morning cortisol) was shown to drive a downregulation of structural immunity, through hormonal assessment. There was a strong correlation between the high baseline cortisol levels with reduced percentage of CD4 + cells and decreased immunoglobulin levels, as well as higher serum levels of hs-CRP and MDA (Table 7).

Table 7 Cross-Sectional Associations of Basal Cortisol Secretions with Immune and Stress Biomarkers.

Cortisol (ng/mL)	CD4+ (%)	IgG (mg/dL)	CRP (mg/L)	MDA (μmol/L)
Low (≤14)	45 ± 5	1180 ± 95	2.0 ± 0.7	2.2 ± 0.4
Medium (15–18)	40 ± 4	1050 ± 90	3.5 ± 1.0	3.0 ± 0.5
High (≥19)	34 ± 3	900 ± 85	4.7 ± 1.2	3.9 ± 0.6

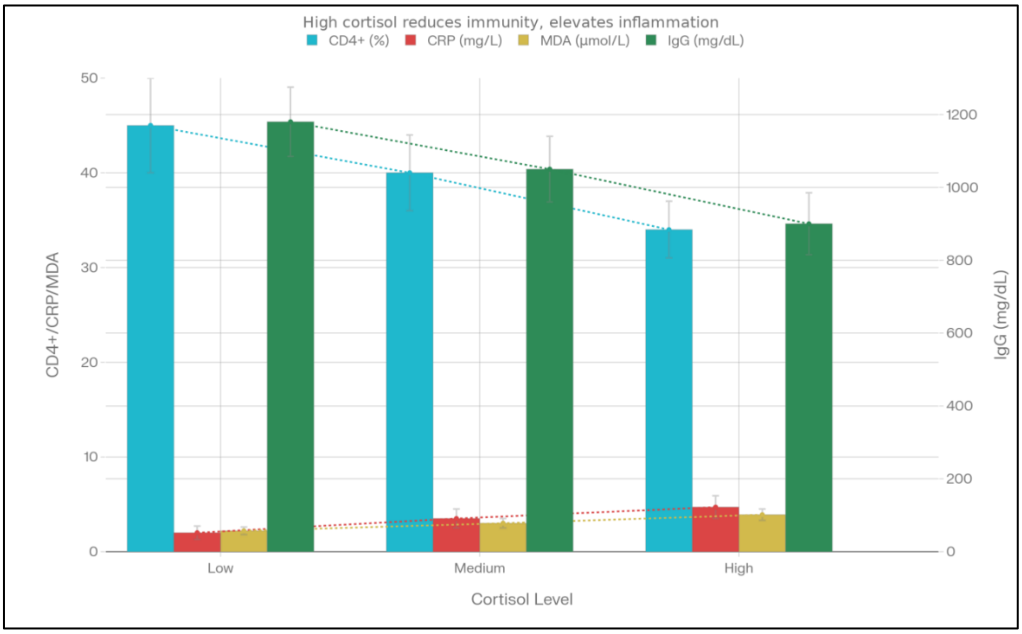

Figure 8 Cortisol Levels vs. Immune/Inflammatory Markers

5.8. Physical Activity and Diet Interactions on Molecular Oxidative States

The joint effect of physical activity and nutrition on the biochemical defense system was evaluated by monitoring the molecular kinetic of the tissue and confirmed the best results for the biochemical defence system (highest glutathione and SOD levels, lowest lipid peroxidation) were obtained when both healthy modalities were combined (Table 8).

Table 8 Combined effect of PA and nutrition tiers on the intracellular antioxidant dynamics.

Physical Activity	Diet Quality	Glutathione (µmol/L)	SOD (U/mg)	MDA (µmol/L)
High	High	8.5 ± 1.0	8.2 ± 1.0	2.2 ± 0.4
High	Low	7.0 ± 0.9	6.8 ± 0.9	3.0 ± 0.5
Low	High	6.5 ± 0.8	6.5 ± 0.8	3.2 ± 0.5
Low	Low	5.2 ± 0.7	5.2 ± 0.8	3.9 ± 0.6

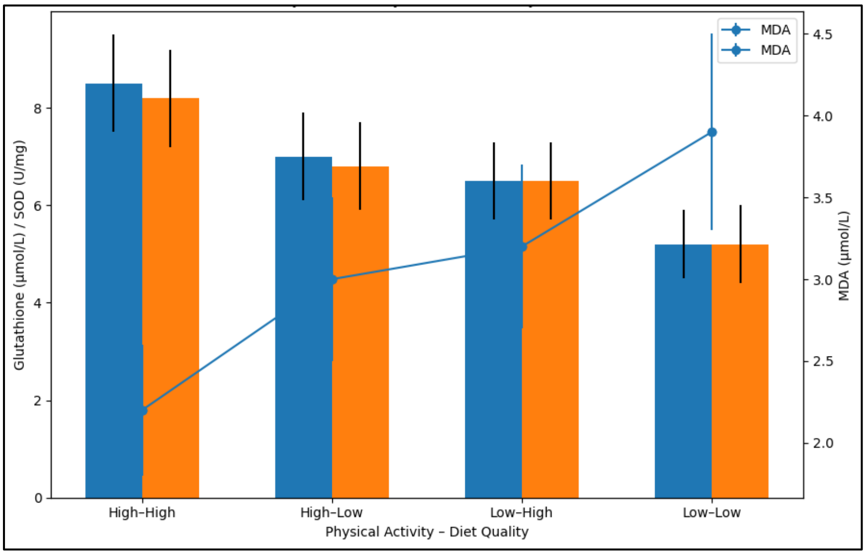

Figure 9 Combined influence of physical activity and diet quality on oxidative stress markers

6. Discussion

Nutrition, physical activity, sleep quality and daily behaviours are factors that affect not only the general markers of immunity but also the cellular and molecular mechanisms that control immune responses [34]. Participants who had higher quality diets presented with elevated white blood cell counts, higher IgG and IgA production and reduced CRP and IL-6 concentrations indicating higher innate and adaptive immunity [35]. Biologically this can be understood by the fact that micronutrients (vitamin A, C, D and E), zinc, and selenium play an important role in regulating lymphocyte and B-cell activity, helping maintain expression of antibodies on immune system cells, and protecting them against reactive oxidants because of their antioxidant properties (reducing activation of the NF-(kB), an important pathway responsible for the production of inflammatory cytokines) [36].

Regarding PA, study showed a significant increase of NK cells, CD4+, and CD8+ T cells as well as an increase of SOD and glutathione and a decrease of MDA reflection of improvement in innate and adaptive immunity and reduction of oxidative stress, respectively [37]. On the molecular level, exercise is known to increase the production of IL-10 and reduce the production of pro-inflammatory cytokines, while improving the blood flow and microvascular circulation (which enables the movement of immune cells to sites of infection) [38]. In addition, the regular physical activity improves mitochondria functioning among immune cells for energy production and antimicrobial capacity [39].

Sleep quality was also associated with the immune indicators. Good sleep was linked to greater IgG, IgA, and CD4+ levels and lower CRP, IL-6 and cortisol levels [40] Physiologically, adequate sleep helps to regulate the hypothalamic--pituitary--adrenal (HPA) axis decreasing secretion of cortisol and suppressive on immunity [41]. Sleep is also shown to improve B-cell activation and antibody production, while sleep deprivation causes an imbalance in the expression of NF-kB signaling and an increase in inflammatory cytokines, which would be a reason for the decrease in CD4+ and IgG observed in those with poor sleep [42]. Unhealthy behaviors such as heavy smoking, excessive alcohol and chronic psychological stress demonstrated a clear negative effect on immune function [43]. These behaviors were correlated with higher CRP, IL-6, MDA levels and less NK cells, CD4+ T cells and IgG levels [44]. Biologically, toxins found in tobacco smoke and alcohol lead to an increase in reactive oxygen species (ROS) which leads to suppression of immune signaling pathways and imbalance in favor of pro- and anti-inflammatory cytokines [45]. Chronic stress activates the HPA axis and leads to increased cortisol, which will inhibit the production of IL-2 and reduce the activity of T-cells and NK-cells, thereby diminishing both innate and adaptive immunity [46].

The study also highlights the cumulative interactions of lifestyle factors. Participants who ate a high-quality diet and practiced regular physical activity or good sleep in addition to healthy behaviors had the best immune profiles with lowest levels of inflammation [47]. This synergistic effect could be explained on the increased gene expression of immune receptors as well as production of anti-inflammatory cytokines and decreased activation of NF saga and MAP kinase pathways which affect the increased immune efficiency and decreased oxidative stress [48].

Besides this, the findings indicated that there was a strong correlation between the hormonal stress, the oxidative stress, and the immune functionality [49]. An increase in cortisol was associated with a reduction in CD4+ and IgG as well as an increase in CRP and MDA concerning the higher levels of DHEA that were positively correlated with improved immune responses [50]. This suggests that hormonal balance has a critical role in modulating the equilibrium between inflammatory and anti-inflammatory pathways with less oxidative damage to immune cells to facilitate the whole-body defense capacity [51].

7. Conclusions

The study concludes that a healthy lifestyle: proper nutrition, physical activity, adequate sleep and positive daily behaviors improve the immune system at the cellular and molecular level by increasing white blood cell levels, increasing antibody production, activating lymphocytes, and reducing inflammatory proteins and oxidative stress. The results of the study suggest that diets high in micronutrients and anti-oxidants boost antibody production and safeguard immune cells and that regular physical activity boosts the ability of immune cells to recognize pathogens and destroy infected cells. Adequate sleep was also shown to regulate the hypothalamic pituitary adrenals (HPA), and decrease cortisol levels, therefore keeping the immune system in balance and reducing inflammation.

In the opposite, unhealthy behaviors such as smoking, alcohol or psychological stress which weakens immune responses and increases inflammation and oxidative stress. The study goes on to confirm that these lifestyle factors have a cumulative effect and using a combination of good nutrition, physical activity, healthy sleep and positive behaviors can improve immunity more than when only one is present. Additionally, the results show that hormonal balance is responsible for decreasing oxidative stress and boosting immune responses. Therefore, living a multidimensional balanced life becomes an effective way of improving immunity and warding off chronic diseases caused by sustained inflammation.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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