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A healthier daily diet is associated with greater immune fitness

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ABSTRACT

Introduction: The aim of this cross-sectional study was to investigate the association between a healthier diet, perceived immune fitness, and biomarkers of the immune system.
Methods: N = 108 participants (31 men and 77 women), 18–30 years old, completed a questionnaire, comprising the Healthy Diet Scale (HDS) and a 1-item scale assessing perceived immune fitness. In addition, saliva samples were collected and C-reactive protein (CRP) and cytokine concentrations of interleukin (IL)− 1β and IL-8 were determined.
Results: Overall, a significant correlation was found between the HDS score and perceived immune fitness ($r = 0.221$, $p = 0.021$), suggesting that a healthier diet was associated with a better immune fitness. The HSD score correlated significantly and negatively with saliva CRP concentrations ($r = -0.240$, $p = 0.013$). No significant correlations were found with other biomarkers. In women, the HDS correlated significantly with perceived immune fitness ($r = 0.247$, $p = 0.030$) and CRP levels ($r = -0.281$, $p = 0.014$). In men, correlations with perceived immune fitness ($r = -0.219$, $p = 0.237$) and the biomarkers were not significant.
Discussion: Significant associations between attaining a healthy diet, perceived immune fitness and CRP were found. More research is needed to investigate the observed sex differences and underlying mechanisms.

1. Introduction

The immune system protects the body from pathogens such as bacteria and viruses, and therefore is playing a vital role in maintaining health and preventing disease [1]. An adequate immune functioning, i.e., immune fitness, means that appropriate responses are initiated when the body encounters pathogens and that after adequate removal of pathogens the immune system returns back to a steady state [2]. Conversely, when the immune system fails to respond effectively, there is an increased risk of uncontrolled infections and illnesses [3]. At the same time, it is essential that the immune system does not remain activated, as this might cause excessive damage to self-tissues and lead to low grade inflammation associated with the development of allergies, chronic inflammation, or even autoimmune disorders [4].

The immune system is involved in the development of many non-communicable diseases (NCDs), including cardiovascular diseases, cancers, respiratory diseases, and diabetes [5,6]. These NCDs are associated with considerable mortality rates. Annual cardiovascular, cancer, respiratory diseases and diabetes accounts for 17.9, 9.3, 4.1 and 1.5 million deaths respectively. Collectively, these NCDs are responsible for 71 % of all deaths worldwide [7]. Given this, it is important to investigate factors that influence a healthy and fit immune system, i.e., immune fitness.

There are various factors that potentially may influence the immune function and they broadly can be classified under modifiable and non-modifiable factors. Examples of non-modifiable factors are aging [8] and sex [9]. In terms of possible interventions, the modifiable factors are highly relevant, as these can be adjusted to improve immune fitness.

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Examples of modifiable factors are lifestyle factors, such as sleep quality, physical activity, stress, smoking and nutrition [10]. The current study will focus on the relationship between compliance to healthy diet consumption and immune fitness.

A healthy diet helps to protect against the development of malnutrition in all its forms, as well as non-communicable diseases (NCDs), including such as diabetes, heart disease, stroke and cancer [11]. Beneficial nutrients include macronutrients, such as carbohydrates, fats and proteins, and micronutrients, such as vitamins, minerals, and trace elements. Macronutrients deliver the energy required for cellular processes, while micronutrients are necessary for normal physiological functioning [12]. The World Health Organization (WHO) advises at least

Fig. 1. The Healthy Diet Scale (HDS).

400 g of fruit and vegetables per day. Furthermore, the WHO recommends less than 10 % of the total energy intake from free sugars and less than 30 % from fats, in which the intake of saturated fats should be reduced to less than 10 % of the total energy intake and the intake of trans-fats to less than 1 % [11]. However, there are various other ways to infer to what extent a diet can be considered healthy (e.g., dietary patterns [10] or the amount of processed foods that are consumed [13]).

The direct interplay between a healthy diet and immune fitness is evident from the fact that food consumption affects the gut microbiome and the immune system. Approximately 70 % of the immune system is located in the gut [14]. Prior findings show that in the gut, nutrients can have a profound impact on the composition and function of the microbiota, which in turn influences immune functioning [15]. These effects have been reported at both the dietary and nutrient level [16–23].

Whereas a relationship between compliance to a healthier diet and markers of systemic inflammation has been demonstrated previously [16,17], this is the first study that examines the relationship between compliance to a healthy diet and perceived immune fitness. For this purpose, a survey was conducted among young adults in the Netherlands. The Healthy Diet Scale (HDS) [24] was used to measure the percentage of the daily diet that comprises healthy food. Instead of evaluating dietary intake of nutrients or foods, the single item HDS is an overarching measure of an overall healthy diet. In addition, perceived immune fitness was assessed via a single item scale [25,26]. Along with the survey, saliva samples were collected to assess markers of systemic inflammation, including C-reactive protein (CRP) and the cytokines interleukin (IL)− 1β, IL6, IL-8, IL10 and tumor necrosis factor (TNF)-alpha. These biomarkers were selected as in previous research they could be detected in saliva [27]. Based on prior studies looking at single nutrients and specific types of diets, it was hypothesized that attaining a healthier diet is associated with a better immune fitness.

2. Materials and methods

In December 2021, a convenience sample of N = 110 young adults, students of the department of pharmaceutical sciences of Utrecht University, The Netherlands, were invited via email to participate in this cross sectional study. The participants were aged 18–30 years old, and students or postdocs of Utrecht University. The study was approved by the Science-Geo Ethics Review Board of Utrecht University (protocol code: S-21525, date of approval: November 21, 2021) and every participant provided written informed consent. The study comprised a single visit to Utrecht University, where participants were asked to complete a survey. Besides the assessment of demographic data (age and sex), measurements of the HDS were conducted and perceived immune fitness was assessed. In addition, a saliva sample was collected. Participants received 20,- euros for participating in the study.

2.1. The healthy diet scale (HDS)

The HDS is a single-item scale used to estimate the percentage of the daily diet that comprises healthy food [24] (See Fig. 1). The scale ranges from 0 % (unhealthy) to 100 % (healthy), in steps of 10 %. To make it easier for the participants to give an accurate estimation, pictures and examples of healthy and unhealthy food items are included in the scale. The test-retest reliability of the HDS is 0.98 [24]. The HDS correlated significantly with the 8-item Start The Conversation diet scale, which aims to assess healthful and unhealthful dietary behaviors [24].

2.2. Perceived immune fitness

Perceived immune fitness was assessed with the question 'Please rate how you feel at this moment, on a scale ranging from 0 (very poor) to 10 (excellent): perceived immune fitness' [25,26]. Higher scores indicated a better perceived immune fitness. Test-retest reliability of the scale (intraclass correlation) is 0.89 and previous studies successfully related perceived immune fitness to various outcomes of health and disease [25,26,28–32].

2.3. Markers of systemic inflammation

A saliva sample was collected by the passive drool method in a 4-mL polypropylene cryovials, using SalivaBio's Saliva Collection Aid (Salimetrics, State College, PA, USA). Participants were not allowed to eat or drink for at least 30 min before donating the saliva. Each saliva sample was transferred to an EDTA collection tube (Greiner Bio one, Kremsmünster, Austria) with Protease Inhibitor Cocktail (Merck, Darmstadt, Germany). The saliva samples were aliquoted and stored at a temperature of − 80 Celsius. Saliva cytokine concentrations of CRP, IL-1β, IL-6, IL-8, IL-10 and TNF-α were determined by multiplex immunoassay (customized ProcartaPlex Immunoassay, ThermoFisher Scientific, Waltham, USA). All incubations were conducted at room temperature. First, 25 μL of each saliva sample was pipetted into a dilution plate; 25 μL of assay buffer was added to dilute the samples (2x). 25 μL magnetic beads were pipetted into a 96-well plate. The beads were washed once before 50 μL standards and samples were added to the plate and incubated for 30 min. The plate was washed three times and detection antibody was added, which was then incubated for 30 min. The plate was washed three times and diluted streptavidin– phycoerythrin (used as detection substrate) was added and incubated for 30 min. The plates were washed three times and reading buffer was added to each well. Fluorescence was read within 30 min with the Luminex LX200 (Luminex Corp., Austin, USA). Biomarker concentrations were expressed in pg/mL saliva. For each cytokine determination, each multiplex plate had a unique lower limit of detection (LOD). For those cytokine concentrations below the LOD of the assay, the LOD value was divided by 2 to enable inclusion of these assessments in the statistical analyses. If more than 30 % of the cytokine assessments were below the LOD value, the results for that cytokine were considered to be unreliable and not used for final data analysis.

2.4. Statistical analysis

Statistical analyses were conducted with SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY, USA: IBM Corp.). The mean and standard deviation (SD) were computed for all survey variables. Saliva sample analysis revealed that IL-6, IL-10 and TNF-α could not be reliably detected (> 30 % of the samples were below the limit of detection). These cytokines were therefore not further considered. As the data were not normally distributed, possible sex differences were investigated using the Independent-Samples Mann–Whitney U-Test. Differences were considered statistically significant if $p < 0.05$ (two-sided). Spearman's correlations were computed between the HDS outcome and measures of immune fitness. Correlations were considered significant if $p < 0.05$. The sample size of the study was based on a test-retest assessment (discussed elsewhere) for which a sample size > 100 is considered as excellent [33]. Ten additional participants were invited to account for possible no show or dropout on the test day.

3. Results

N = 108 participants, with an age range of 18–30 years old, participated in the study. The mean (SD) age was 21.5 (2.6) years old, and the male/female ratio was 31/77. Saliva of one female participant was not suitable for processing (due to thickness of the sample), and thus the final dataset of saliva assessments comprised N = 107 participants. The study outcomes are summarized in Table 1. No significant sex differences were found.

Table 2 and Fig. 2 summarize the relationship between the HDS, perceived immune fitness, and markers of systemic inflammation. The analysis revealed a significant correlation between the HDS and

Table 1
Healthy diet score, perceived immune fitness, and biomarker concentrations.

	Overall	Men	Women	p-value
N	108	31	77	
Healthy Diet Scale	68.1 (14.3)	64.5 (13.9)	69.5 (14.3)	0.097
Perceived immune fitness	7.6 (1.2)	7.8 (1.3)	7.5 (1.2)	0.338
CRP (pg/mL)	155.9 (193.7)	142.9 (172.7)	161.2 (202.5)	0.631
IL-1β (pg/mL)	174.3 (212.0)	183.2 (243.4)	170.7 (199.5)	0.940
IL-8 (pg/mL)	232.1 (166.8)	250.7 (174.0)	224.5 (164.3)	0.410

Mean and standard deviation (SD) are shown. Sex differences are considered significant if $p < 0.05$ and indicated by * . Abbreviations: IL = interleukin, CRP = C-reactive protein. Saliva of one female participant was not suitable for processing, and thus the final dataset of saliva assessments comprised N = 107 participants.

Table 2
Relationship between immune fitness and healthy diet.

Correlations with the Healthy Diet Scale	r	p-value
Perceived immune fitness	0.221	0.021 *
CRP	-0.240	0.013 *
IL-1β	-0.030	0.756
IL-8	-0.123	0.207

Spearman's correlations are shown. Correlations are considered significant if $p < 0.05$ and indicated by * . Abbreviations: IL = interleukin, CRP = C-reactive protein.

perceived immune fitness (See Fig. 2a). Whereas the saliva CRP concentration correlated significantly with the HDS (See Fig. 2b), no significant correlations were found for IL-1β and IL-8.

Table 3 summarizes the separate outcomes for men and women. In women, the HDS correlated significantly with perceived immune fitness and CRP. In men, the correlations of HDS with perceived immune fitness and the biomarkers were not significant.

4. Discussion

The current study demonstrated a significant relationship between adherence to a healthy diet and immune fitness. Overall, significant correlations were found with both perceived immune fitness and CRP. These findings are consistent with scientific literature. For example, several studies found that a higher intake of fruit and vegetables, which are part of a healthy diet, was associated with lower concentrations of CRP in blood [34–36]. Moreover, other studies reported that the intake of certain vitamins (e.g. vitamin C, D and E), which can be found in healthy food products, negatively correlated with CRP concentrations [37–39]. Furthermore, King et al. [40] revealed that the consumption of saturated fats, which can mainly be found in unhealthy diets, was moderately associated with higher levels of CRP. These studies support the notion that dietary composition determines the extent to which a diet can be considered healthy. Therefore, future studies, using food frequency questionnaires (FFQs), food diaries, or 24 h dietary recall, should compare performance of the HDS with specific nutrient or food group intake. Future studies should link the HDS outcomes to its relationship with activity of the gut, brain, and the immune system, in order to gain mechanistic insights in their interactions, and how compliance to a healthier diet influences immune fitness.

The current study revealed sex differences in the relationship between immune fitness and healthy diet. That is, the correlation between healthy diet and immune fitness was significant in women, but not in men. One possible explanation is that the sample of men (N = 31) was too small to demonstrate statistical significance. This is a likely

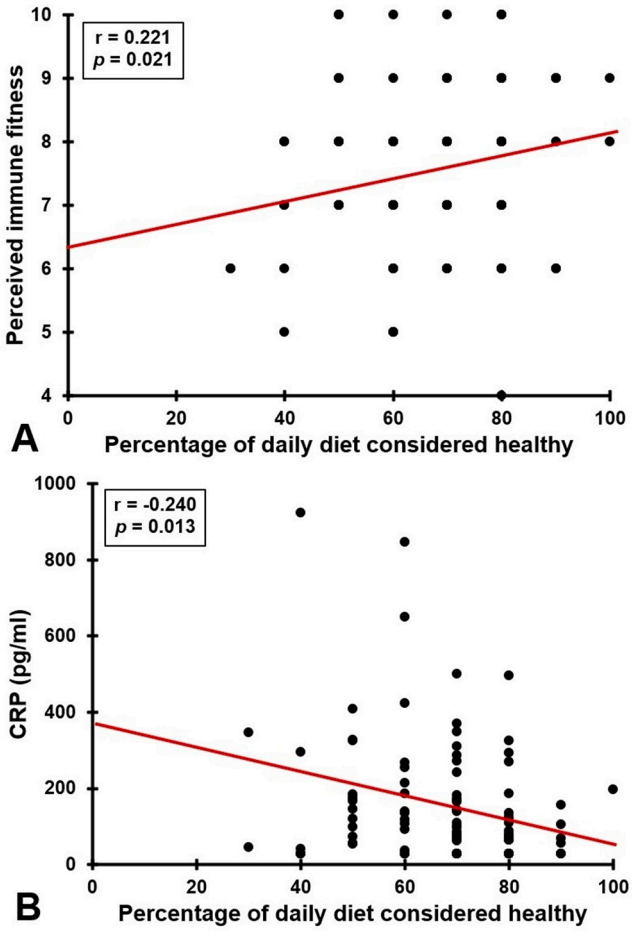

Fig. 2. The relationship between immune fitness and healthy diet. Fig. 2a shows the correlation between the Healthy Diet Scale (HDS) score and perceived immune fitness; Fig. 2b shows the correlation between the HDS score and saliva C-reactive protein (CRP) concentration.

Table 3
Relationship between immune fitness and healthy diet for men and women.

	Men (N = 31)		Women (N = 77)	
Correlations with the HDS	r	p-value	r	p-value
Perceived immune fitness	0.219	0.237	0.247	0.030 *
CRP	-0.184	0.322	-0.281	0.014 *
IL-1β	0.118	0.526	-0.067	0.567
IL-8	-0.097	0.605	-0.115	0.321

Spearman's correlations are shown. Correlations are considered significant if $p < 0.05$ and indicated by * . Abbreviations: HDS = Healthy Diet Scale, IL = interleukin, CRP = C-reactive protein.

explanation, as the correlations found for perceived immune fitness were of the same magnitude in men and women. Regarding CRP, the correlation in men was less robust and did not reach statistical significance, but was also in the same negative direction as observed in women.

Several limitations of the current study should be considered when interpreting the results. First of all, it is hard to determine the exact association between a healthy diet and immune fitness, as there are other factors (e.g., sleep and physical activity) affecting immune fitness as well [10]. These factors were not considered in the current analysis. In future studies these factors could be included as covariates in a regression model. Second of all, the HDS relies on retrospective self-report. Consequently, recall bias may have affected the outcomes of

the study. Thirdly, the research population involved young, higher educated, and healthy individuals. Therefore, it is uncertain to what extent the results of the current study are representative for other age groups such as elderly people, samples with a different socioeconomic or education background, or people with underlying disease. In future, studies should also be conducted in these cohorts. It might also be interesting to further investigate the interplay between healthy diet and other lifestyle factors in influencing immune fitness, disease risk, mood, and quality of life.

Taken together, the results are in line with scientific literature and demonstrate the efficacy of the HDS to estimate the percentage of daily diet that can be considered as healthy. The link between healthy diet and immune fitness shown in this study is important as it underlines that attaining a healthy diet is beneficial for maintaining an adequate immune fitness. Prevention campaigns should therefore create awareness about the importance of attaining healthy dietary habits, in order to maintain immune fitness and reduce the chances of developing non-communicable diseases among the general population.

5. Conclusions

The current study examined the impact of a healthy diet on both perceived immune fitness and markers of systemic inflammation, among young adults in the Netherlands. A significant positive correlation was found between compliance to healthy diet consumption and perceived immune fitness, and a significant negative correlation was found between compliance to healthy diet consumption and saliva CRP concentration. The observed sex differences in the associations require further investigation, while taking into account other factors such as age, sleep and stress.

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CRediT authorship contribution statement

Evi C. van Oostrom: Conceptualization, Investigation, Writing – review & editing. **Kiki EW Mulder:** Conceptualization, Investigation, Writing – review & editing. **Marjolijn CE Verheul:** Conceptualization, Investigation, Writing – review & editing. **Pauline A. Hendriksen:** Conceptualization, Investigation, Writing – review & editing. **Suzan Thijssen:** Conceptualization, Methodology, Writing – review & editing. **Aletta D. Kraneveld:** Conceptualization, Writing – review & editing. **Berber Vlieg-Boerstra:** Conceptualization, Writing – review & editing. **Johan Garssen:** Conceptualization, Writing – review & editing. **Joris C. Verster:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Science-Geo Ethics Review Board of Utrecht University (approval code: S-21525, date of approval: November 21, 2021).

Informed Consent Statement

Informed consent was obtained from all participants.

Conflicts of Interest

Over the past 3 years, J.C.V. has acted as a consultant/advisor for KNMP, Mentis, Red Bull, Sen-Jam Pharmaceutical, and Toast!. J.G. is part-time employee of Nutricia Research and received research grants from Nutricia research foundation, Top Institute Pharma, Top Institute Food and Nutrition, GSK, STW, NWO, Friesland Campina, CCC, Raak-Pro, and EU. Over the past 36 months, A.D.K. has held research grants from H2020, Nutricia-Danone, Netherlands Center of Translational Research, Lung Fund, SGF/Health Holland and NWO. The other authors have no potential conflicts of interest to disclose.

Data Availability

The data is available from the corresponding author upon reasonable request.

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