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Original Article

Self-reported sleep duration, white blood cell counts and cytokine profiles in European adolescents: the HELENA study

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ABSTRACT

Background: Sleep patterns face important changes during adolescence. This can have implications for the immune system, which is regulated by the sleep–wake cycle; however, most studies relating sleep and immune system have been conducted on adults.
Objective: To study the relationships between sleep duration, immune cell counts, and cytokines in European adolescents participating in the HELENA Cross-Sectional Study.
Methods: Adolescents (12.5–17.5 years; $n = 933$; 53.9% girls) were grouped according to self-reported sleep duration into <8, 8–8.9 and ≥9 h/night. Blood samples were collected in the morning after an overnight fast to analyze counts of white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, the lymphocyte subsets $CD3^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, $CD45RO^+$, $CD3^-CD16^+56^+$ and $CD19^+$, and concentrations of cortisol, CRP, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α and IFN-γ. Pro-/anti-inflammatory and Th1/Th2 cytokine ratios were calculated. Immune parameters were correlated to sleep duration and compared between the three groups.
Results: Sleep duration was negatively associated with cortisol levels and WBC, neutrophil, monocyte, $CD4^+$ and $CD4^+CD45RO^+$ counts; in girls it is also negatively associated with IL-5 and IL-6 levels. The 8–8.9 h/night group presented the highest IL-4 values and the lowest pro-/anti-inflammatory and Th1/Th2 cytokine ratios.
Conclusion: A sleep duration of 8–8.9 h/night was associated with a healthier immune profile in our adolescents.

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1. Introduction

Most functions within the human body present a rhythmicity of ~24 h (circadian rhythms); the circulating levels of our hormones, cytokines and metabolites experience regular daily variations that are controlled by a central clock [1]. Environmental and behavioral changes such as alternation between light and darkness, intake and fasting, or activity and repose are key regulators of this central clock. The breakdown of the normal phase relationship between the internal circadian rhythms and the 24 h environmental cycles is named chronodisruption. In our modern society, chronodisruption can be the result of several conditions such as jet lag, shift working, or night eating [2]. Among adolescents, the main agent of chronodisruption is lack of sleep. Sleep patterns are often altered in this age period; however, adequate sleep is a critical factor for adolescents' health and the establishment of health-related behaviors [3].

In recent years, sleep duration has decreased significantly in the adult population, a situation that has been related to various metabolic disorders [4]. Lack of sleep is associated with higher risk of suffering from infections and worse recovery, and with increased risk of developing non-infectious diseases and chronic low-grade inflammation [5]. These conditions are linked to alterations in the immune response, which may be secondary to the activation of the stress axis and the release of cortisol, a well-known regulator of immune activity; however, it cannot be ruled out that changes in immune parameters respond to a direct cross-talk between sleep and the immune system [6–8].

The immune system shows circadian rhythms in its cell counts and in its functions [9]. In addition, peripheral circadian clocks have been found in various white blood cell (WBC) types [7,10,11]. Different experiments have been conducted in the laboratory setting to study the effects of sleep on immune function. The majority explore the effects of short-term (acute) lack of sleep, and adopt one of two strategies: controlled sleep restriction, in which sleep duration is limited to a few hours (frequently five or less), and controlled sleep deprivation, in which subjects experience continuous wakefulness for a certain period of time (generally 24–48 h). These experiments have shown that lack of sleep is followed by elevations in WBC counts, notably in neutrophils and/or monocytes [12–15]. Sleep is believed to stimulate the specific (adaptive) immune response, whereas wakefulness seems to favor non-specific responses (innate immunity) mediated mainly by neutrophils, monocytes and natural killer (NK) cells (Table 1) [5,7]. The expression and production of cytokines from immune cells follow a circadian pattern that can be synchronized with sleep and wakefulness [9]. Therefore, the sleep–wake cycle may influence immune function.

There is a limitation, however, to the interpretation of the information available on the relationship between sleep (especially lack of sleep) and immune function. While sleep restriction and sleep deprivation studies provide very useful information on the effects of both acute and more prolonged lack of sleep, we cannot be certain that, in a real life-setting, less dramatic but habitual lack of sleep will trigger the same response in the body. In other words, adaptive responses may differ with time, and so may their impact on the individual's health.

The vast majority of studies have been conducted on adults and little information is available on adolescents. However, as mentioned above, adolescence is a period of great interest, due to the many physiological and behavioral changes taking place; these include changes in sleep habits, characterized mainly by delayed sleep onset leading to shortening of sleep duration. For these reasons, we aimed to study whether sleep duration is related to immune parameters in a representative sample of European adolescents.

2. Methods

2.1. Study design and sample selection

A European multicenter cross-sectional study (CSS) was performed with the objective to assess a "healthy lifestyle in Europe by nutrition in adolescence" (HELENA). The HELENA-CSS aimed to obtain reliable and comparable data on nutrition and other health indicators such as physical activity and fitness, body composition, cardiovascular disease risk factors, vitamin and mineral status, and immunological and genetic markers in European adolescents [16]. The methodology used in this study has been published elsewhere [17]. The study was performed according to the ethical guidelines of the Edinburgh revision of the 1964 Declaration of Helsinki (2000), the International Conferences on Harmonization for Good Clinical Practice and the legislation on clinical research from each of the participating countries. The protocol was approved by the Research Ethics Committees of each center involved. Written informed consent was obtained from the adolescents and their parents [18].

Briefly, subjects aged 12.5–17.5 years were recruited from 10 cities belonging to nine countries across Europe (Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pècs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria, and Zaragoza in Spain). The total eligible HELENA-CSS population consisted of 3528 adolescents, with inclusion criteria being not participating simultaneously in another clinical trial and being free of any acute infection occurring within the week prior to the study.

2.2. Sleep hours determination

Habitual sleep time was estimated by a questionnaire. The adolescents were asked the following questions: "How many hours (and minutes) do you usually sleep during week days?" and "How many hours (and minutes) do you usually sleep during weekend days?" A total weekly sleep score was calculated as: [(min weekday × 5) + (min weekend day × 2)]/7.

Table 1
Summary of the different white blood cell (WBC) types analyzed.

WBC types	Lymphocyte subsets	Membrane markers	Immune function
Neutrophils			Innate or non-specific
Monocytes			Innate or non-specific
Lymphocytes	Mature T-cells	$CD3^+$	Adaptive or specific
	Naive T-helper	$CD3^+CD4^+CD45RA^+$	Adaptive or specific
	Memory T-helper	$CD3^+CD4^+CD45RO^+$	Adaptive or specific
	Naive T-cytotoxic	$CD3^+CD8^+CD45RA^+$	Adaptive or specific
	Memory T-cytotoxic	$CD3^+CD8^+CD45RO^+$	Adaptive or specific
	B-cells	$CD3^-CD19^+$	Adaptive or specific
	Natural killer cells	$CD3^-CD16^+56^+$	Innate or non-specific
Eosinophils			Innate or non-specific
Basophils			Innate or non-specific