**Pérez-de-Heredia, F, Gómez-Martínez, S, Díaz, L-E, Veses, AM, Nova, E, Marcos, A, Wärnberg, J, Wastlund, A, Sjöstrom, M, Huybrechts, I, Vyncke, K, Androutsos, O, Manios, Y, Ferrari, M, Palacios, G, González-Gross, M, Kovács, É, Molnár, D, Gottrand, F, Castillo, MJ, Kafatos, A, Widhalm, K and Moreno, LA**

# Influence of sex, age, pubertal maturation and body mass index on circulating white blood cell counts in healthy European adolescents—the HELENA study

**http://researchonline.ljmu.ac.uk/2938/**

**Article**

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Pérez-de-Heredia, F, Gómez-Martínez, S, Díaz, L-E, Veses, AM, Nova, E, Marcos, A, Wärnberg, J, Wastlund, A, Sjöstrom, M, Huybrechts, I, Vyncke, K, Androutsos, O, Manios, Y, Ferrari, M, Palacios, G, González-Gross, M, Kovács, É, Molnár, D, Gottrand, F, Castillo, MJ, Kafatos, A, Widhalm, K and**

# Influence of sex, age, pubertal maturation and body mass index on circulating white blood cell counts in healthy European adolescents - The HELENA STUDY

Fátima Pérez-de-Heredia[1,2*†], Sonia Gómez-Martínez[2†,] Ligia-Esperanza Díaz[2], Ana M. Veses[2], Esther Nova[2], Julia Wärnberg[3], Inge Huybrechts[4,5], Krishna Vyncke[4], Odysseas Androutsos[6], Marika Ferrari[7], Gonzalo Palacios[8], Acki Wastlund[3], Éva Kovács[13], Frédéric Gottrand[9], Marcela González-Gross[8,10], Manuel J. Castillo[11], Michael Sjöstrom[3], Yannis Manios[6], Anthony Kafatos[12], Denes Molnár[13], Kurt Widhalm[14], Luis A. Moreno[15], and Ascensión Marcos[2], on behalf of the HELENA Study Group.

F.PerezDeHerediaBenedicte@ljmu.ac.uk; sgomez@ictan.csic.es; ldiaz@ictan.csic.es; amveses@ictan.csic.es; enova@ictan.csic.es; jwarnberg@uma.es; Inge.Huybrechts@UGent.be; Krishna.Vyncke@UGent.be; oandrou@hua.gr; marika.ferrari@entecra.it; gonzalopalacios88@gmail.com; acki@fms.se; e.k.kovacs@gmail.com; Frederic.GOTTRAND@CHRU-LILLE.FR; marcela.gonzalez.gross@upm.es; mcgarzon@ugr.es; Michael.Sjostrom@ki.se; manios@hua.gr; kafatos@med.uoc.gr; denes.molnar@aok.pte.hu; kurt.widhalm@meduniwien.ac.at; lmoreno@unizar.es; amarcos@ictan.csic.es

---

[1] School of Natural Sciences and Psychology, Liverpool John Moores University, UK.
[2] Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain
[3] Unit for Preventive Nutrition, Department of Biosciences and Nutrition at Novum, Karolinska Institutet, Stockholm, Sweden
[4] Department of Public Health, Faculty of Medicine and Health Sciences, University of Ghent, Belgium
[5] International Agency for Research on Cancer, Dietary Exposure Assessment Group, Lyon, France
[6] Department of Nutrition & Dietetics, Harokopio University, Athens, Greece
[7] CRA-NUT, Food and Nutrition Research Center, Agricultural Research Council Roma, Italy
[8] ImFine Research Group, Faculty of Physical Activity and Sport Sciences, Technical University of Madrid, Spain
[9] Hôpital J de Flandre CHRU de Lille, Inserm U995, IFR114, Faculté de Médecine, Université de Lille 2, France
[10] Department of Nutrition and Food Sciences – Nutritional Physiology, University of Bonn, Germany
[11] Department of Medical Physiology, Faculty of Medicine, University of Granada, Spain
[12] University of Crete School of Medicine, Department of Social Medicine, Preventive Medicine and Nutrition Clinic, Heraklion, Crete, Greece
[13] Department of Pediatrics, University of Pécs, Hungary
[14] Private Medical University Salzburg, Dept. of Pediatrics, Austria
[15] GENUD (Growth, Exercise, Nutrition and Development) Research Group, University of Zaragoza, Spain

[†]These authors have equally contributed to the elaboration of this manuscript.

*Corresponding author:
Fátima Pérez de Heredia, PhD
School of Natural Sciences and Psychology, Liverpool John Moores University
James Parsons Building, Byrom Street, Liverpool, L3 3AF, United Kingdom
Tel: +44 151 231 2003; Fax: +44 151 231 2338
E-mail: F.PerezDeHerediaBenedicte@ljmu.ac.uk

## Abstract

Percentiles 10th, 25th, 50th, 75th and 90th are presented for total circulating white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils and basophils in healthy European adolescents (12.5-17.5 years, n=405, 48.9% boys), considering age, sex, puberty and body mass index (BMI). $CD3^+$ (mature T cells), $CD4^+$ (T helper), $CD8^+$ (T cytotoxic), $CD16^+56^+$ (natural killer), $CD19^+$ (B cells), $CD45RA^+$ (naïve) and $CD45RO^+$ (memory) lymphocytes were also analysed by immunophenotyping. Girls presented higher WBC, neutrophil, $CD3^+CD45RO^+$ and $CD4^+CD45RO^+$ cell counts and $CD3^+/CD19^+$ ratio, and lower $CD3^+CD45RA^+$ and $CD4^+CD45RA^+$ counts than boys. Age was associated with higher neutrophil counts and $CD3^+/CD19^+$ ratio and lower $CD19^+$ counts; in boys, with lower $CD3^+CD45RA^+$, $CD4^+CD45RA^+$ and $CD8^+CD45RA^+$ counts as well; in girls, with higher WBC, $CD3^+CD45RO^+$, and $CD4^+CD45RO^+$ counts. Pubertal maturation in boys was associated with lower WBC and lymphocyte counts; in girls, with higher basophil, $CD3^+CD45RO^+$ and $CD4^+CD45RO^+$ values. BMI was associated with higher WBC counts; in boys, also with higher lymphocyte counts; in girls, with higher neutrophil, $CD4^+$, $CD3^+CD45RO^+$ and $CD4^+CD45RO^+$ counts. Conclusions: Our study provides normative values for circulating immune cells in adolescents, highlighting the importance of considering sex, age, pubertal maturation and BMI when establishing reference values for WBC in paediatric populations.

**Key words:** adolescents; immune cells; immunophenotyping; sex; puberty; body mass index.

**Abbreviations used**

BMI – body mass index

WBC – white blood cells

**What is known?**

Reference values for white blood cell counts and immunophenotyping of lymphocyte subsets can constitute useful clinical tools and health indicators in both adult and paediatric populations. Like other health indicators, they can vary according to a number of factors, such as age or sex.

**What is new?**

Specific data from WBC in European adolescents are limited, and even less is known about changes in lymphocyte subsets during adolescence. This work provides normative values obtained from adolescents from 9 European countries, considering the influence of sex, age, pubertal maturation and BMI and finding that:

- Girls had higher WBC, neutrophil and $CD45RO^+$ (memory) cell values, while boys had higher percentages of lymphocytes, monocytes and eosinophils and $CD45RA^+$ (naive) cell counts.
- Age was associated with higher WBC, neutrophil, $CD45RO^+$ and $CD3^+/CD19^+$ values, and lower percentage of total lymphocytes and $CD45RA^+$ cell counts.
- Pubertal maturation was associated, on the contrary, with lower WBC and lymphocytes, but only in boys. In girls, pubertal maturation was linked to higher $CD45RO^+$ cell counts.
- BMI was associated with higher WBC, mainly due to greater lymphocyte counts in boys, and to neutrophil and $CD45RO^+$ cell counts in girls.

## Introduction

Total white blood cell (WBC) counts and the evaluation of the different subtypes of white blood cells are useful clinical indicators and are frequently used as diagnostic tools for adults as well as for children. Besides providing information on acute inflammatory and infectious states, the immunological status serves as an indicator of many other physiological processes, and immune markers are becoming increasingly used to study alterations other than infections – for example, as early as in the 70s, Friedman and colleagues reported an association between WBC count and myocardial infarction [9]. Immune function is closely related to overall health and nutritional status [31], and during the last decade, WBC count has been related to different metabolic alterations, such as impaired glucose tolerance, type 2 diabetes mellitus, obesity, or the metabolic syndrome, and this has been observed in adults [10, 25] as well as in children and adolescents [5, 36]. The analysis of lymphocyte subsets by flow cytometry (known as immunophenotyping) is an ever more widely used tool not only for assessing health status but also specifically in nutritional evaluation, and helps identify subjects at risk of disease.

Adequate and reliable reference values from healthy populations become a key point in the clinical use of any biological parameter. Variations can occur as a consequence of physiological, ethnic or environmental factors; therefore, normative values should be specific for a given population group. For example, childhood and adolescence are growth periods in which all the systems in the body are developing physically and functionally. The immune system itself experiences a series of modifications from birth until adulthood; during childhood and adolescence the immune cells vary in both their number and functionality [1, 6, 8, 13, 16, 18, 28, 32, 34]. Therefore, normative values for immune cells obtained from adult populations can be misleading when applied to children and adolescents. In addition, sex-related and even ethnicity-related variations in the values of circulating immune cells have been acknowledged [1, 4, 7, 17, 28, 29, 34, 38,], highlighting the importance of providing reference values for specific population groups and geographical areas. However, information about WBC counts and specific lymphocyte subsets on healthy adolescents is still scarce [1, 8, 16, 28, 32, 34], as most available data belong to populations with particular immune-related diseases, or the studies, although useful and informative, have usually been conducted on relatively modest sample sizes for this particular age group.

The present study aims to provide normative ranges for total and differential WBC counts and for selected lymphocyte subsets in a representative sample of healthy European adolescents, attending to variations due to sex, age, degree of pubertal maturation, and body mass index.

## METHODS

### Study design and sample selection

A European multicentre cross-sectional study (CSS) was performed with the objective of assessing 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 [21]. The methodology used in this study has been published elsewhere [20]. 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 the participating centres. Written informed consent was obtained from the parents of the adolescents and from the adolescents themselves [2].

Briefly, subjects aged 12.5-17.5 years were recruited randomly from schools in ten 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 3,528 adolescents. Blood samples were obtained in one third of participants, resulting in a representative subpopulation of 1,089 adolescents (approximately 100 boys and girls per city). The size of this subpopulation was previously calculated as sufficient to account for the expected variability in blood measurements.

## Data exclusion

Those subjects with conditions that might interfere with or imply stimulation of normal immune function were excluded from the analysis. Exclusion criteria included: suffering from allergies, suffering from fever on the 24 hours prior to blood sampling, having had a cold or any infection during the week prior to the day of blood sampling, having taken any medication in the previous 24 hours or for more than 7 days in the previous 30 days, having taken any vitamin or mineral supplement during the previous month, and having been vaccinated in the two weeks prior to the day of blood sampling. After applying these exclusion criteria, the final study population consisted of 405 subjects (48.9% boys).

## Measurements of pubertal maturation and body mass index

Evaluation of the degree of pubertal maturation was assessed by a medical doctor, according to the Tanner and Whitehouse classification [33]. Anthropometric data were also collected following harmonized protocol procedures previously described [23]. Body mass index (BMI) was calculated as: body weight (kg) / [height (m)]$^2$. Standardized BMI values (z-scores) were calculated and the sample was classified according to quartiles of standardized BMI values.

## Blood sampling, white blood cell profiling and immunophenotyping

Venous blood samples were collected in EDTA Monovette (Sarstedt, Germany) tubes between 8.00 and 10.00 a.m. after a 12-hour overnight fast. WBC counts and percentages were determined in each participating city with automated blood cell counters. For immunophenotyping of lymphocyte subsets, blood samples were collected in EDTA-K3E Vacutainer (BD Biosciences) tubes. Blood aliquots were taken into 1.5 ml plastic tubes and diluted 1:1 with Cytochex™ Reagent (Streck Laboratories, Omaha, NE, USA). The samples were all sent to the CSIC group laboratory (Madrid, Spain) within 7 days from collection. The methodology for WBC determination and for collection, preparation and shipping of the blood samples to Madrid was standardized amongst all participating cities [11].

Immunophenotyping

Blood aliquots were incubated for 30 minutes at room temperature in the dark with fluorochrome-conjugated monoclonal antibodies (BD Biosciences, San José, CA, USA), to differentially label those cells positive for the surface markers CD45 (the pan-leukocyte marker), CD3 (T mature cells), CD4 (helper T cells), CD8 (cytotoxic T cells), CD19 (B cells), $CD16^+56$ (natural killer cells), CD45RO (memory cells) and CD45RA (naïve cells). A quadruple immunostaining procedure was performed as follows: CD3/CD8/CD45/CD4, CD45RA/CD45RO/CD8/CD3, CD45RA/CD45RO/CD4/CD3 and CD3/$CD16^+56$/CD45/ /CD19. After lysis of red blood cells, lymphocytes were analysed by flow cytometry (FACScan Plus Dual Laser, Becton Dickinson Sunnyvale, CA). The lympho-gate was defined on the forward and side scatter patterns of lymphocytes. The analysis protocol gated on lymphocytes stained with PerCP and/or APC and the selected population was then analysed with the two remaining colours (FITC and PE) to obtain percentages of cell expressing the specific antigens.

Percentages of the following lymphocyte subsets were obtained: total mature T cells ($CD45^+CD3^+$, or $CD3^+$ for simplicity), helper T cells ($CD45^+CD3^+CD4^+$, or $CD4^+$), cytotoxic T cells ($CD45^+CD3^+CD8^+$, or $CD8^+$), natural killer (NK) cells ($CD45^+CD3^-16^+56^+$, or $CD16^+56^+$), B cells ($CD45^+CD3^-CD19^+$, or $CD19^+$), naïve T cells ($CD3^+CD45RA^+$, $CD4^+CD45RA^+$ and $CD8^+CD45RA^+$, or $CD3RA^+$, $CD4RA^+$ and $CD8RA^+$), and memory T cells ($CD3^+CD45RO^+$, $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$, or $CD3RO^+$, $CD4RO^+$ and $CD8RO^+$). Absolute cell counts were calculated from total lymphocyte numbers, which were determined with automated blood cell counters in each participating city. The $CD4^+/CD8^+$ and the $CD3^+/CD19^+$ ratios were also calculated.

## Statistical analysis

All the analyses conducted on the HELENA-CSS data were adjusted by a weighing factor to balance the studied population according to the age and sex distribution of the theoretical sample. Adolescents were grouped into four age categories: 12.5-13.9 years (from 12.5 years to the day before the 14th birthday), 14-14.9 years (from the 14th birthday to the day before the 15th birthday), 15-15.9 years (from the 15th birthday to the day before the 16th birthday), and 16-17.5 years (from the 16th birthday to 17.5 years). The Chi-squared ($X^2$) test was used to compare frequencies of sex, age categories, and Tanner stages between the studied and the excluded groups, as well as to compare frequencies of age categories and Tanner stages between sexes.

Absolute counts and percentages of cells are presented as percentiles 10th, 25th, 50th (median), 75th and 90th for description of the population classified by sex and age. Data were then studied according to sex, age, Tanner stage and standardized BMI categories. Normality of variables was checked by the Kolmogorov-Smirnov test, and those not normally distributed were appropriately transformed when necessary.

Differences between sexes were analysed with the Mann-Whitney U test. Subsequent tests to assess the influence of age, pubertal maturation and BMI were conducted separately in boys and girls. Associations between cell values and age and between cell values and BMI were assessed with Pearson partial correlation test, controlling for city of origin (centre). Differences in cell values between Tanner stages and BMI categories were assessed by analysis of covariance (ANCOVA), adjusting for centre (and age when comparing Tanner stages). Due to the small number of individuals found within Tanner stages I and II, these two groups were combined to allow statistical analysis.

Statistical significance was set at $P<0.05$. All statistical analyses were performed with IBM® SPSS® Statistics v.19 for Windows.

## RESULTS

### Characteristics of the population

There were no differences in the proportions of sexes between included and excluded adolescents (48.9 vs. 45.5% boys; $X^2 = 1.196$, $P = 0.274$). Similarly, no significant differences were found in the distributions of age categories ($X^2 = 1.146$, $P = 0.766$), Tanner stages ($X^2 = 3.851$, $P = 0.278$) or BMI categories ($X^2 = 7.164$, $P = 0.067$) between the adolescents included in the analysis and their excluded peers.

The average age of the studied population was 14.9 ± 1.2 years (range 12.5-17.4 y). There were significantly fewer adolescents between 16 and 17.5 years (22%) than in the other age groups ($X^2 = 16.392$, $P < 0.01$). In relation to pubertal maturation, most of the adolescents (78.2%) were found at either the IV or V Tanner stages, as could be expected according to the age range. Boys and girls were similar in age, Tanner stage distributions and mean BMI values in each quartile of BMI z-scores (boys: 17.7 ± 1.1 (Q1), 19.8 ± 0.8 (Q2), 21.8 ± 1.2 (Q3), and 27.0 ± 3.2 (Q4) kg/m$^2$; girls: 17.9 ± 1.1 (Q1), 20.0 ± 0.8 (Q2), 22.0 ± 1.1 (Q3), and 26.3 ± 3.0 (Q4) kg/m$^2$).

### Influence of sex on immune cell counts and percentages

Statistical comparison between sexes showed that girls in general presented higher values for total WBC and neutrophil counts (**table 1**). Percentages of neutrophils were also higher in girls, while those of lymphocytes, monocytes and eosinophils were greater in boys (**supplementary table 1**).

**Table 2** shows the percentiles 10th, 25th, 50th (median), 75th and 90th of the absolute counts of the selected lymphocyte subsets, separately by sex and group of age. Percentiles of the lymphocyte subsets percentages can be found in **supplementary table 2**. Boys showed a tendency to higher counts of $CD3^+CD45RA^+$ and $CD4^+CD45RA^+$ naïve cells (differences significant at 14-14.9 y), while girls presented higher counts of $CD3^+CD45RO^+$ (differences being significant at 14-14.9 and 16-17.5 y) and $CD4^+CD45RO^+$ memory cells (significant at 16-17.5 y). The ratio $CD3^+/CD19^+$ was also higher in girls (difference being significant at 15-15.9 years) (**table 2**). Similarly, boys had higher percentages of $CD3^+CD45RA^+$ (significant at 14-14.9 y and 16-17.5 y), $CD4^+CD45RA^+$ and $CD8^+CD45RA^+$ (differences significant at 14-14.9 y); girls in turn had higher percentages of $CD3^+$ and $CD4^+$ (differences significant at 15-15.9 y), $CD3^+CD45RO^+$ (differences significant at 14-14.9 and 16-17.5 y), $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$ (differences significant at 14-14.9 y) (**supplementary table 2**).

### Influence of age on immune cell counts and percentages

Total WBC and neutrophil counts increased with age in the total population ($r = 0.121$, $P = 0.015$ and $r = 0.153$, $P = 0.003$, respectively; and **table 1**). Neutrophil percentages also increased with age, while the percentage of lymphocytes decreased, both in the total population ($r = 0.167$ and $r = -0.173$, respectively, $P = 0.001$) and in boys (**supplementary table 1**).

Age was also associated with increased counts of $CD4^+CD45RO^+$ ($r = 0.179$, $P = 0.001$) and the ratio $CD3^+/CD19^+$ ($r = 0.240$, $P < 0.001$), and decreased counts of $CD19^+$ ($r = -0.250$,

$P < 0.001$), CD3$^+$CD45RA$^+$ ($r = -0.118$, $P = 0.036$), and CD4$^+$CD45RA$^+$ ($r = -0.124$, $P = 0.027$). The percentages of these subsets showed the same relationships with age (%CD19$^+$: $r = -0.255$; %CD3$^+$CD45RA$^+$: $r = -0.184$; %CD4$^+$CD45RA$^+$: $r = -0.238$; %CD3$^+$CD45RO$^+$: $r = 0.206$; %CD4$^+$CD45RO$^+$: $r = 0.235$; all $P < 0.01$). In boys alone, age was inversely correlated with counts of CD19$^+$, CD8$^+$ and naïve cells (CD3$^+$CD45RA$^+$, CD4$^+$CD45RA$^+$ and CD8$^+$CD45RA$^+$), and positively with the ratio CD3$^+$/CD19$^+$ (**table 2**). In girls alone, older age was associated as well with lower CD19$^+$ and higher CD3$^+$/CD19$^+$, but also with increased counts of CD3$^+$CD45RO$^+$ and CD4$^+$CD45RO$^+$ (**table 2**). As a result, in both boys and girls there was a trend to decreased percentages of naïve cells and increased of memory cells with age (**supplementary table 2**).

### Influence of pubertal maturation on immune cell counts and percentages

In boys, pubertal maturation (Tanner stage) was associated with lower WBC and lymphocyte counts, independently of age (**table 3**); lymphocyte percentages were also lower in more developed Tanner stages (**supplementary table 3**). This decrease was reflected in most lymphocyte subsets: CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$, CD3$^+$CD45RA$^+$, CD3$^+$CD45RO$^+$, CD4$^+$CD45RO$^+$, CD8$^+$CD45RA$^+$ and CD8$^+$CD45RO$^+$ cell counts were all lower in more advanced Tanner stages (**table 4**).

In girls, higher basophil counts (**table 3**) and percentages (**supplementary table 3**), and CD3$^+$CD45RO$^+$ and CD4$^+$CD45RO$^+$ cell counts were higher in more advanced pubertal maturation stages (**table 4**).

### Influence of BMI on immune cell counts and percentages

In boys, counts of total WBC, neutrophils and lymphocytes were higher with increasing BMI (**table 5**). The increase in lymphocyte counts was reflected in most subsets (CD3$^+$, CD4$^+$, CD8$^+$, natural killer, CD19$^+$, CD3$^+$CD45RO$^+$, CD4$^+$CD45RO$^+$ and CD8$^+$CD45RO$^+$), whereas the ratio CD4$^+$/CD8$^+$ (**table 6**) and the percentages of naïve cells (CD3$^+$CD45RA$^+$ and CD4$^+$CD45RA$^+$) decreased (**supplementary table 6**).

In girls, BMI showed a positive relationship with total WBC and neutrophil counts (**table 5**), neutrophil percentages (**supplementary table 5**), and memory cell counts (CD3$^+$CD45RO$^+$ and CD4$^+$CD45RO$^+$) (**table 6**), and a negative one with percentages of CD8$^+$CD45RA$^+$ cells (**supplementary table 6**).

## DISCUSSION

The current study describes the immune cell profile of a representative sample of healthy European adolescents. Our results show that sex, age, pubertal maturation and BMI are factors that influence normal circulating counts and percentages of immune cells in adolescents.

There were clear sex-related differences in the percentages and absolute counts of immune cells in our population. In general, girls had higher total WBC, neutrophil and memory T cells values (in particular, CD3$^+$CD45RO$^+$ and CD4$^+$CD45RO$^+$), while boys had higher percentages of lymphocytes and eosinophils, and higher counts of naïve T cells (all three subsets, CD3$^+$CD45RA$^+$, CD4$^+$CD45RA and CD8$^+$CD45RA$^+$). Similar trends for differences in WBC between sexes were observed in a previous study in Spanish adolescents, although the authors did not find statistical significance [27]. With regards to lymphocyte subsets, our observations are also in agreement with previous studies [1, 28, 34]. On the contrary, other authors have found no effect of sex on WBC counts [38] or lymphocyte subsets [32] in adolescents. Sex dimorphism in WBC counts and percentages can be explained by differences in sex hormones, as suggested by Rudy and colleagues [28]. Sex hormones have been shown to modulate immune function at various levels, and a greater immune responsiveness has been observed in girls in general [12, 35].

A trend was observed towards higher WBC counts with age, in agreement with the findings reported by Bartlett and colleagues [1]. In particular, neutrophil counts were elevated in older boys and girls in our study; this led to increasing percentages of this cell type and decreasing percentages of lymphocytes in boys, while no significant changes in cell percentages with age were observed in girls. In contrast, age was associated with decreasing values for B cells ($CD19^+$) leading to a higher $CD3^+/CD19^+$ ratio, and with a significant shift towards lower naïve/memory cells ratios. Lower B cell numbers with age were also observed by Bartlett [1]. Likewise, the changes in naïve and memory T cells are in agreement with previous studies [16, 28], and coherent with an age-related maturation process of the immune system [32]. Age-sex interactions were observed: boys presented higher counts of naïve cells than girls, but the difference was ameliorated by age; in contrast, the counts of memory cells were higher in girls, and the gap increased with age. As a consequence, the changes in percentages of naïve and memory T cells, which were similar in both boys and girls, were the result of decreased naïve cell counts in boys, and of increased memory cell counts in girls. This difference between boys and girls could suggest a more mature or experienced immune system in the girls. As our adolescents were age-matched, the cause must be related to other parameters of growth rather than age, like pubertal maturation.

For that reason, pubertal maturation was considered for the analysis of immune cell profiles in our study. Similarly to age, the effect of pubertal maturation on WBC counts and percentages showed a distinct sex-related pattern. In boys, pubertal maturation was associated with lower WBC counts, lymphocyte counts and percentages; the decrease in lymphocyte counts was reflected in most subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD3^+CD45RA^+$, $CD3^+CD45RO^+$, $CD8^+CD45RA^+$ and surprisingly, also $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$ cells). This could be explained by the physiological increase in androgen levels, mainly testosterone, since studies in men have reported negative associations between testosterone levels and WBC counts [3, 30]. In girls, pubertal maturation was associated with increases in basophil counts and percentages and higher memory T cell counts. The lack of other significant relationships between pubertal maturation and immune cell counts could be related to the fact that the degree of pubertal maturation of the female sample in this study was high, with 82% of them being in the IV and V Tanner stages (in boys this proportion was lower, 73.7%).

Finally, BMI z-scores were also significantly associated with values of circulating immune cells in the studied population, and again the relationship was sex-specific. Higher BMI was in general associated with higher counts of total WBC and memory T cells (all three subsets in boys and $CD3^+CD45RO^+$ and $CD4^+CD45RO^+$ in girls). In boys, both neutrophils and lymphocytes were elevated, but the increase in lymphocytes was proportionally greater. In this line, in addition to memory cells, higher BMI z-scores in boys were also associated to elevated cell counts in most subsets ($CD3^+$, $CD4^+$, $CD8^+$, NK, $CD19^+$, and $CD8^+CD45RA^+$) and with a lower $CD4^+/CD8^+$ ratio. In girls, BMI was mainly linked to counts of neutrophils and memory cells. Alterations in immune parameters have been associated with both insufficient and excessive body weight [reviewed in 19 and 26]. In adults, positive associations were reported between BMI and circulating WBC, neutrophils, lymphocytes and monocytes [15]. Later, other authors have found similar age-independent, positive associations between WBC counts and BMI in children and adolescents [14, 36], in line with our observations. Furthermore, obesity in children and adolescents has been linked to elevated counts of neutrophils, monocytes, total T cells and helper T cells [37]. Our results, like those by Hsieh [14] and Wu [36], show that this increase is not merely the result of obesity, but it takes place in a linear manner with increasing BMI. It is worth highlighting this correlation, as WBC count has been related to features of the metabolic syndrome in both adult [10, 22] and paediatric populations [5, 14, 36], and a follow-up study in Japanese adults concluded that higher WBC counts were associated with higher risk of developing metabolic syndrome in the future [24]. The explanation for the relationship between

BMI and memory T cells or its potential implications is less straightforward. On the one hand, it could suggest that increasing BMI provides a more favorable environment for immune system development, similarly to age and pubertal maturation. On the other hand, we could instead be facing similar outcomes with different origins, and BMI-related changes could indicate abnormal or excessive activation of the immune system. This hypothesis would be supported by the relationships between circulating WBC and the metabolic syndrome mentioned above. However, the underlying processes linking nutritional status, BMI and immune function in children and adolescents clearly requires further research.

In clinical practice to date, sex and age have been routinely taken into account for the establishment of normative ranges, since they constitute easy-to-obtain information. Other physiological features like pubertal maturation or BMI, however, are not frequently available or assessed when analysing blood variables. In light of ours and other authors' results, we recommend considering these characteristics when setting reference values for or performing blood measurements in paediatric populations.

Finally, two considerations should be made in relation to the present study. On the one hand, the number of subjects suitable for statistical analysis was not balanced in relation to the actual population size of each city. On the other, the sample studied included adolescents from different ethnic origins, and as it was mentioned above, ethnicity can be an influential factor for differences in WBC counts [1, 17, 38]. Despite these caveats, our work contributes to the development of a database of haematological reference values in healthy European adolescents. This is particularly strengthened by the use of standardized protocols and methods across all centres participating in the study.

In conclusion, the present work provides data on normal values for white blood cell counts and percentages from healthy European adolescents, and highlights the importance of taking into account the influence of sex, age, the degree of pubertal maturation and BMI when comparing or using white blood cell counts for clinical and research purposes in paediatric populations.

## Acknowledgements

Authors wished to dedicate this work to the memory of Dr. Javier Romeo, and to acknowledge his personal and professional contribution to our group.

The present study was performed as part of the HELENA study (www.helenastudy.com). Authors gratefully acknowledge the participation of all HELENA Study members (listed in the Appendix), as well as the financial support of the European Community 6th RTD Framework Programme (contract FOOD-CT-2005-007034). F.P.d.H. acknowledges funding from a JAE-Doc contract cofounded by the Spanish National Research Council (CSIC) and the European Social Fund. K.V. acknowledges funding from the Research Foundation Flanders (Brussels, Belgium). L.A.M., F.G., M.G.G., E.N. and A.M. designed the research. S.G.M., L.E.D., A.M.V., J.W., and E.N. performed the sample processing and database building. F.P.d.H. performed the data cleaning and the statistical analysis. F.P.d.H., S.G.M., L.E.D., A.M.V. and E.N. wrote the paper and A.M. edited the manuscript. F.P.d.H. and S.G.M. have primary responsibility for the final content. All authors read and approved the final manuscript.

## Conflict of interest

None of the authors had a personal or financial conflict of interest.

## REFERENCES

1. Bartlett JA, Schleifer SJ, Demetrikopoulos MK, Delaney BR, Shiflett SC, Keller SE (1998) Immune function in healthy adolescents. Clin Diagn Lab Immunol 5:105-113

2. Béghin L, Castera M, Manios Y, Gilbert CC, Kersting M, De Henauw S, Kafatos A, Gottrand F, Molnar D, Sjöström M, Leclercq C, Widhalm K, Mesana MI, Moreno LA, Libersa C, on behalf of the HELENA Study Group (2008) Quality assurance of ethical issues and regulatory aspects relating to good practices in the HELENA Cross-Sectional Study. Int J Obes 32:S12-S18. DOI: 10.1038/ijo.2008.179

3. Brand JS, van der Schouw YT, Dowsett M, Folkerd E, Luben RN, Wareham NJ, Khaw KT (2012) Testosterone, SHBG and differential white blood cell count in middle-aged and older men. Maturitas 71(3):274-278. DOI: 10.1016/j.maturitas.2011.12.007.

4. Bunders M, Cortina-Borja M, Newell ML; European Collaborative Study (2005) Age-related standards for total lymphocyte, CD4+ and CD8+ T cell counts in children born in Europe. Pediatr Infect Dis J 24(7):595-600

5. Capuano V, Bambacaro A, Darminio T, Mazzot G (2001) White blood cell count is related to arterial pressure and cholesterolemia in normal children. Ital Heart J 2(9):685-689

6. Cheng CK, Chan J, Cembrowski GS, van Assendelft OW (2004) Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. Lab Hematol 10(1):42-53

7. Choong ML, Ton SH, Cheong SK (1995) Influence of race, age and sex on the lymphocyte subsets in peripheral blood of healthy Malaysian adults. Ann Clin Biochem 32 (6):532-539

8. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WCJ, Groeneveld K, Hooijkaas H, van Dongen JJM (1997) Immunophenotyping of blood lymphocytes in childhood. J Pediatr 130:388-393. DOI: 10.1016/S0022-3476(97)70200-2

9. Friedman GD, Klatsky AL, Siegelaub AB (1974) The leukocyte count as a predictor of myocardial infarction. N Engl J Med 290:1275-1278. DOI: 10.1056/NEJM197406062902302

10. Ford ES (2003) The metabolic syndrome and C-reactive protein, fibrinogen, and leukocyte count: findings from the Third National Health and Nutrition Examination Survey. Atherosclerosis 168:351-358. DOI: 10.1016/S0021-9150(03)00134-5

11. González-Gross M, Breidenassel C, Gómez-Martínez S, Ferrari M, Béghin L, Spinneker A, Díaz LE, Maiani G, Demailly A, Al-Tahan J, Albers U, Wärnberg J, Stoffel-Wagner B, Jiménez-Pavón D, Libersa C, Pietrzik K, Marcos A, Stehle P; the HELENA Study Group (2008) Sampling and processing of fresh blood samples within a European multicenter nutritional study: evaluation of biomarker stability during transport and storage. Int J Obes 32:S66-S75. DOI: 10.1038/ijo.2008.185

12. Grossman C (1989) Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. J Steroid Biochem 34:241-251

13. Heldrup J, Kalm O, Prellner K (1992) Blood T and B lymphocyte subpopulations in healthy infants and children. Acta Paediatr 81(2):125-132

14. Hsieh CH, Pei D, Kuo SW, Chen CY, Tsai SL, Lai CL, Lee CH, Wu CZ, Hung YJ, Hsieh MC (2007) Correlation between white blood cell count and metabolic syndrome in adolescence. Pediatr Int 49(6):827-832. DOI: 10.1111/j.1442-200X.2007.02450.x

15. Huang ZS, Chien KL, Yang CY, Tsai KS, Wang CH (2001) Peripheral differential leukocyte counts in humans vary with hyperlipidemia, smoking, and body mass index. Lipids 36(3):237-245

16. Huenecke S, Behl M, Fadler C, Zimmermann SY, Bochennek K, Tramsen L, Esser R, Klarmann D, Kamper M, Sattler A, von Laer D, Klingebiel T, Lehrnbecher T, Koehl U (2008) Age-matched lymphocyte subpopulation reference values in childhood and adolescence: application of exponential regression analysis. Eur J Haematol 80:532-539. DOI: 10.1111/j.1600-0609.2008.01052.x

17. Lim EM, Cembrowski G, Cembrowski M, Clarke G (2010) Race-specific WBC and neutrophil count reference intervals. Int J Lab Hematol 32(6):590-597. DOI: 10.1111/j.1751-553X.2010.01223.x

18. Lin SC, Chou CC, Tsai MJ, Wu KH, Huang MT, Wang LH, Chiang BL (1998) Age-related changes in blood lymphocyte subsets of Chinese children. Pediatr Allergy Immunol 9(4):215-220

19. Marcos A, Nova E, Montero A (2003) Changes in the immune system are conditioned by nutrition. Eur J Clin Nutr 57(Suppl 1):S66-S69. DOI: 10.1038/sj.ejcn.1601819

20. Moreno LA, De Henauw S, González-Gross M, Kersting M, Molnár D, Gottrand F, Barrios L, Sjöström M, Manios Y, Gilbert CC, Leclercq C, Widhalm K, Kafatos A and Marcos A, on behalf of the HELENA Study Group (2008) Design and implementation of the Health Lifestyle in Europe by Nutrition in Adolescence Cross-Sectional Study. Int J Obes 32:S4-S11. DOI: 10.1038/ijo.2008.177

21. Moreno LA , González-Gross M, Kersting M, Molnár D, De Henauw S, Béghin L, Sjöström M, Hagströmer M, Manios Y, Gilbert CC, Ortega FB, Dallongeville J, Arcella D, Wärnberg J, Hallberg M, Fredriksson H, Maes L, Widhalm K, Kafatos AG, Marcos A (2008) Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr 11(3):288-299. DOI: 10.1017/S1368980007000535

22. Nagasawa N, Tamakoshi K, Yatsuya H, Hori Y, Ishikawa M, Murata C, Zhang H, Wada K, Otsuka R, Mabuchi T, Kondo T, Toyoshima H (2004) Association of white blood cell count and clustered components of metabolic syndrome in Japanese men. Circ J 68(10):892-897. DOI: 10.1253/circj.68.892

23. Nagy E, Vicente-Rodriguez G, Manios Y, Beghin L, Iliescu C, Censi L, Dietrich S, Ortega Porcel FB, De Vriendt T, Plada M, Moreno LA, Molnar D, the HELENA Study Group (2008) Harmonization process and reliability assessment of anthropometric measurements in a multicenter study in adolescents. Int J Obes 32:S58-S65. DOI: 10.1038/ijo.2008.184

24. Odagiri K, Uehara A, Mizuta I, Yamamoto M, Kurata C (2011) Longitudinal study on white blood cell count and the incidence of metabolic syndrome. Intern Med 50:2491-2498. DOI: 10.2169/internalmedicine.50.5877

25. Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, Hara H, Kohno N (2004) Elevated white blood cell count in subjects with impaired glucose tolerance. Diabetes Care 27(2):491-496. DOI: 10.2337/diacare.27.2.491

26. Pérez de Heredia F, Gómez-Martínez S, Marcos A (2012) Obesity, inflammation and the immune system. Proc Nutr Soc 71:332-338. DOI: 10.1017/S0029665112000092

27. Romeo J, Wärnberg J, Gómez-Martínez S, Díaz LE, Moreno LA, Castillo MJ, Redondo C, Baraza JC, Sola R, Zamora S and Marcos A, on behalf of the AVENA group (2009) Haematological reference values in Spanish adolescents: the AVENA study. Eur J Haematol 83:586-594. DOI: 10.1111/j.1600-0609.2009.01326.x

28. Rudy BJ, Wilson CM, Durako S, Moscicki AB, Muenz L, Douglas SD (2002) Peripheral blood lymphocyte subsets in adolescents: a longitudinal analysis from the REACH project. Clin Diagn Lab Immunol 9(5):959-965. DOI: 10.1128%2FCDLI.9.5.959-965.2002

29. Santagostino A, Gargaccio G, Pistorio A, Bolis V, Camisasca G, Pagliaro P, Girotto M (1999) An Italian national multicenter study for the definition of a reference ranges for normal values of peripheral blood lymphocyte subsets in healthy adults. Haematologica 84:499-504

30. Schooling CM (2013) Androgen activity and markers of inflammation among men in NHANES III. Am J Hum Biol 25(5):622-628. DOI: 10.1002/ajhb.22421.

31. Scrimshaw NS (2007) Immunonutrition in health and disease. Br J Nutr 98 (Suppl 1):S3-S4. DOI: 10.1017/S0007114507833034

32. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, Wara DW, Douglas SD, Luzuriaga K, McFarland EJ, Yogev R, Rathore MH, Levy W, Graham BL, Spector SA; Pediatric AIDS Clinical Trials Group (2003) Lymphocyte subsets in healthy children from birth through 18 years of age: The Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 112:973-980. DOI: 10.1016/j.jaci.2003.07.003

33. Tanner JM, Whitehouse RH (1976) Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170-179

34. Tollerud DJ, Ildstad ST, Brown LM, Clark JW, Blattner WA, Mann DL, Neuland CY, Pankiw-Trost L, Hoover RN (1990) T-cell subsets in healthy teenagers: transition to the adult phenotype. Clin Immunol Immunopathol 56(1):88-96

35. Verthelyi D (2001) Sex hormones as immunomodulators in health and disease. Int Immunopharmacol 1:983-993. DOI: 10.1016/S1567-5769(01)00044-3

36. Wu CZ, Hsiao FC, Lin JD, Su CC, Wang KS, Chu YM, Lee LH, Wang K, Hsia TL, Pei D (2010) Relationship between white blood cell count and components of the metabolic syndrome among young adolescents. Acta Diabetol 47:65-71. DOI: 10.1007/s00592-009-0101-z

37. Zaldivar F, McMurray RG, Nemet D, Galassetti P, Mills PJ, Cooper DM (2006) Body fat and circulating leukocytes in children. Int J Obes (Lond) 30(6):906-911. DOI: 10.1038/sj.ijo.0803227

38. Zeh C, Amornkul PN, Inzaule S, Ondoa P, Oyaro B, Mwaengo DM, Vandenhoudt H, Gichangi A, Williamson J, Thomas T, Decock KM, Hart C, Nkengasong J, Laserson K (2011) Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in Western Kenya. PLoS One 6(6):e21040. doi:10.1371/journal.pone.0021040

**APPENDIX: HELENA Study Group**

Co-ordinator: Luis A. Moreno.

Core Group members: Luis A. Moreno, Fréderic Gottrand, Stefaan De Henauw, Marcela González-Gross, Chantal Gilbert.

Steering Committee: Anthony Kafatos (President), Luis A. Moreno, Christian Libersa, Stefaan De Henauw, Sara Castelló, Fréderic Gottrand, Mathilde Kersting, Michael Sjöstrom, Dénes Molnár, Marcela González-Gross, Jean Dallongeville, Chantal Gilbert, Gunnar Hall, Lea Maes, Luca Scalfi.

Project Manager: Pilar Meléndez.

1. Universidad de Zaragoza (Spain): Luis A. Moreno, Jesús Fleta, José A. Casajús, Gerardo Rodríguez, Concepción Tomás, María I. Mesana, Germán Vicente-Rodríguez, Adoración Villarroya, Carlos M. Gil, Ignacio Ara, Juan Revenga, Carmen Lachen, Juan Fernández Alvira, Gloria Bueno, Aurora Lázaro, Olga Bueno, Juan F. León, Jesús Mª Garagorri, Manuel Bueno, Juan Pablo Rey López, Iris Iglesia, Paula Velasco, Silvia Bel, Luis A. Gracia Marco, Theodora Mouratidou.
2. Consejo Superior de Investigaciones Científicas (Spain): Ascensión Marcos, Esther Nova, Sonia Gómez-Martínez, Ligia Esperanza Díaz, Javier Romeo, Ana Veses, Belén Zapatera, Tamara Pozo, David Martínez.
3. Université de Lille 2 (France): Laurent Beghin, Christian Libersa, Frédéric Gottrand, Catalina Iliescu, Juliana Von Berlepsch.
4. Research Institute of Child Nutrition Dortmund, Rheinische Friedrich-Wilhelms-Universität Bonn (Germany): Mathilde Kersting, Wolfgang Sichert-Hellert, Ellen Koeppen.
5. Pécsi Tudományegyetem (University of Pécs) (Hungary): Dénes Molnar, Eva Erhardt, Katalin Csernus, Katalin Török, Szilvia Bokor, Enikö Nagy, Orsolya Kovács, Judit Répasi.

6. University of Crete School of Medicine (Greece): Anthony Kafatos, Caroline Codrington, María Plada, Angeliki Papadaki, Katerina Sarri, Anna Viskadourou, Christos Hatzis, Michael Kiriakakis, George Tsibinos, Constantine Vardavas, Manolis Sbokos, Eva Protoyeraki, Maria Fasoulaki.
7. Institut für Ernährungs- und Lebensmittelwissenschaften – Ernährungphysiologie, Rheinische Friedrich-Wilhelms-Universität (Germany): Peter Stehle, Klaus Pietrzik, Marcela González-Gross, Christina Breidenassel, Andre Spinneker, Jasmin Al-Tahan, Miriam Segoviano, Anke Berchtold, Christine Bierschbach, Erika Blatzheim, Adelheid Schuch, Petra Pickert.
8. University of Granada (Spain): Manuel J. Castillo, Ángel Gutiérrez, Francisco B. Ortega, Jonatan R. Ruiz, Enrique G. Artero, Vanesa España, David Jiménez-Pavón, Palma Chillón, Cristóbal Sánchez-Muñoz, Magdalena Cuenca.
9. Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (Italy): Davide Arcella, Elena Azzini, Emma Barison, Noemi Bevilacqua, Pasquale Buonocore, Giovina Catasta, Laura Censi, Donatella Ciarapica, Paola D’Acapito, Marika Ferrari, Myriam Galfo, Cinzia Le Donne, Catherine Leclercq, Giuseppe Maiani, Beatrice Mauro, Lorenza Mistura, Antonella Pasquali, Raffaela Piccinelli, Angela Polito, Raffaella Spada, Stefania Sette, Maria Zaccaria, Romana Roccaldo.
10. University of Napoli ‘Federico II’ Department of Food Science (Italy): Luca Scalfi, Paola Vitaglione, Concetta Montagnese.
11. Ghent University (Belgium): Ilse De Bourdeaudhuij, Stefaan De Henauw, Tineke De Vriendt, Lea Maes, Christophe Matthys, Carine Vereecken, Mieke de Maeyer, Charlene Ottevaere, Inge Huybrechts.
12. Medical University of Vienna (Austria): Kurt Widhalm, Katharina Phillipp, Sabine Dietrich, Birgit Kubelka, Marion Boriss-Riedl.

13. Harokopio University (Greece): Yannis Manios, Eva Grammatikaki, Zoi Bouloubasi, Tina Louisa Cook, Sofia Eleutheriou, Orsalia Consta, George Moschonis, Ioanna Katsaroli, George Kraniou, Stalo Papoutsou, Despoina Keke, Ioanna Petraki, Elena Bellou, Sofia Tanagra, Kostalenia Kallianoti, Dionysia Argyropoulou, Katerina Kondaki, Stamatoula Tsikrika, Christos Karaiskos.
14. Institut Pasteur de Lille (France): Jean Dallongeville, Aline Meirhaeghe.
15. Karolinska Institutet (Sweden): Michael Sjöstrom, Jonatan R. Ruiz, Francisco B. Ortega, María Hagströmer, Anita Hurtig Wennlöf, Lena Hallström, Emma Patterson, Lydia Kwak, Julia Wärnberg, Nico Rizzo.
16. Asociación de Investigación de la Industria Agroalimentaria (Spain): Jackie Sánchez-Molero, Sara Castelló, Elena Picó, Maite Navarro, Blanca Viadel, José Enrique Carreres, Gema Merino, Rosa Sanjuán, María Lorente, María José Sánchez.
17. Campden BRI (United Kingdom): Chantal Gilbert, Sarah Thomas, Elaine Allchurch, Peter Burgess.
18. SIK – Institutet foer Livsmedel och Bioteknik (Sweden): Gunnar Hall, Annika Astrom, Anna Sverkén, Agneta Broberg.
19. Meurice Recherche & Development asbl (Belgium): Annick Masson, Claire Lehoux, Pascal Brabant, Philippe Pate, Laurence Fontaine.
20. Campden and Chorleywood Food Development Institute (Hungary): Andras Sebok, Tunde Kuti, Adrienn Hegyi.
21. Productos Aditivos SA (Spain): Cristina Maldonado, Ana Llorente.
22. Cárnicas Serrano SL (Spain): Emilio García.
23. Cederroth International AB (Sweden): Holger von Fircks, Marianne Lilja Hallberg, Maria Messerer.

24. Lantmännen Food R&D (Sweden): Mats Larsson, Helena Fredriksson, Viola Adamsson, Ingmar Börjesson.
25. European Food Information Council (Belgium): Laura Fernández, Laura Smillie, Josephine Wills.
26. Universidad Politécnica de Madrid (Spain): Marcela González-Gross, Jara Valtueña, David Jiménez-Pavón, Ulrike Albers, Raquel Pedrero, Gonzalo Palacios, Agustín Meléndez, Pedro J. Benito, Juan José Gómez Lorente, David Cañada, Alejandro Urzanqui, Juan Carlos Ortiz, Francisco Fuentes, Rosa María Torres, Paloma Navarro.

**Table 1.** White blood cell (WBC) counts (cell/μl) in European adolescents, according to age categories and stratified for sex.

| | | Boys | | | | | Girls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age range (years) | | 12.5-13.9 | 14-14.9 | 15-15.9 | 16-17.5 | | 12.5-13.9 | 14-14.9 | 15-15.9 | 16-17.5 | |
| N | | 48 | 55 | 46 | 49 | R | 56 | 48 | 63 | 40 | R |
| WBC | 10th | 4,106 | 4,382 | 3,787$^{\S\S}$ | 4,630 | 0.107 | **4,493** | **4,368** | **4,934**$^{\S\S}$ | **5,230** | **0.154*** |
| | 25th | 4,854 | 5,011 | 5,214$^{\S\S}$ | 5,055 | | **5,151** | **5,202** | **5,670**$^{\S\S}$ | **5,682** | |
| | 50th | 5,721 | 5,706 | 6,100$^{\S\S}$ | 6,105 | | **6,010** | **6,404** | **6,741**$^{\S\S}$ | **6,520** | |
| | 75th | 6,572 | 6,579 | 6,804$^{\S\S}$ | 6,810 | | **6,929** | **7,823** | **8,011**$^{\S\S}$ | **7,702** | |
| | 90th | 7,605 | 8,000 | 7,568$^{\S\S}$ | 7,490 | | **8,457** | **9,573** | **8,753**$^{\S\S}$ | **8,350** | |
| Neutrophils | 10th | **1,786**$^{\S}$ | **1,906**$^{\S\S}$ | **1,787**$^{\S}$ | **2,197**$^{\S\S}$ | **0.184*** | **2,310**$^{\S}$ | **1,870**$^{\S\S}$ | **2,138**$^{\S}$ | **2,700**$^{\S\S}$ | **0.162*** |
| | 25th | **2,117**$^{\S}$ | **2,328**$^{\S\S}$ | **2,371**$^{\S}$ | **2,576**$^{\S\S}$ | | **2,834**$^{\S}$ | **2,587**$^{\S\S}$ | **2,860**$^{\S}$ | **3,153**$^{\S\S}$ | |
| | 50th | **2,940**$^{\S}$ | **2,681**$^{\S\S}$ | **3,310**$^{\S}$ | **3,190**$^{\S\S}$ | | **3,227**$^{\S}$ | **3,555**$^{\S\S}$ | **3,990**$^{\S}$ | **3,835**$^{\S\S}$ | |
| | 75th | **3,448**$^{\S}$ | **3,641**$^{\S\S}$ | **3,984**$^{\S}$ | **3,635**$^{\S\S}$ | | **3,898**$^{\S}$ | **4,873**$^{\S\S}$ | **4,900**$^{\S}$ | **4,370**$^{\S\S}$ | |
| | 90th | **4,771**$^{\S}$ | **4,458**$^{\S\S}$ | **4,737**$^{\S}$ | **4,700**$^{\S\S}$ | | **5,158**$^{\S}$ | **6,357**$^{\S\S}$ | **5,591**$^{\S}$ | **4,930**$^{\S\S}$ | |
| Lymphocytes | 10th | 1,688 | 1,539 | 1,450 | 1,460 | –0.130 | 1,483 | 1,418 | 1,570 | 1,700 | 0.054 |
| | 25th | 1,895 | 1,757 | 1,700 | 1,918 | | 1,800 | 1,691 | 1,870 | 1,846 | |
| | 50th | 2,200 | 2,241 | 2,040 | 2,120 | | 2,100 | 2,141 | 2,140 | 1,960 | |
| | 75th | 2,411 | 2,561 | 2,334 | 2,283 | | 2,408 | 2,535 | 2,500 | 2,488 | |
| | 90th | 3,106 | 2,887 | 2,768 | 2,960 | | 2,663 | 3,088 | 3,003 | 2,940 | |
| Monocytes | 10th | 295 | 300 | 330 | 300 | 0.074 | 324 | 280 | 350 | 280 | 0.092 |
| | 25th | 360 | 370 | 376 | 399 | | 395 | 340 | 400 | 376 | |
| | 50th | 420 | 461 | 445 | 460 | | 440 | 430 | 500 | 510 | |
| | 75th | 522 | 600 | 543 | 510 | | 514 | 551 | 570 | 578 | |
| | 90th | 700 | 769 | 648 | 620 | | 700 | 743 | 714 | 610 | |
| Eosinophils | 10th | 80 | 80 | 40 | 60 | –0.056 | 57 | 49 | 64 | 60 | -0.074 |
| | 25th | 100 | 100 | 100 | 100 | | 100 | 80 | 100 | 70 | |
| | 50th | 119 | 160 | 140 | 135 | | 137 | 130 | 125 | 110 | |
| | 75th | 200 | 224 | 232 | 191 | | 230 | 200 | 200 | 186 | |
| | 90th | 300 | 400 | 455 | 310 | | 424 | 255 | 334 | 280 | |
| Basophils | 10th | 0 | 0 | 0 | 0 | 0.029 | 0 | 1 | 0 | 0 | 0.090 |
| | 25th | 0 | 10 | 8 | 20 | | 0 | 10 | 10 | 20 | |
| | 50th | 20 | 20 | 20 | 30 | | 20 | 30 | 30 | 20 | |
| | 75th | 40 | 45 | 30 | 31 | | 33 | 40 | 50 | 40 | |
| | 90th | 79 | 92 | 64 | 60 | | 58 | 76 | 100 | 50 | |

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. $^{\S}$Significant differences between boys and girls for a given age category, as assessed by the Mann-Whitney U test, $^{\S}P<0.05$, $^{\S\S}P<0.01$. R is the partial correlation coefficient between cell counts and age, controlling for centre; bold rows indicate significant correlations, $^{*}P<0.05$, $^{**}P<0.01$.

**Table 2.** Estimated cell counts (cell/µl) of selected lymphocyte subsets in adolescents, according to age categories and stratified for sex.

| | | Boys | | | | | Girls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age range (years) | | 12.5-13.9 | 14-14.9 | 15-15.9 | 16-17.5 | | 12.5-13.9 | 14-14.9 | 15-15.9 | 16-17.5 | |
| N | | 36 | 49 | 41 | 45 | R | 39 | 42 | 50 | 26 | R |
| $CD3^+$ | 10th | 1,147 | 1,005 | 952 | 943 | -0.149 | 1,041 | 922 | 1,117 | 1,151 | 0.081 |
| | 25th | 1,239 | 1,208 | 1,139 | 1,145 | | 1,278 | 1,138 | 1,291 | 1,275 | |
| | 50th | 1,477 | 1,524 | 1,355 | 1,464 | | 1,384 | 1,498 | 1,536 | 1,374 | |
| | 75th | 1,758 | 1,737 | 1,695 | 1,598 | | 1,573 | 1,772 | 1,951 | 1,819 | |
| | 90th | 2,330 | 1,974 | 2,002 | 2,127 | | 1,889 | 2,124 | 2,180 | 1,930 | |
| $CD4^+$ | 10th | 580 | 535 | 491 | 487 | -0.139 | 573 | 459 | 616 | 567 | 0.109 |
| | 25th | 652 | 660 | 628 | 581 | | 651 | 612 | 706 | 687 | |
| | 50th | 806 | 824 | 771 | 791 | | 779 | 789 | 842 | 785 | |
| | 75th | 1,055 | 962 | 902 | 972 | | 888 | 950 | 1,054 | 1,095 | |
| | 90th | 1,154 | 1,150 | 1,044 | 1,070 | | 1,057 | 1,181 | 1,292 | 1,212 | |
| $CD8^+$ | 10th | **372** | **349** | **343** | **309** | **-0.154*** | 393 | 333 | 392 | 383 | 0.009 |
| | 25th | **490** | **443** | **441** | **358** | | 442 | 425 | 463 | 472 | |
| | 50th | **541** | **601** | **531** | **539** | | 581 | 558 | 584 | 515 | |
| | 75th | **770** | **724** | **704** | **683** | | 672 | 735 | 725 | 633 | |
| | 90th | **1,016** | **915** | **850** | **888** | | 757 | 970 | 983 | 725 | |
| $CD3^-CD16^+56^+$ | 10th | 164 | 119 | 145 | 165 | -0.030 | 148 | 172 | 168 | 138 | 0.022 |
| | 25th | 248 | 201 | 222 | 217 | | 191 | 242 | 238 | 180 | |
| | 50th | 333 | 298 | 350 | 376 | | 275 | 312 | 312 | 322 | |
| | 75th | 410 | 417 | 447 | 447 | | 434 | 418 | 388 | 400 | |
| | 90th | 754 | 647 | 562 | 542 | | 579 | 531 | 507 | 784 | |
| $CD3^-CD19^+$ | 10th | **160** | **157** | **153** | **131** | **-0.263**** | **181** | **140** | **150** | **99** | **-0.266**** |
| | 25th | **221** | **198** | **195** | **174** | | **231** | **196** | **174** | **127** | |
| | 50th | **286** | **283** | **251** | **219** | | **267** | **244** | **238** | **241** | |
| | 75th | **333** | **377** | **311** | **279** | | **385** | **304** | **299** | **285** | |
| | 90th | **514** | **514** | **439** | **375** | | **501** | **409** | **354** | **321** | |
| $CD3^+CD45RA^+$ | 10th | **637** | **643§** | **485** | **461** | **-0.199*** | 570 | 510§ | 609 | 535 | -0.016 |
| | 25th | **770** | **735§** | **711** | **612** | | 718 | 660§ | 678 | 673 | |
| | 50th | **958** | **1,000§** | **809** | **860** | | 870 | 815§ | 887 | 824 | |
| | 75th | **1,113** | **1,138§** | **1,016** | **1,001** | | 1,024 | 1,015§ | 1,149 | 911 | |
| | 90th | **1,448** | **1,218§** | **1,213** | **1,195** | | 1,236 | 1,386§ | 1,408 | 1,193 | |
| $CD3^+CD45RO^+$ | 10th | 346 | 332§ | 317 | 385§ | -0.016 | **347** | **322§** | **437** | **517§** | **0.237**** |
| | 25th | 451 | 437§ | 392 | 463§ | | **440** | **475§** | **530** | **572§** | |
| | 50th | 526 | 482§ | 564 | 575§ | | **520** | **630§** | **625** | **660§** | |
| | 75th | 735 | 674§ | 705 | 672§ | | **619** | **778§** | **749** | **791§** | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 90th | 867 | 807§ | 847 | 832§ | | **818** | **897§** | **928** | **942§** | |
| CD4$^{+}$CD45RA$^{+}$ | 10th | **306** | **311§** | **248** | **201** | **-0.196*** | 306 | 232§ | 287 | 197 | -0.042 |
| | 25th | **382** | **381§** | **322** | **304** | | 364 | 288§ | 343 | 347 | |
| | 50th | **497** | **524§** | **416** | **418** | | 479 | 422§ | 448 | 451 | |
| | 75th | **626** | **629§** | **482** | **596** | | 570 | 590§ | 643 | 512 | |
| | 90th | **752** | **709§** | **640** | **708** | | 716 | 694§ | 744 | 590 | |
| CD4$^{+}$CD45RO$^{+}$ | 10th | 227 | 194 | 194 | 256§ | 0.006 | **205** | **187** | **279** | **261§** | **0.344**** |
| | 25th | 245 | 251 | 234 | 292§ | | **252** | **269** | **313** | **338§** | |
| | 50th | 313 | 304 | 338 | 337§ | | **291** | **358** | **371** | **389§** | |
| | 75th | 414 | 430 | 442 | 406§ | | **343** | **428** | **483** | **566§** | |
| | 90th | 487 | 556 | 508 | 466§ | | **401** | **522** | **540** | **644§** | |
| CD8$^{+}$CD45RA$^{+}$ | 10th | **268** | **249** | **221** | **206** | **-0.186*** | 200 | 203 | 255 | 230 | 0.021 |
| | 25th | **311** | **298** | **285** | **231** | | 282 | 271 | 299 | 301 | |
| | 50th | **384** | **414** | **362** | **367** | | 380 | 342 | 391 | 334 | |
| | 75th | **489** | **523** | **505** | **446** | | 471 | 450 | 543 | 441 | |
| | 90th | **645** | **663** | **619** | **603** | | 574 | 558 | 678 | 497 | |
| CD8$^{+}$CD45RO$^{+}$ | 10th | 90 | 65 | 79 | 78 | -0.036 | 84 | 85 | 92 | 94 | 0.032 |
| | 25th | 107 | 98 | 102 | 110 | | 118 | 130 | 128 | 139 | |
| | 50th | 164 | 159 | 152 | 155 | | 146 | 182 | 180 | 173 | |
| | 75th | 224 | 225 | 199 | 224 | | 217 | 290 | 231 | 213 | |
| | 90th | 264 | 290 | 249 | 328 | | 313 | 354 | 300 | 240 | |
| CD4$^{+}$/CD8$^{+}$ | 10th | 1.04 | 0.85 | 0.98 | 0.87 | 0.051 | 1.03 | 0.89 | 1.00 | 1.14 | 0.104 |
| | 25th | 1.16 | 1.21 | 1.16 | 1.13 | | 1.22 | 1.11 | 1.19 | 1.24 | |
| | 50th | 1.29 | 1.49 | 1.36 | 1.62 | | 1.44 | 1.46 | 1.55 | 1.56 | |
| | 75th | 1.58 | 1.86 | 1.78 | 1.97 | | 1.64 | 1.64 | 1.78 | 1.87 | |
| | 90th | 2.18 | 2.23 | 2.22 | 2.36 | | 1.98 | 2.02 | 2.11 | 2.86 | |
| CD3$^{+}$/CD19$^{+}$ | 10th | **3.08** | **3.10** | **3.44§** | **3.90** | **0.189*** | **3.04** | **3.62** | **3.99§** | **4.74** | **0.311**** |
| | 25th | **4.32** | **3.75** | **4.02§** | **5.24** | | **3.87** | **4.06** | **5.27§** | **6.10** | |
| | 50th | **5.95** | **5.25** | **5.82§** | **6.11** | | **4.75** | **5.69** | **6.61§** | **6.89** | |
| | 75th | **7.10** | **7.14** | **6.90§** | **7.74** | | **6.68** | **7.14** | **8.31§** | **9.93** | |
| | 90th | **8.71** | **9.24** | **8.58§** | **10.76** | | **7.87** | **11.00** | **12.04§** | **13.79** | |

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Lymphocyte populations are designated by their cell markers. § Significant differences between boys and girls for a given age category, as assessed by the Mann-Whitney U test; §*P*<0.05, §§*P*<0.01. R is the partial correlation coefficient between cell counts and age, controlling for centre; bold rows indicate significant correlations,* *P*<0.05, ** *P*<0.01.

**Table 3.** White blood cell (WBC) counts (cell/µl) in European adolescents, according to Tanner stages and stratified for sex.

| | | Boys | | | | | Girls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tanner stage | | I+II | III | IV | V | | I+II | III | IV | V | |
| N | | 12 | 34 | 77 | 55 | *P* | 7 | 26 | 97 | 60 | *P* |
| WBC | 10th | **4,073** | **4,803** | **4,160** | **4,357** | **0.037** | 3,920 | 4,353 | 4,696 | 4,995 | 0.404 |
| | 25th | **4,751** | **5,469** | **4,940** | **4,946** | | 4,423 | 5,030 | 5,481 | 5,357 | |
| | 50th | **6,050** | **5,965** | **5,706** | **5,938** | | 5,091 | 6,065 | 6,541 | 6,500 | |
| | 75th | **7,271** | **6,769** | **6,581** | **6,811** | | 6,878 | 8,179 | 7,612 | 8,003 | |
| | 90th | **9,129** | **8,273** | **7,454** | **7,300** | | - | 9,581 | 8,693 | 8,400 | |
| Neutrophils | 10th | 1,243 | 2,129 | 1,766 | 2,021 | 0.068 | 1,290 | 1,908 | 2,153 | 2,444 | 0.089 |
| | 25th | 2,158 | 2,833 | 2,169 | 2,447 | | 1,543 | 2,361 | 2,864 | 2,975 | |
| | 50th | 3,095 | 3,206 | 2,891 | 3,210 | | 2,259 | 3,515 | 3,599 | 3,662 | |
| | 75th | 4,057 | 3,636 | 3,504 | 4,000 | | 3,601 | 5,056 | 4,486 | 4,699 | |
| | 90th | 4,794 | 5,097 | 4,197 | 4,710 | | - | 7,111 | 5,335 | 5,476 | |
| Lymphocytes | 10th | **1,803** | **1,650** | **1,537** | **1,298** | **0.002** | 1,550 | 1,385 | 1,566 | 1,500 | 0.981 |
| | 25th | **1,898** | **1,880** | **1,903** | **1,575** | | 1,917 | 1,658 | 1,850 | 1,828 | |
| | 50th | **2,247** | **2,138** | **2,155** | **1,935** | | 2,122 | 2,097 | 2,120 | 2,140 | |
| | 75th | **2,984** | **2,567** | **2,397** | **2,310** | | 2,339 | 2,468 | 2,510 | 2,500 | |
| | 90th | **3,845** | **3,275** | **2,870** | **2,728** | | - | 2,790 | 2,991 | 2,893 | |
| Monocytes | 10th | 350 | 299 | 290 | 316 | 0.825 | 340 | 279 | 331 | 291 | 0.873 |
| | 25th | 367 | 360 | 350 | 382 | | 346 | 362 | 400 | 370 | |
| | 50th | 420 | 408 | 420 | 469 | | 392 | 452 | 494 | 439 | |
| | 75th | 540 | 540 | 521 | 510 | | 702 | 570 | 570 | 526 | |
| | 90th | 661 | 720 | 618 | 632 | | - | 735 | 671 | 627 | |
| Eosinophils | 10th | 85 | 54 | 50 | 69 | 0.922 | 51 | 61 | 60 | 74 | 0.206 |
| | 25th | 111 | 100 | 100 | 100 | | 98 | 94 | 80 | 100 | |
| | 50th | 164 | 150 | 130 | 130 | | 130 | 158 | 110 | 140 | |
| | 75th | 219 | 220 | 223 | 203 | | 165 | 263 | 200 | 200 | |
| | 90th | 428 | 426 | 311 | 358 | | - | 513 | 280 | 354 | |
| Basophils | 10th | 5 | 0 | 4 | 0 | 0.061 | **10** | **1** | **0** | **0** | **0.011** |
| | 25th | 22 | 16 | 10 | 9 | | **15** | **10** | **10** | **20** | |
| | 50th | 38 | 20 | 20 | 20 | | **30** | **29** | **20** | **30** | |
| | 75th | 41 | 40 | 30 | 40 | | **40** | **32** | **40** | **60** | |
| | 90th | 60 | 74 | 46 | 60 | | **-** | **72** | **50** | **100** | |

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Bold rows indicate significant differences between Tanner stages, as assessed by analysis of covariance (ANCOVA), controlling for centre and age, *P*<0.05.

**Table 4.** Estimated cell counts (cell/μl) of selected lymphocyte subsets in European adolescents, according to Tanner stages and stratified for sex.

| | | Boys | | | | | Girls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Tanner stage | | I+II | III | IV | V | | I+II | III | IV | V | |
| N | | 9 | 26 | 67 | 52 | *P* | 7 | 19 | 65 | 55 | *P* |
| CD3$^{+}$ | 10th | **1,157** | **1,039** | **1,066** | **876** | **0.002** | 1,135 | 819 | 1,112 | 1,089 | 0.362 |
| | 25th | **1,259** | **1,401** | **1,237** | **1,007** | | 1,295 | 990 | 1,236 | 1,202 | |
| | 50th | **1,694** | **1,517** | **1,467** | **1,316** | | 1,366 | 1,364 | 1,470 | 1,549 | |
| | 75th | **2,095** | **1,903** | **1,672** | **1,526** | | 1,466 | 1,575 | 1,835 | 1,858 | |
| | 90th | **-** | **2,254** | **1,991** | **1,909** | | - | 1,938 | 2,205 | 2,051 | |
| CD4$^{+}$ | 10th | **584** | **542** | **551** | **479** | **0.010** | 632 | 477 | 585 | 572 | 0.308 |
| | 25th | **644** | **735** | **681** | **575** | | 727 | 593 | 684 | 652 | |
| | 50th | **780** | **894** | **835** | **737** | | 815 | 689 | 827 | 834 | |
| | 75th | **1,084** | **960** | **994** | **882** | | 881 | 862 | 1,080 | 1,015 | |
| | 90th | **1,457** | **1,168** | **1,143** | **1,014** | | - | 1,069 | 1,252 | 1,223 | |
| CD8$^{+}$ | 10th | **492** | **383** | **347** | **279** | **0.012** | 330 | 291 | 382 | 392 | 0.325 |
| | 25th | **510** | **454** | **430** | **358** | | 412 | 388 | 447 | 455 | |
| | 50th | **671** | **602** | **554** | **464** | | 528 | 478 | 559 | 590 | |
| | 75th | **822** | **843** | **692** | **653** | | 606 | 632 | 676 | 733 | |
| | 90th | **1,026** | **1,038** | **780** | **846** | | - | 815 | 883 | 938 | |
| CD3$^{-}$CD16$^{+}$56$^{+}$ | 10th | 247 | 161 | 136 | 149 | 0.067 | 119 | 127 | 168 | 170 | 0.536 |
| | 25th | 276 | 249 | 210 | 210 | | 185 | 183 | 241 | 221 | |
| | 50th | 319 | 357 | 342 | 323 | | 326 | 304 | 339 | 286 | |
| | 75th | 506 | 430 | 446 | 455 | | 496 | 360 | 440 | 375 | |
| | 90th | - | 687 | 540 | 590 | | - | 540 | 554 | 571 | |
| CD3$^{-}$CD19$^{+}$ | 10th | **162** | **182** | **159** | **116** | **0.049** | 144 | 152 | 137 | 128 | 0.078 |
| | 25th | **210** | **209** | **200** | **172** | | 169 | 185 | 217 | 171 | |
| | 50th | **282** | **276** | **256** | **225** | | 342 | 240 | 273 | 232 | |
| | 75th | **337** | **325** | **359** | **287** | | 418 | 293 | 321 | 289 | |
| | 90th | **-** | **586** | **460** | **398** | | - | 466 | 417 | 355 | |
| CD3$^{+}$CD45RA$^{+}$ | 10th | **737** | **649** | **591** | **476** | **0.024** | 745 | 515 | 528 | 533 | 0.698 |
| | 25th | **814** | **754** | **732** | **582** | | 832 | 631 | 666 | 674 | |
| | 50th | **1,005** | **980** | **939** | **777** | | 901 | 727 | 846 | 901 | |
| | 75th | **1,405** | **1,189** | **1,094** | **975** | | 1,016 | 1,039 | 1,056 | 1,076 | |
| | 90th | **-** | **1,294** | **1,193** | **1,129** | | - | 1,329 | 1,427 | 1,297 | |
| CD3$^{+}$CD45RO$^{+}$ | 10th | **354** | **394** | **356** | **297** | **<0.001** | **304** | **284** | **422** | **465** | **0.021** |
| | 25th | **477** | **486** | **459** | **373** | | **357** | **354** | **474** | **528** | |
| | 50th | **549** | **592** | **559** | **467** | | **454** | **479** | **634** | **636** | |
| | 75th | **722** | **775** | **682** | **585** | | **536** | **684** | **784** | **753** | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 90th | **-** | **907** | **777** | **776** | | **-** | **813** | **918** | **942** | |
| $CD4^+CD45RA^+$ | 10th | 318 | 331 | 227 | 238 | 0.102 | 388 | 263 | 245 | 235 | 0.824 |
| | 25th | 408 | 355 | 370 | 296 | | 472 | 332 | 363 | 332 | |
| | 50th | 487 | 449 | 491 | 384 | | 538 | 390 | 475 | 426 | |
| | 75th | 639 | 667 | 629 | 506 | | 565 | 608 | 598 | 580 | |
| | 90th | 783 | 769 | 701 | 646 | | - | 720 | 740 | 729 | |
| $CD4^+CD45RO^+$ | 10th | **213** | **198** | **227** | **191** | **0.005** | **186** | **182** | **241** | **270** | **0.025** |
| | 25th | **235** | **293** | **280** | **233** | | **239** | **203** | **289** | **314** | |
| | 50th | **303** | **400** | **327** | **303** | | **281** | **283** | **363** | **387** | |
| | 75th | **352** | **457** | **419** | **361** | | **306** | **312** | **445** | **493** | |
| | 90th | **851** | **487** | **505** | **485** | | **-** | **398** | **558** | **619** | |
| $CD8^+CD45RA^+$ | 10th | **336** | **282** | **222** | **219** | **0.032** | 193 | 201 | 228 | 257 | 0.473 |
| | 25th | **367** | **316** | **297** | **245** | | 247 | 256 | 301 | 288 | |
| | 50th | **458** | **413** | **383** | **325** | | 407 | 325 | 363 | 412 | |
| | 75th | **622** | **540** | **451** | **446** | | 509 | 440 | 469 | 499 | |
| | 90th | **-** | **684** | **593** | **624** | | - | 560 | 631 | 649 | |
| $CD8^+CD45RO^+$ | 10th | **107** | **88** | **79** | **49** | **0.008** | 61 | 60 | 105 | 94 | 0.069 |
| | 25th | **120** | **117** | **109** | **89** | | 82 | 103 | 128 | 138 | |
| | 50th | **159** | **160** | **164** | **130** | | 109 | 150 | 171 | 186 | |
| | 75th | **211** | **242** | **199** | **197** | | 166 | 236 | 229 | 268 | |
| | 90th | **-** | **366** | **261** | **246** | | - | 320 | 303 | 332 | |
| $CD4^+/CD8^+$ | 10th | 1.09 | 0.98 | 0.99 | 0.92 | 0.478 | 1.04 | 0.90 | 1.16 | 0.94 | 0.349 |
| | 25th | 1.13 | 1.12 | 1.22 | 1.14 | | 1.26 | 1.21 | 1.27 | 1.14 | |
| | 50th | 1.20 | 1.28 | 1.55 | 1.43 | | 1.78 | 1.52 | 1.50 | 1.48 | |
| | 75th | 1.39 | 1.72 | 1.93 | 1.91 | | 2.14 | 1.79 | 1.76 | 1.77 | |
| | 90th | 1.48 | 2.07 | 2.15 | 2.47 | | - | 2.03 | 2.11 | 1.99 | |
| $CD3^+/CD19^+$ | 10th | 3.09 | 3.40 | 3.28 | 3.54 | 0.417 | 2.99 | 3.71 | 3.64 | 3.86 | 0.257 |
| | 25th | 5.34 | 4.81 | 4.37 | 4.13 | | 3.22 | 4.04 | 4.73 | 4.97 | |
| | 50th | 6.38 | 5.38 | 5.79 | 5.79 | | 4.47 | 5.12 | 5.56 | 6.77 | |
| | 75th | 7.28 | 7.28 | 6.97 | 7.68 | | 7.09 | 7.35 | 7.32 | 9.68 | |
| | 90th | - | 8.08 | 8.11 | 10.96 | | - | 10.56 | 10.54 | 11.78 | |

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Lymphocyte populations are designated by their cell markers. Bold rows indicate significant differences between Tanner stages, as assessed by analysis of covariance (ANCOVA), controlling for centre and age, *P*<0.05.

**Table 5.** White blood cell (WBC) counts (cell/µl) in European adolescents, according to BMI z-scores and stratified for sex.

| | | Boys | | | | | | Girls | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI z-scores | | Q1 | Q2 | Q3 | Q4 | | | Q1 | Q2 | Q3 | Q4 | | |
| N | | 43 | 42 | 42 | 43 | *P* | R | 41 | 41 | 38 | 39 | *P* | R |
| WBC | 10th | **4,629** | **4,180** | **4,160** | **5,175** | **0.001** | 0.195** | **4,392** | **4,484** | **5,208** | **5,221** | **0.005** | 0.208** |
| | 25th | **5,223** | **4,700** | **4,909** | **5,775** | | | **5,054** | **5,101** | **5,716** | **5,772** | | |
| | 50th | **6,079** | **5,463** | **5,711** | **6,403** | | | **6,260** | **6,300** | **6,804** | **6,574** | | |
| | 75th | **6,512** | **6,504** | **6,781** | **7,200** | | | **7,161** | **7,751** | **7,938** | **8,321** | | |
| | 90th | **7,709** | **7,150** | **7,487** | **8,825** | | | **8,221** | **8,480** | **9,068** | **9,836** | | |
| Neutrophils | 10th | **2,032** | **1,769** | **1,893** | **2,211** | **0.020** | 0.115 | **1,799** | **2,225** | **2,529** | **2,644** | **0.003** | 0.224** |
| | 25th | **2,381** | **2,275** | **2,111** | **2,800** | | | **2,693** | **2,636** | **3,000** | **3,170** | | |
| | 50th | **3,084** | **2,799** | **2,910** | **3,269** | | | **3,190** | **3,512** | **3,806** | **4,146** | | |
| | 75th | **3,791** | **3,539** | **3,537** | **4,009** | | | **4,200** | **4,384** | **4,902** | **5,070** | | |
| | 90th | **4,658** | **4,387** | **4,308** | **5,042** | | | **4,666** | **5,646** | **6,021** | **6,287** | | |
| Lymphocytes | 10th | **1,373** | **1,456** | **1,551** | **1,879** | **<0.001** | 0.331** | 1,590 | 1,497 | 1,488 | 1,367 | 0.806 | 0.029 |
| | 25th | **1,703** | **1,671** | **1,729** | **2,170** | | | 1,829 | 1,694 | 1,856 | 1,870 | | |
| | 50th | **2,060** | **1,992** | **2,089** | **2,380** | | | 2,093 | 2,035 | 2,195 | 2,122 | | |
| | 75th | **2,378** | **2,190** | **2,331** | **2,817** | | | 2,482 | 2,456 | 2,439 | 2,552 | | |
| | 90th | **2,676** | **2,553** | **2,862** | **3,157** | | | 2,834 | 2,940 | 2,802 | 3,092 | | |
| Monocytes | 10th | 300 | 300 | 319 | 300 | 0.261 | 0.115 | 294 | 284 | 349 | 300 | 0.224 | 0.071 |
| | 25th | 363 | 388 | 370 | 380 | | | 390 | 363 | 413 | 370 | | |
| | 50th | 420 | 420 | 460 | 500 | | | 476 | 432 | 500 | 463 | | |
| | 75th | 511 | 500 | 583 | 580 | | | 559 | 553 | 555 | 600 | | |
| | 90th | 672 | 613 | 660 | 709 | | | 665 | 654 | 707 | 733 | | |
| Eosinophils | 10th | 80 | 90 | 50 | 50 | 0.066 | –0.095 | 70 | 60 | 50 | 50 | 0.742 | 0.034 |
| | 25th | 100 | 100 | 100 | 100 | | | 90 | 95 | 81 | 100 | | |
| | 50th | 139 | 130 | 190 | 120 | | | 120 | 105 | 143 | 100 | | |
| | 75th | 209 | 190 | 277 | 180 | | | 200 | 200 | 220 | 200 | | |
| | 90th | 307 | 320 | 516 | 246 | | | 316 | 272 | 300 | 398 | | |
| Basophils | 10th | 0 | 0 | 0 | 0 | 0.506 | –0.126 | 0 | 0 | 0 | 0 | 0.527 | –0.045 |
| | 25th | 10 | 10 | 10 | 10 | | | 11 | 10 | 10 | 0 | | |
| | 50th | 30 | 20 | 20 | 20 | | | 30 | 21 | 21 | 20 | | |
| | 75th | 50 | 30 | 40 | 30 | | | 47 | 35 | 50 | 40 | | |
| | 90th | 70 | 45 | 60 | 60 | | | 100 | 50 | 80 | 100 | | |

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Bold rows indicate significant differences between quartiles of standardized body mass index values (BMI z-scores), as assessed by analysis of covariance (ANCOVA), controlling for centre, *P*<0.05. R is the partial correlation coefficient between cell counts and BMI z-scores, controlling for centre, *$P$<0.05, **$P$<0.01.

**Table 6.** Estimated cell counts (cell/µl) of lymphocyte subsets in European adolescents, according to BMI z-scores and stratified for sex.

| | | **Boys** | | | | | | **Girls** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI z-scores | | Q1 | Q2 | Q3 | Q4 | | | Q1 | Q2 | Q3 | Q4 | | |
| N | | 43 | 42 | 42 | 43 | *P* | R | 41 | 41 | 38 | 39 | *P* | R |
| CD3$^{+}$ | 10th | **850** | **994** | **958** | **1,244** | **0.014** | **0.238**** | 1,084 | 1,017 | 946 | 1,126 | 0.301 | 0.122 |
| | 25th | **1,083** | **1,088** | **1,162** | **1,411** | | | 1,158 | 1,183 | 1,269 | 1,314 | | |
| | 50th | **1,457** | **1,238** | **1,476** | **1,696** | | | 1,382 | 1,364 | 1,523 | 1,517 | | |
| | 75th | **1,657** | **1,590** | **1,622** | **1,978** | | | 1,578 | 1,756 | 1,741 | 1,881 | | |
| | 90th | **2,038** | **1,740** | **1,979** | **2,269** | | | 1,986 | 2,165 | 1,930 | 2,301 | | |
| CD4$^{+}$ | 10th | 443 | 529 | 551 | 591 | 0.085 | **0.174*** | 579 | 522 | 572 | 621 | 0.265 | **0.164*** |
| | 25th | 632 | 600 | 617 | 749 | | | 620 | 649 | 671 | 723 | | |
| | 50th | 808 | 748 | 788 | 942 | | | 711 | 783 | 871 | 845 | | |
| | 75th | 957 | 858 | 895 | 1,086 | | | 903 | 960 | 1,020 | 1,075 | | |
| | 90th | 1,139 | 1,053 | 982 | 1,178 | | | 1,248 | 1,224 | 1,145 | 1,237 | | |
| CD8$^{+}$ | 10th | **280** | **300** | **346** | **425** | **0.009** | **0.266**** | 385 | 359 | 381 | 373 | 0.634 | 0.067 |
| | 25th | **386** | **380** | **413** | **537** | | | 413 | 455 | 434 | 463 | | |
| | 50th | **507** | **492** | **554** | **659** | | | 526 | 552 | 557 | 578 | | |
| | 75th | **649** | **643** | **684** | **864** | | | 700 | 681 | 711 | 713 | | |
| | 90th | **852** | **758** | **809** | **1,013** | | | 863 | 787 | 782 | 1,046 | | |
| CD3$^{-}$CD16$^{+}$56$^{+}$ | 10th | **149** | **149** | **137** | **192** | **0.042** | **0.192*** | 119 | 150 | 154 | 181 | 0.766 | 0.064 |
| | 25th | **222** | **204** | **198** | **303** | | | 191 | 216 | 199 | 240 | | |
| | 50th | **307** | **323** | **282** | **389** | | | 320 | 275 | 307 | 320 | | |
| | 75th | **374** | **422** | **437** | **558** | | | 464 | 361 | 400 | 403 | | |
| | 90th | **463** | **491** | **579** | **704** | | | 565 | 496 | 531 | 570 | | |
| CD3$^{-}$CD19$^{+}$ | 10th | **131** | **153** | **152** | **186** | **0.013** | **0.221**** | 145 | 105 | 153 | 132 | 0.700 | –0.001 |
| | 25th | **185** | **169** | **202** | **224** | | | 185 | 167 | 202 | 188 | | |
| | 50th | **239** | **219** | **286** | **287** | | | 257 | 239 | 266 | 241 | | |
| | 75th | **291** | **281** | **352** | **433** | | | 316 | 288 | 327 | 310 | | |
| | 90th | **417** | **336** | **482** | **502** | | | 430 | 431 | 397 | 422 | | |
| CD3$^{+}$CD45RA$^{+}$ | 10th | 469 | 505 | 513 | 642 | 0.413 | 0.133 | 577 | 513 | 522 | 556 | 0.962 | 0.036 |
| | 25th | 699 | 649 | 700 | 789 | | | 664 | 633 | 717 | 695 | | |
| | 50th | 928 | 808 | 905 | 1,015 | | | 805 | 880 | 878 | 857 | | |
| | 75th | 1,113 | 976 | 1,040 | 1,215 | | | 1,003 | 1,097 | 1,031 | 1,073 | | |
| | 90th | 1,192 | 1,112 | 1,193 | 1,365 | | | 1,282 | 1,455 | 1,267 | 1,429 | | |
| CD3$^{+}$CD45RO$^{+}$ | 10th | **292** | **356** | **339** | **479** | **0.003** | **0.266**** | **367** | **426** | **393** | **456** | **0.036** | **0.171*** |
| | 25th | **375** | **401** | **441** | **572** | | | **454** | **486** | **456** | **556** | | |
| | 50th | **475** | **499** | **514** | **690** | | | **556** | **569** | **576** | **656** | | |
| | 75th | **636** | **589** | **649** | **780** | | | **696** | **731** | **745** | **775** | | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 90th | **819** | **654** | **874** | **914** | | | **880** | **903** | **861** | **940** | | |
| CD4$^{+}$CD45RA$^{+}$ | 10th | 216 | 263 | 259 | 234 | 0.932 | 0.045 | 223 | 247 | 244 | 266 | 0.917 | 0.034 |
| | 25th | 357 | 314 | 328 | 368 | | | 336 | 330 | 362 | 348 | | |
| | 50th | 483 | 391 | 453 | 475 | | | 428 | 449 | 488 | 424 | | |
| | 75th | 613 | 501 | 543 | 637 | | | 567 | 596 | 577 | 598 | | |
| | 90th | 718 | 705 | 667 | 719 | | | 655 | 828 | 709 | 690 | | |
| CD4$^{+}$CD45RO$^{+}$ | 10th | **191** | **204** | **196** | **278** | **0.002** | **0.261**** | **225** | **225** | **219** | **261** | **0.012** | **0.254**** |
| | 25th | **227** | **256** | **254** | **308** | | | **274** | **286** | **289** | **306** | | |
| | 50th | **291** | **302** | **328** | **408** | | | **313** | **362** | **341** | **398** | | |
| | 75th | **410** | **373** | **399** | **487** | | | **387** | **406** | **438** | **493** | | |
| | 90th | **487** | **436** | **466** | **556** | | | **533** | **485** | **612** | **620** | | |
| CD8$^{+}$CD45RA$^{+}$ | 10th | 220 | 227 | 222 | 291 | 0.131 | **0.191*** | 248 | 199 | 230 | 199 | 0.854 | 0.033 |
| | 25th | 246 | 252 | 287 | 348 | | | 279 | 292 | 298 | 297 | | |
| | 50th | 382 | 343 | 370 | 431 | | | 341 | 373 | 380 | 373 | | |
| | 75th | 471 | 446 | 450 | 609 | | | 474 | 462 | 486 | 472 | | |
| | 90th | 549 | 489 | 638 | 713 | | | 570 | 568 | 635 | 697 | | |
| CD8$^{+}$CD45RO$^{+}$ | 10th | **51** | **74** | **89** | **110** | **0.019** | **0.248**** | 84 | 113 | 88 | 92 | 0.060 | 0.095 |
| | 25th | **85** | **97** | **107** | **150** | | | 127 | 135 | 110 | 155 | | |
| | 50th | **144** | **133** | **150** | **206** | | | 158 | 172 | 155 | 209 | | |
| | 75th | **190** | **196** | **197** | **236** | | | 229 | 233 | 226 | 266 | | |
| | 90th | **242** | **244** | **312** | **300** | | | 290 | 334 | 290 | 336 | | |
| CD4$^{+}$/CD8$^{+}$ | 10th | 0.91 | 1.07 | 1.08 | 0.91 | 0.286 | **–0.161*** | 0.89 | 1.03 | 1.15 | 0.99 | 0.713 | 0.100 |
| | 25th | 1.16 | 1.20 | 1.21 | 1.05 | | | 1.09 | 1.23 | 1.24 | 1.20 | | |
| | 50th | 1.54 | 1.41 | 1.36 | 1.30 | | | 1.43 | 1.43 | 1.55 | 1.51 | | |
| | 75th | 2.03 | 1.91 | 1.70 | 1.71 | | | 1.84 | 1.72 | 1.75 | 1.83 | | |
| | 90th | 2.48 | 2.36 | 2.04 | 2.19 | | | 2.05 | 2.10 | 1.95 | 2.15 | | |
| CD3$^{+}$/CD19$^{+}$ | 10th | 3.58 | 3.74 | 3.12 | 3.44 | 0.250 | –0.057 | 3.74 | 3.71 | 3.71 | 3.70 | 0.336 | 0.094 |
| | 25th | 4.02 | 5.34 | 3.74 | 4.56 | | | 4.03 | 4.60 | 4.75 | 5.44 | | |
| | 50th | 5.89 | 6.07 | 4.73 | 5.46 | | | 5.33 | 6.31 | 5.57 | 6.77 | | |
| | 75th | 7.85 | 7.12 | 6.74 | 7.11 | | | 7.87 | 8.54 | 6.68 | 7.63 | | |
| | 90th | 9.90 | 7.67 | 10.24 | 8.61 | | | 10.90 | 13.48 | 9.29 | 11.50 | | |

Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Lymphocyte populations are designated by their cell membrane markers. Bold rows indicate significant differences between quartiles of standardized body mass index (BMI z-scores), as assessed by analysis of covariance (ANCOVA), controlling for centre, *P*<0.05. R is the partial correlation coefficient between cell counts and BMI z-scores, controlling for centre; **P*<0.05, ***P*<0.01.