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

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

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Abstract

Percentiles 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} 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 immuno-phenotyping 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 14^{th} birthday), 14-14.9 years (from the 14^{th} birthday to the day before the 15^{th} birthday), 15-15.9 years (from the 15^{th} birthday to the day before the 16^{th} birthday), and 16-17.5 years (from the 16^{th} 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 10^{th}, 25^{th}, 50^{th} (median), 75^{th} and 90^{th} 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 10^{th}, 25^{th}, 50^{th} (median), 75^{th} and 90^{th} 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.

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Conflict of interest

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

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APPENDIX: HELENA Study Group

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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§§	4,630	0.107	**4,493**	**4,368**	**4,934§§**	**5,230**	**0.154***
	25th	4,854	5,011	5,214§§	5,055		**5,151**	**5,202**	**5,670§§**	**5,682**	
	50th	5,721	5,706	6,100§§	6,105		**6,010**	**6,404**	**6,741§§**	**6,520**	
	75th	6,572	6,579	6,804§§	6,810		**6,929**	**7,823**	**8,011§§**	**7,702**	
	90th	7,605	8,000	7,568§§	7,490		**8,457**	**9,573**	**8,753§§**	**8,350**	
Neutrophils	10th	**1,786§**	**1,906§§**	**1,787§**	**2,197§§**	**0.184***	**2,310§**	**1,870§§**	**2,138§**	**2,700§§**	**0.162***
	25th	**2,117§**	**2,328§§**	**2,371§**	**2,576§§**		**2,834§**	**2,587§§**	**2,860§**	**3,153§§**	
	50th	**2,940§**	**2,681§§**	**3,310§**	**3,190§§**		**3,227§**	**3,555§§**	**3,990§**	**3,835§§**	
	75th	**3,448§**	**3,641§§**	**3,984§**	**3,635§§**		**3,898§**	**4,873§§**	**4,900§**	**4,370§§**	
	90th	**4,771§**	**4,458§§**	**4,737§**	**4,700§§**		**5,158§**	**6,357§§**	**5,591§**	**4,930§§**	
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. §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.

598 **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
$CD3^+$	10^{th}	1,147	1,005	952	943	-0.149	1,041	922	1,117	1,151	0.081
	25^{th}	1,239	1,208	1,139	1,145		1,278	1,138	1,291	1,275	
	50^{th}	1,477	1,524	1,355	1,464		1,384	1,498	1,536	1,374	
	75^{th}	1,758	1,737	1,695	1,598		1,573	1,772	1,951	1,819	
	90^{th}	2,330	1,974	2,002	2,127		1,889	2,124	2,180	1,930	
$CD4^+$	10^{th}	580	535	491	487	-0.139	573	459	616	567	0.109
	25^{th}	652	660	628	581		651	612	706	687	
	50^{th}	806	824	771	791		779	789	842	785	
	75^{th}	1,055	962	902	972		888	950	1,054	1,095	
	90^{th}	1,154	1,150	1,044	1,070		1,057	1,181	1,292	1,212	
$CD8^+$	10^{th}	**372**	**349**	**343**	**309**	**-0.154***	393	333	392	383	0.009
	25^{th}	**490**	**443**	**441**	**358**		442	425	463	472	
	50^{th}	**541**	**601**	**531**	**539**		581	558	584	515	
	75^{th}	**770**	**724**	**704**	**683**		672	735	725	633	
	90^{th}	**1,016**	**915**	**850**	**888**		757	970	983	725	
$CD3^-CD16^+56^+$	10^{th}	164	119	145	165	-0.030	148	172	168	138	0.022
	25^{th}	248	201	222	217		191	242	238	180	
	50^{th}	333	298	350	376		275	312	312	322	
	75^{th}	410	417	447	447		434	418	388	400	
	90^{th}	754	647	562	542		579	531	507	784	
$CD3^-CD19^+$	10^{th}	**160**	**157**	**153**	**131**	**-0.263****	**181**	**140**	**150**	**99**	**-0.266****
	25^{th}	**221**	**198**	**195**	**174**		**231**	**196**	**174**	**127**	
	50^{th}	**286**	**283**	**251**	**219**		**267**	**244**	**238**	**241**	
	75^{th}	**333**	**377**	**311**	**279**		**385**	**304**	**299**	**285**	
	90^{th}	**514**	**514**	**439**	**375**		**501**	**409**	**354**	**321**	
$CD3^+CD45RA^+$	10^{th}	**637**	**643**[§]	**485**	**461**	**-0.199***	570	510[§]	609	535	-0.016
	25^{th}	**770**	**735**[§]	**711**	**612**		718	660[§]	678	673	
	50^{th}	**958**	**1,000**[§]	**809**	**860**		870	815[§]	887	824	
	75^{th}	**1,113**	**1,138**[§]	**1,016**	**1,001**		1,024	1,015[§]	1,149	911	
	90^{th}	**1,448**	**1,218**[§]	**1,213**	**1,195**		1,236	1,386[§]	1,408	1,193	
$CD3^+CD45RO^+$	10^{th}	346	332[§]	317	385[§]	-0.016	**347**	**322**[§]	**437**	**517**[§]	**0.237****
	25^{th}	451	437[§]	392	463[§]		**440**	**475**[§]	**530**	**572**[§]	
	50^{th}	526	482[§]	564	575[§]		**520**	**630**[§]	**625**	**660**[§]	
	75^{th}	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, $P<0.05$.

606 **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	

607 Data are presented as percentiles 10th, 25th, 50th (median), 75th, and 90th. Lymphocyte populations are designated by their cell markers. Bold rows indicate significant
608 differences between Tanner stages, as assessed by analysis of covariance (ANCOVA), controlling for centre and age, $P<0.05$.
609

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, *$P<0.05$, **$P<0.01$.

614 **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
$CD3^+$	10^{th}	**850**	**994**	**958**	**1,244**	**0.014**	**0.238****	1,084	1,017	946	1,126	0.301	0.122
	25^{th}	**1,083**	**1,088**	**1,162**	**1,411**			1,158	1,183	1,269	1,314		
	50^{th}	**1,457**	**1,238**	**1,476**	**1,696**			1,382	1,364	1,523	1,517		
	75^{th}	**1,657**	**1,590**	**1,622**	**1,978**			1,578	1,756	1,741	1,881		
	90^{th}	**2,038**	**1,740**	**1,979**	**2,269**			1,986	2,165	1,930	2,301		
$CD4^+$	10^{th}	443	529	551	591	0.085	**0.174***	579	522	572	621	0.265	**0.164***
	25^{th}	632	600	617	749			620	649	671	723		
	50^{th}	808	748	788	942			711	783	871	845		
	75^{th}	957	858	895	1,086			903	960	1,020	1,075		
	90^{th}	1,139	1,053	982	1,178			1,248	1,224	1,145	1,237		
$CD8^+$	10^{th}	**280**	**300**	**346**	**425**	**0.009**	**0.266****	385	359	381	373	0.634	0.067
	25^{th}	**386**	**380**	**413**	**537**			413	455	434	463		
	50^{th}	**507**	**492**	**554**	**659**			526	552	557	578		
	75^{th}	**649**	**643**	**684**	**864**			700	681	711	713		
	90^{th}	**852**	**758**	**809**	**1,013**			863	787	782	1,046		
$CD3^-CD16^+56^+$	10^{th}	**149**	**149**	**137**	**192**	**0.042**	**0.192***	119	150	154	181	0.766	0.064
	25^{th}	**222**	**204**	**198**	**303**			191	216	199	240		
	50^{th}	**307**	**323**	**282**	**389**			320	275	307	320		
	75^{th}	**374**	**422**	**437**	**558**			464	361	400	403		
	90^{th}	**463**	**491**	**579**	**704**			565	496	531	570		
$CD3^-CD19^+$	10^{th}	**131**	**153**	**152**	**186**	**0.013**	**0.221****	145	105	153	132	0.700	–0.001
	25^{th}	**185**	**169**	**202**	**224**			185	167	202	188		
	50^{th}	**239**	**219**	**286**	**287**			257	239	266	241		
	75^{th}	**291**	**281**	**352**	**433**			316	288	327	310		
	90^{th}	**417**	**336**	**482**	**502**			430	431	397	422		
$CD3^+CD45RA^+$	10^{th}	469	505	513	642	0.413	0.133	577	513	522	556	0.962	0.036
	25^{th}	699	649	700	789			664	633	717	695		
	50^{th}	928	808	905	1,015			805	880	878	857		
	75^{th}	1,113	976	1,040	1,215			1,003	1,097	1,031	1,073		
	90^{th}	1,192	1,112	1,193	1,365			1,282	1,455	1,267	1,429		
$CD3^+CD45RO^+$	10^{th}	**292**	**356**	**339**	**479**	**0.003**	**0.266****	**367**	**426**	**393**	**456**	**0.036**	**0.171***
	25^{th}	**375**	**401**	**441**	**572**			**454**	**486**	**456**	**556**		
	50^{th}	**475**	**499**	**514**	**690**			**556**	**569**	**576**	**656**		
	75^{th}	**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; *$P<0.05$, **$P<0.01$.