Biochemical Marker Reference Values across Pediatric, Adult, and Geriatric Ages: Establishment of Robust Pediatric and Adult Reference Intervals on the Basis of the Canadian Health Measures Survey

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BACKGROUND: Biological covariates such as age and sex can markedly influence biochemical marker reference values, but no comprehensive study has examined such changes across pediatric, adult, and geriatric ages. The Canadian Health Measures Survey (CHMS) collected comprehensive nationwide health information and blood samples from children and adults in the household population and, in collaboration with the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER), examined biological changes in biochemical markers from pediatric to geriatric age, establishing a comprehensive reference interval database for routine disease biomarkers.

METHODS: The CHMS collected health information, physical measurements, and biosamples (blood and urine) from approximately 12,000 Canadians aged 3–79 years and measured 24 biochemical markers with the Ortho Vitros 5600 FS analyzer or a manual microplate. By use of CLSI C28-A3 guidelines, we determined age- and sex-specific reference intervals, including corresponding 90% CIs, on the basis of specific exclusion criteria.

RESULTS: Biochemical marker reference values exhibited dynamic changes from pediatric to geriatric age. Most biochemical markers required some combination of age and/or sex partitioning. Two or more age partitions were required for all analytes except bicarbonate, which remained constant throughout life. Additional sex partitioning was required for most biomarkers, except bicarbonate, total cholesterol, total protein, urine iodine, and potassium.

CONCLUSIONS: Understanding the fluctuations in biochemical markers over a wide age range provides important insight into biological processes and facilitates clinical application of biochemical markers to monitor manifestation of various disease states. The CHMS-CALIPER collaboration addresses this important evidence gap and allows the establishment of robust pediatric and adult reference intervals.

Evidence-based clinical diagnostics and health care delivery are critically dependent on appropriate selection and monitoring of a wide range of biochemical markers. To ensure appropriate interpretation of test results, accurate reference intervals determined from a healthy population and stratified by key covariates, including age and sex, are essential. However, determining population reference values is a major challenge, requiring selection and recruitment of large numbers of healthy individuals representing relevant demographic groups. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER)5 is a national program aimed at addressing the gaps in pediatric reference intervals (1–3). CALIPER has been establishing a comprehensive database of age- and sex-stratified reference intervals for pediatric disease biomarkers. To date, pediatric reference values for >85 biomarkers have been established (by use of biochemical, immunochemical, and tandem mass spectrometric assays). To expand the CALIPER database, we collaborated with Statistics Canada to develop a robust national database of both pediatric and adult reference intervals. The Canadian Health Measures Survey (CHMS) is a

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Nonstandard abbreviations: CALIPER, Canadian Laboratory Initiative on Pediatric Reference Intervals; CHMS, Canadian Health Measures Survey; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; LDH, lactate dehydrogenase; BMI, body mass index.
Canada-wide initiative to close data gaps by addressing limitations within Canada’s health information system. The CHMS collected 11,999 biosamples representing approximately 96% of Canada’s population (4, 5). Although CALIPER has previously focused on pediatric reference values, analyte concentrations can fluctuate considerably with age, warranting a comprehensive analysis of key biomarkers over a wide age range. Here, we present comprehensive reference values for 24 routine chemistry biomarkers and show the fluctuations in concentration that occur with biological and physiological development over a wide age range. The sampling procedure and methodology were consistent across the study population, enabling direct comparison of results across pediatric and adult age ranges.

**Materials and Methods**

The study protocol was approved by the Institutional Review Board at the Hospital for Sick Children, Toronto, Canada.

**CHMS RECRUITMENT AND SAMPLE ACQUISITION**

From March 2007 through February 2009, Cycle 1 of the CHMS collected information from respondents aged 6–79. From August 2009 through November 2011, Cycle 2 collected data from respondents aged 3–79. Data were collected from >500 females and >500 males in each of the following age groups: 3–5 (cycle 2), 6–11, 12–19, 20–39, 40–59, and 60–79 years (4). Sampling was representative of >96% of the Canadian population and excluded full-time members of the Canadian Forces and people living on reserves or in other aboriginal settlements, in institutions, and in some remote regions (5). Selection of dwellings and eligible participants at each collection location has been described (4). The survey consisted of an initial in-home interview to collect general health information including nutrition, smoking habits, and medical history, followed by a visit to a mobile examination center to obtain biological samples and direct physical measures including height, weight, and blood pressure. Response to the survey was voluntary. Further details on survey design and sampling procedure are available at www.statcan.gc.ca/chms.

**SAMPLE ANALYSIS**

Whole blood was collected by certified phlebotomists into serum separator tubes or fluoride tubes containing glycolytic inhibitor for plasma glucose, then centrifuged to separate serum/plasma (6). All blood samples were centrifuged within 2 h of collection, then immediately aliquoted and stored in the mobile examination center laboratory in either the refrigerator or freezer, depending on the test (6). A midstream urine sample was also collected on site, refrigerated immediately, and frozen within 4 h (6). The Vitros 5600 FS analyzer (Ortho Clinical Diagnostics) was used to measure alanine aminotransferase (ALT), albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), bicarbonate, bilirubin (total), calcium (total), chloride, cholesterol (total), creatinine (serum), creatinine (urine), γ-glutamyltransferase (GGT), glucose, HDL cholesterol, lactate dehydrogenase (LDH), LDL cholesterol, phosphate, potassium, protein (total), sodium, triglycerides, urea, and uric acid. Manual microplate analysis was used to measure urine iodine. For analytical methods and traceability, see Supplemental Table 1, which accompanies the online version of this article at http://www.clinchem.org/content/vol61/issue8.

**STATISTICAL ANALYSIS AND DETERMINATION OF REFERENCE INTERVALS**

Participants were assigned survey weights corresponding to the number of people that each participant represented on the basis of age, sex, and other demographic information, ensuring that the survey data accurately represented the Canadian population. Briefly, selection weights for collection sites were multiplied by the selection weights for dwellings (households) and adjusted for nonresponse. The household weights were then converted to person weights and further adjusted for nonresponse. Further details on the weighting procedure can be found elsewhere (6). Data were collected from all respondents, and we used an a posteriori approach to apply exclusion criteria before calculation of reference intervals. Figs. 1–4 and online Supplemental Figs. 1–24 show scatterplots of data from all respondents before application of exclusion criteria, with each data point representing the mean of ≥11 closely associated results to maintain respondent confidentiality. We also calculated the weighted frequencies and percentage of respondents in each body mass index (BMI) category from 4 different age groups (3–17, 18–39, 40–59, and 60–79 years) before applying exclusion criteria (see online Supplemental Table 2). Participants were excluded from the statistical analysis on the basis of pregnancy, diagnosed serious medical illness or chronic conditions, or use of prescription medication (see online Supplemental Table 3). Statistical analysis was performed with SAS and R software in accordance with CLSI C28-A3 guidelines. First, we removed extreme outliers by visual inspection of scatterplots. Next, age and sex partitions were determined by visual inspection, then statistically verified with the Harris and Boyd method (7). We used Q–Q plots to assess the normality of each partition and transformed skewed data with the Box–Cox method (8). The Tukey test (9) and adjusted Tukey test (10) were used to remove outliers in normally distributed partitions or partitions that remained skewed, respectively. The nonparametric rank method was used to calculate reference intervals. The median of each par-
tition was then calculated, along with the 90% CIs for the upper and lower limits of each reference interval, using weighted data. We also calculated the average portion of weighted observations removed on the basis of exclusion criteria and outlier removal for 3 age groups: pediatric (3–18 years), adult (19–59 years), and geriatric (60–79 years).

**Results**

Figs. 1–4 show distributions of key biochemical markers across pediatric, adult, and geriatric ages. Data for all 24 markers are shown in online Supplemental Figs. 1–24. Age- and sex-specific reference intervals for 24 chemistry analytes were calculated and are provided in Table 1 and online Supplemental Table 4.

Bicarbonate and potassium remained relatively stable throughout the age range, with only 1 broad age partition required for bicarbonate and 2 for potassium (Fig. 1). Urine iodine concentrations also remained relatively consistent; after an initial decrease at 5 years of age, concentrations remained stable throughout the age range. Albumin, total protein, total calcium, total bilirubin, sodium, and chloride all required 4–5 age partitions, although changes in concentration were relatively minor. Total protein required 4 age partitions and no sex partitions, with upper limit concentrations peaking at 20–29 years. Albumin, total calcium, and total bilirubin all required additional sex partitions, with significantly lower concentrations in women during adulthood. Sex partitions were also required for sodium and chloride in the 16- to 49-year and 12- to 29-year partitions, respectively, owing to differences in reference interval width between sexes.

Phosphate, ALKP, LDH, and AST all decreased in concentration across the pediatric age range. Phosphate decreased at 16 years of age in both sexes, and ALKP decreased at the same age in boys; in girls, however, ALKP decreased at an earlier age of 11 years. Both phosphate and ALKP concentrations then remained relatively stable throughout adulthood and geriatric ages (Fig. 2). LDH values were highest in early childhood and required 5 age partitions, with declining concentrations over the pediatric age range, which then remained relatively stable into adulthood. Sex differences were also observed, with slightly lower LDH concentrations in females in the 11- to 15-year and 21- to 39-year partitions. AST declined steadily until 18 years of age, at which time enzyme activities in women remained relatively stable into adult and geriatric ages. Men, however, reached peak enzyme activities at 20–54 years, after which activities dropped and were consistent with women’s values throughout the remainder of the age range.

Creatinine (serum and urine), total cholesterol, LDL cholesterol, triglycerides, uric acid, and urea all had increased concentrations in adulthood vs childhood. As expected, both serum and urine creatinine concentrations increased substantially throughout the pediatric age range. Males had increased urine and serum creatinine concentrations after 30 and 12 years of age, respectively, compared to females (Fig. 3A). Males also had higher concentrations of urea and uric acid between 8–59 and 13–79 years of age, respectively (Fig. 3, B and C). Triglycerides required only 2 age partitions, with higher concentrations after 30 years of age. A sex difference was also observed, as men had higher triglycerides than women from 30 to 79 years of age. LDL required 3 age partitions, with increased enzyme activities for both sexes from 25 to 49 years of age; however, this increase was more pronounced in men compared with women. No sex differences were observed for total cholesterol, but 6 age partitions were required, with concentrations decreasing during adolescence and then increasing progressively at 20–79 years of age. In females, HDL concentration increased with age; however, male HDL concentrations peaked at 6–14 years of age and subsequently decreased to concentrations lower than observed in childhood.

GGT, ALT, and glucose all required 5–6 age partitions, with lower limits that remained relatively unchanged throughout the age range but increasing upper limits in adult and geriatric ages (Fig. 4). The upper limit of ALT peaked for men at 18–49 years; however, female upper limits remained relatively constant throughout the age range. For GGT and glucose, upper limits increased steadily from pediatric to geriatric ages; however, this was observed to a greater extent in males for both analytes.

**Discussion**

The collaboration between CALIPER and Statistics Canada has enabled generation of an expansive reference interval database, representative of the Canadian household population. The reference intervals were established from a representative cohort of the Canadian population, enabling insight into trends that reflect common lifestyle and environmental factors, subclinical disease, the “normal” aging process, and the current health status of Canadians. It is important to note that for most analytes, <5% of observations were removed as outliers. However, a larger percentage were removed once exclusion criteria were applied. The average portion of observations removed was lowest in children and highest in the geriatric population, reflecting the declining health status of Canadians with advancing age. On the basis of a combination of outlier detection and application of all exclusion criteria, 21% of observations in 3- to 18-year-olds, 43% in 19- to 39-year-olds, and 79% in 60- to 79-year-olds were excluded.

The large sample size collected by the CHMS has allowed for calculation of narrow confidence intervals and detection of subtle biochemical changes between
Table 1. Age- and sex-specific reference intervals for 24 biochemical markers.

| Analyte       | Male | Female |
|---------------|------|--------|
|               | Age range, years | Lower limit | Upper limit | Median | Lower 90% CI | Upper 90% CI | Median | Samples, n | Lower 90% CI | Upper 90% CI | Median | Samples, n | Lower 90% CI | Upper 90% CI | Median |
| ALT, U/L      | 3-5  | 15     | 33     | 23     | 390   | 13–16     | 32–34     | 3-5  | 15     | 33     | 23     | 390   | 13–16     | 32–34     |       |
|               | 6-8  | 16     | 37     | 24     | 630   | 14–18     | 36–38     | 6-8  | 16     | 37     | 24     | 630   | 14–18     | 36–38     |       |
|               | 9-11 | 18     | 39     | 26     | 750   | 18–19     | 37–40     | 9-11 | 18     | 39     | 26     | 750   | 18–19     | 37–40     |       |
|               | 12-17 | 17    | 50     | 26     | 630   | 16–18     | 47–53     | 12-17 | 14     | 41     | 24     | 1760  | 14–15     | 39–42     |       |
|               | 18-49 | 18    | 78     | 35     | 1250  | 16–20     | 72–84     | 18-49 | 14     | 41     | 24     | 1760  | 14–15     | 39–42     |       |
|               | 50-79 | 20    | 62     | 34     | 410   | 18–21     | 56–68     | 50-79 | 16     | 44     | 27     | 440   | 14–17     | 42–45     |       |
| Albumin, g/dL | 3-5  | 3.9    | 5.0    | 4.5    | 430   | 3.9–4.0   | 5.0–5.1   | 3-5  | 3.9    | 5.0    | 4.5    | 430   | 3.9–4.0   | 5.0–5.1   |       |
|               | 6-15 | 4.1    | 5.1    | 4.6    | 2320  | 4.1–4.1   | 5.1–5.2   | 6-15 | 4.1    | 5.1    | 4.6    | 2320  | 4.1–4.1   | 5.1–5.2   |       |
|               | 16-29 | 4.6   | 5.3    | 4.8    | 560   | 4.6–4.6   | 5.3–5.3   | 16-29 | 3.9    | 5.0    | 4.4    | 1650  | 3.9–3.9   | 5.0–5.0   |       |
|               | 30-54 | 4.4   | 5.1    | 4.7    | 850   | 4.4–4.4   | 5.1–5.2   | 30-54 | 4.4    | 5.1    | 4.7    | 850   | 4.4–4.4   | 5.1–5.2   |       |
|               | 55-79 | 4.2   | 5.0    | 4.4    | 570   | 4.2–4.2   | 4.9–5.0   | 55-79 | 4.2    | 5.0    | 4.4    | 570   | 4.2–4.2   | 4.9–5.0   |       |
| ALKP, U/L     | 3-5  | 144   | 327    | 214    | 420   | 138–150   | 307–347   | 3-5  | 144   | 327    | 214    | 420   | 138–150   | 307–347   |       |
|               | 6-10 | 153   | 367    | 235    | 1190  | 149–156   | 357–376   | 6-10 | 153   | 367    | 235    | 1190  | 149–156   | 357–376   |       |
|               | 11-15 | 113   | 438    | 247    | 570   | 104–122   | 418–459   | 11-15 | 64    | 359    | 157    | 530   | 57–71     | 346–373   |       |
|               | 16-21 | 56    | 167    | 92     | 400   | 53–58     | 150–184   | 16-21 | 44    | 107    | 67     | 600   | 41–46     | 102–112   |       |
|               | 22-79 | 50    | 116    | 76     | 1510  | 48–52     | 109–122   | 22-79 | 46    | 122    | 72     | 1400  | 44–48     | 116–129   |       |
| AST, U/L      | 3-5  | 28    | 52     | 37     | 420   | 27–29     | 51–54     | 3-5  | 28    | 52     | 37     | 420   | 27–29     | 51–54     |       |
|               | 6-11 | 25    | 47     | 33     | 720   | 25–26     | 45–50     | 6-11 | 23    | 44     | 32     | 720   | 23–24     | 43–46     |       |
|               | 12-17 | 18    | 36     | 27     | 610   | 17–19     | 35–36     | 12-17 | 15    | 34     | 22     | 700   | 15–16     | 32–36     |       |
|               | 18-54 | 18    | 54     | 28     | 1420  | 17–19     | 48–60     | 18-54 | 18    | 34     | 23     | 1190  | 18–18     | 32–36     |       |
|               | 55-79 | 18    | 39     | 27     | 650   | 17–19     | 38–40     | 55-79 | 18    | 39     | 27     | 650   | 17–19     | 38–40     |       |
| Bicarbonate, mEq/L | 6-79 | 19    | 26     | 23     | 2930  | 19–19     | 26–26     | 6-79 | 19    | 26     | 23     | 2930  | 19–19     | 26–26     |       |

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Table 1. Age- and sex-specific reference intervals for 24 biochemical markers. (Continued from page 1052)

| Analyte                      | Male                          | Female                        |
|------------------------------|-------------------------------|-------------------------------|
|                              | Age range, years | Lower limit | Upper limit | Median | Samples, n | Lower 90% CI | Higher 90% CI | Age, years | Lower limit | Upper limit | Median | Samples, n | Lower 90% CI | Higher 90% CI |
| Bilirubin, total, mg/dL      | 3-5                          | 0.1          | 0.5         | 0.1    | 430        | 0.1-0.1      | 0.4-0.6      | 3-5        | 0.1          | 0.5         | 0.1    | 430        | 0.1-0.1      | 0.4-0.6      |
|                              | 6-15                          | 0.1          | 0.9         | 0.2    | 2290       | 0.1-0.1      | 0.9-1.0      | 6-15       | 0.1          | 0.9         | 0.2    | 2290       | 0.1-0.1      | 0.9-1.0      |
|                              | 16-48a                        | 0.2          | 1.1         | 0.4    | 1300       | 0.2-0.2      | 1.0-1.1      | 16-48a     | 0.1          | 0.9         | 0.3    | 1480       | 0.1-0.1      | 0.8-1.1      |
|                              | 49-79a                        | 0.1          | 1.2         | 0.4    | 440        | 0.1-0.1      | 1.1-1.3      | 49-79a     | 0.1          | 1.0         | 0.3    | 510        | 0.1-0.1      | 0.8-1.1      |
| Calcium, total, mg/dL        | 3-5                          | 9.4          | 10.6        | 10.0   | 430        | 9.3-9.5      | 10.5-10.6    | 3-5        | 9.4          | 10.6        | 10.0   | 430        | 9.3-9.5      | 10.5-10.6    |
|                              | 6-15                          | 9.3          | 10.5        | 9.9    | 2310       | 9.3-9.4      | 10.4-10.5    | 6-15       | 9.3          | 10.5        | 9.9    | 2310       | 9.3-9.4      | 10.4-10.5    |
|                              | 16-19                         | 9.2          | 10.4        | 9.8    | 680        | 9.2-9.3      | 10.3-10.5    | 16-19      | 9.2          | 10.4        | 9.8    | 680        | 9.2-9.3      | 10.3-10.5    |
|                              | 20-39a                        | 9.1          | 10.4        | 9.7    | 740        | 9.1-9.2      | 10.3-10.5    | 20-39a     | 9.0          | 10.1        | 9.5    | 830        | 8.9-9.1      | 10.0-10.2    |
|                              | 40-79                         | 9.0          | 10.2        | 9.6    | 1700       | 8.9-9.0      | 10.2-10.3    | 40-79      | 9.0          | 10.2        | 9.6    | 1700       | 8.9-9.0      | 10.2-10.3    |
| Chloride, mmol/L             | 3-5                          | 100          | 107         | 104    | 430        | 100-101      | 106-107      | 3-5        | 100          | 107         | 104    | 430        | 100-101      | 106-107      |
|                              | 6-11                          | 101          | 107         | 104    | 1490       | 100-101      | 107-107      | 6-11       | 101          | 107         | 104    | 1490       | 100-101      | 107-107      |
|                              | 12-29a                        | 101          | 106         | 103    | 1000       | 101-101      | 106-106      | 12-29a     | 100          | 107         | 104    | 930        | 100-101      | 107-107      |
|                              | 30-79                         | 102          | 108         | 104    | 2500       | 102-102      | 108-108      | 30-79      | 102          | 108         | 104    | 2500       | 102-102      | 108-108      |
| Cholesterol, total, mg/dL    | 3-5                          | 120          | 216         | 162    | 420        | 112-124      | 201-228      | 3-5        | 120          | 216         | 162    | 420        | 112-124      | 201-228      |
|                              | 6-15                          | 116          | 205         | 154    | 2230       | 112-116      | 201-205      | 6-15       | 116          | 205         | 154    | 2230       | 112-116      | 201-205      |
|                              | 16-19                         | 100          | 182         | 154    | 590        | 93-112       | 182-185      | 16-19      | 100          | 182         | 154    | 590        | 93-112       | 182-185      |
|                              | 20-29                         | 116          | 228         | 166    | 580        | 112-124      | 220-236      | 20-29      | 116          | 228         | 166    | 580        | 112-124      | 220-236      |
|                              | 30-39                         | 147          | 266         | 185    | 900        | 147-151      | 255-280      | 30-39      | 147          | 266         | 185    | 900        | 147-151      | 255-280      |
|                              | 40-79                         | 139          | 274         | 201    | 1710       | 127-151      | 270-282      | 40-79      | 139          | 274         | 201    | 1710       | 127-151      | 270-282      |

Continued on page 1054
| Analyte               | Male                  | Female               |
|----------------------|-----------------------|----------------------|
|                      | Age range, years      | Lower limit | Upper limit | Median | Samples, n | Lower 90% CI | Upper 90% CI | Median | Samples, n | Lower 90% CI | Upper 90% CI |
| Creatinine (serum), mg/dL | 3-5 0.3 0.5 0.4 410 0.3-0.3 0.5-0.5 | 3-5 0.3 0.5 0.4 410 0.3-0.3 0.5-0.5 |
|                      | 6-7 0.4 0.6 0.5 410 0.4-0.4 0.5-0.6 | 6-7 0.4 0.6 0.5 410 0.4-0.4 0.5-0.6 |
|                      | 8-9 0.4 0.6 0.5 500 0.3-0.4 0.6-0.7 | 8-9 0.4 0.6 0.5 500 0.3-0.4 0.6-0.7 |
|                      | 10-11 0.4 0.7 0.5 570 0.4-0.4 0.7-0.7 | 10-11 0.4 0.7 0.5 570 0.4-0.4 0.7-0.7 |
|                      | 12-15a 0.5 0.9 0.7 440 0.5-0.5 0.9-0.9 | 12-15a 0.5 0.9 0.7 440 0.5-0.5 0.9-0.9 |
|                      | 16-79a 0.7 1.2 0.9 1910 0.7-0.7 1.2-1.2 | 16-79a 0.6 1.0 0.7 1940 0.6-0.6 1.0-1.0 |
| Creatinine (urine), mg/dL | 3-5 14.7 151.6 63.3 470 11.3-18.1 138.0-165.2 | 3-5 14.7 151.6 63.3 470 11.3-18.1 138.0-165.2 |
|                      | 6-11 13.6 195.7 86.0 1640 10.2-15.8 158.5-205.9 | 6-11 13.6 195.7 86.0 1640 10.2-15.8 158.5-205.9 |
|                      | 12-13 21.5 214.9 124.4 450 15.8-27.1 205.9-224.0 | 12-13 21.5 214.9 124.4 450 15.8-27.1 205.9-224.0 |
|                      | 14-29 19.2 305.4 147.1 1680 12.4-26.0 291.9-319.0 | 14-29 19.2 305.4 147.1 1680 12.4-26.0 291.9-319.0 |
|                      | 30-79a 14.7 294.1 134.6 1300 9.0-21.5 286.2-302.0 | 30-79a 12.4 229.6 79.2 1430 11.3-13.6 219.5-239.8 |
| GGT, U/L             | 3-5 14.7 151.6 63.3 470 11.3-18.1 138.0-165.2 | 3-5 14.7 151.6 63.3 470 11.3-18.1 138.0-165.2 |
|                      | 6-14a 10 20 14 390 11-11 18-21 | 6-14a 10 20 14 390 11-11 18-21 |
|                      | 15-19a 10 33 17 450 9-11 31-36 | 15-19a 10 33 17 450 9-11 31-36 |
|                      | 20-35a 12 62 23 540 12-13 60-65 | 20-35a 12 62 23 540 12-13 60-65 |
|                      | 36-79a 13 109 29 1050 13-14 97-122 36-79a 10 54 18 1090 10-11 48-61 | 36-79a 13 109 29 1050 13-14 97-122 36-79a 10 54 18 1090 10-11 48-61 |
| Glucose, mg/dL       | 3-5 75 111 84 360 75-75 104-120 | 3-5 75 111 84 360 75-75 104-120 |
|                      | 6-11 73 91 82 1300 73-73 91-93 | 6-11 73 91 82 1300 73-73 91-93 |
|                      | 12-19 75 93 84 1290 75-75 93-93 | 12-19 75 93 84 1290 75-75 93-93 |
|                      | 20-54a 78 106 87 1180 78-78 102-109 20-39a 73 91 82 710 73-73 91-93 | 20-54a 78 106 87 1180 78-78 102-109 20-39a 73 91 82 710 73-73 91-93 |
|                      | 55-79a 76 115 93 300 73-80 111-120 40-79a 80 107 87 780 80-80 104-109 | 55-79a 76 115 93 300 73-80 111-120 40-79a 80 107 87 780 80-80 104-109 |
| Analyte                  | Male                                      | Female                                   |
|-------------------------|-------------------------------------------|------------------------------------------|
|                         | Age range, years | Lower limit | Upper limit | Median | Samples, n | Lower 90% CI | Higher 90% CI |
| HDL cholesterol, mg/dL  | 3-5            | 31          | 73          | 54     | 430        | 27-39        | 73-77        |
|                         | 6-14           | 35          | 81          | 54     | 2110       | 35-35        | 77-81        |
|                         | 15-79a         | 31          | 70          | 46     | 2030       | 27-31        | 70-73        |
| Iodine, μg/dL           | 3-5            | 5           | 83          | 26     | 450        | 4-6          | 49-118       |
|                         | 6-79           | 1           | 49          | 13     | 6320       | 1-1          | 47-51        |
| LDH, U/L                | 6-10           | 405         | 646         | 515    | 530        | 387-422      | 633-660      |
|                         | 11-15a         | 319         | 612         | 455    | 240        | 309-329      | 578-646      |
|                         | 16-20          | 259         | 472         | 353    | 320        | 243-275      | 452-493      |
|                         | 21-39a         | 267         | 477         | 378    | 300        | 246-288      | 461-493      |
|                         | 40-79          | 271         | 497         | 375    | 740        | 269-272      | 483-510      |
| LDL cholesterol, mg/dL  | 6-24           | 46          | 143         | 89     | 1590       | 43-50        | 140-147      |
|                         | 25-49a         | 62          | 189         | 124    | 480        | 54-70        | 182-193      |
|                         | 50-79          | 73          | 189         | 127    | 450        | 70-77        | 182-197      |
| Phosphate, mg/dL        | 3-5            | 4.4         | 6.0         | 5.2    | 420        | 4.2-4.5      | 5.9-6.1      |
|                         | 6-10           | 4.4         | 5.7         | 5.0    | 1180       | 4.3-4.4      | 5.6-5.9      |
|                         | 11-15a         | 3.8         | 5.9         | 4.9    | 580        | 3.8-3.9      | 5.8-6.0      |
|                         | 16-47          | 2.9         | 4.7         | 3.8    | 2890       | 2.9-3.0      | 4.6-4.7      |
|                         | 48-79a         | 2.8         | 4.7         | 3.6    | 490        | 2.7-2.9      | 4.7-4.9      |
| Potassium, mmol/L       | 3-5            | 3.9         | 4.6         | 4.3    | 350        | 3.9-4.0      | 4.5-4.6      |
|                         | 6-79           | 3.8         | 4.9         | 4.3    | 6280       | 3.8-3.8      | 4.9-5.0      |

*Continued on page 1056*
| Analyte                  | Male | Female |
|-------------------------|------|--------|
| Age, years              |      |        |
| Lower limit             | 3.5  | 2.0    |
| Upper limit             | 5.9  | 4.9    |
| Median                  | 4.4  | 3.2    |
| Samples, n              | 430  | 430    |
| Lower 90% CI            | 6.2  | 6.2    |
| Higher 90% CI           | 7.9  | 7.9    |
| Age, years              | 6–19 | 6–19   |
| Lower limit             | 1.8  | 1.8    |
| Upper limit             | 5.1  | 5.1    |
| Median                  | 3.3  | 3.3    |
| Samples, n              | 2840 | 2840   |
| Lower 90% CI            | 6.8  | 6.8    |
| Higher 90% CI           | 7.4  | 7.4    |
| Age, years              | 30–79| 30–79  |
| Lower limit             | 1.8  | 1.8    |
| Upper limit             | 5.1  | 5.1    |
| Median                  | 3.3  | 3.3    |
| Samples, n              | 2470 | 2470   |
| Lower 90% CI            | 6.8  | 6.8    |
| Higher 90% CI           | 7.4  | 7.4    |
| Protein, total, g/dL    |      |        |
| 3–5                     | 6.3  | 6.3    |
| 5–9                     | 6.8  | 6.8    |
| 10–14                   | 7.3  | 7.3    |
| 15–19                   | 7.8  | 7.8    |
| Analysed, year of age   |      |        |
| Female                  |      |        |
| 90% CI Higher limit     | Lower | Median |
| 7.7 11.7               | Lower | Upper  |
| Male                    |      |        |
| 90% CI Higher limit     | Lower | Median |
| 7.7 11.7               | Lower | Upper  |
sexes and throughout life. For instance, inspection of the scatterplots for the electrolytes, chloride, potassium, and sodium, showed narrow intervals with minimal changes, supporting the concept that electrolytes have feedback mechanisms that remain stable throughout life. However, calculation of reference intervals revealed that several age and sex partitions were required to reflect minor fluctuations in electrolyte concentrations, i.e., chloride and sodium each required 4 age partitions, whereas potassium required 2. Although our data agree with the range of reference values that are typical for healthy individuals, the robust dataset demonstrates an even more complex pattern than previously observed (11–13). Another large study of >7000 Chinese adults aged 20–79 years showed a similar electrolyte profile, in which at least 6 age partitions were required for electrolytes (14), further supporting the requirement of a large sample population to fully capture such minor fluctua-

![Fig. 1. Scatterplot distributions for bicarbonate (A) and potassium (B) over the 3- to 79-year age range.](image)

To maintain participant confidentiality (on the basis of Statistics Canada policy), each data point represents the mean of ≥11 closely associated data points. Black horizontal lines depict lower and upper reference limits for both sexes. Blue and pink horizontal lines depict male-specific and female-specific lower and upper reference limits, respectively.
tions. The slight increases in electrolyte concentrations observed with advancing age likely reflect different stages of kidney function and regulation during aging. This is particularly evident for potassium, for which the rise in the upper limit from 4.6 nmol/L in the 3- to 5-year group to 4.9 mmol/L in the 6- to 79-year group is probably attributable to changes in kidney excretion and hormones that regulate its clearance (Fig. 1B). Interestingly, sex differences were observed in the 12- to 29-year group for chloride and the 16- to 49-year group for sodium. It is unclear why these sex differences exist, but differences in dietary habits and/or hormonal changes are possible reasons. In contrast, bicarbonate required only 1 interval spanning the entire age range, demonstrating its tight regulation during all stages of development, maturation, and aging.

Most of the analytes related to bone metabolism demonstrated the expected patterns, reflecting typical

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**Fig. 2.** Scatterplot distributions for phosphate (A) and ALKP (B) over the 3- to 79-year age range.

To maintain participant confidentiality (on the basis of Statistics Canada policy), each data point represents the mean of ≥11 closely associated data points. Black horizontal lines depict lower and upper reference limits for both sexes. Blue and pink horizontal lines depict male-specific and female-specific lower and upper reference limits, respectively.
Bone development and turnover during aging. Total calcium decreased over time, with the lowest concentrations observed in the 40- to 79-year partition in males; however, for females, the lowest values were observed in the 20- to 39-year partition. Phosphate showed similar trends: the highest values in childhood and adolescence correlated with growth spurts and bone development (Fig. 2A). Declining phosphate concentrations likely reflect both the cessation of bone growth after puberty and various stages of bone mineralization, as exemplified by the low values observed from 48 to 79 years of age. High total calcium and phosphate in childhood have previously been observed in the CALIPER study (1), and the downward trends with age are corroborated by other studies (13, 15). Interestingly, the lower calcium and phosphate values observed for females have also been reported by some groups (11) but not others (14). Also as expected, fluctuations in ALKP (Fig. 2B) closely mirrored bone growth. Enzyme activity levels initially increased slightly during childhood, with the highest levels during the main growth period. After cessation of growth following puberty, ALKP subsequently declined, paralleling the decreases observed for calcium and phosphate. These trends have also been reported by others (16). A total of 5 age partitions were required for ALKP, 4 of which occurred between 3 and 22 years of age, reflecting the period of most profound bone changes. Sex differences were also observed for all partitions between 11 and 79 years, which is suggestive of unique sex-specific bone changes that occur throughout most of life.

Albumin and total protein followed a similar trend, with rising concentrations during the pediatric range that peaked in adolescence or early adulthood, then declined later in life. Sex differences were observed for albumin between 16 and 54 years of age, with lower concentrations in females. Decreased albumin in adolescent females has previously been noted in the CALIPER study (1) and could be attributed to net losses that occur from menstruation (17). Indeed, this sex difference disappeared postmenopause. The slight increase in albumin throughout the pediatric age range, which peaked in males from 16 to 29 years and in females from 6 to 15 years, may reflect growth and maturation of the liver. This trend was also observed in CALIPER (1), and a
similar pattern was noted for other indicators of liver synthetic function such as apolipoproteins B and AI in our companion manuscript (18). The upper limits of the liver function markers ALT and GGT increased with age (Fig. 4), whereas AST generally decreased. These trends are consistent with previous CALIPER reports for the pediatric population (1). The rise in ALT and GGT in the adult and geriatric cohorts may reflect the increased development of fatty liver and the metabolic syndrome, which generally occur later in life. These trends were mirrored by the increasing incidence of obesity with age in the CHMS respondents (see online Supplemental Table 2). For GGT in particular, the lower limit and median remained relatively stable in comparison to the significant increases in the upper limit concentrations, especially for males, further indicating that the rise in the upper-limit concentrations represents only a subset of the population. Whereas GGT is specific for the liver, ALT

Fig. 4. Scatterplot distributions for ALT (A) and GGT (B) over the 3- to 79-year age range.
To maintain participant confidentiality (on the basis of Statistics Canada policy), each data point represents the mean of ≥11 closely associated data points. Black horizontal lines depict lower and upper reference limits for both sexes. Blue and pink horizontal lines depict male-specific and female-specific lower and upper reference limits, respectively.
also reflects inflammation. Thus, increased ALT may also indicate subacute inflammation with advancing age. AST is expressed in other tissues such as the heart, red blood cells, and kidney, and this differential expression pattern may explain why AST activities were higher in childhood and declined into adulthood, as this trend may parallel growth and development of organ systems other than the liver at an early age. Sex differences appeared around adolescence for all liver markers and persisted over the subsequent age partitions. Females generally showed lower values than males, in agreement with previous reports for GGT and AST (11, 19) but not for ALT (20). GGT showed the largest sex differences in adulthood, with women’s enzyme activities less than half of those observed for men in the 36- to 79-year group. LDH was highest in early childhood, possibly reflecting its release from cells during rapid cell turnover with growth and development; however, the reason for multiple sex-specific partitions at specific age groups remains unclear and requires further investigation.

Not surprisingly, total bilirubin increased with age, with the highest concentrations occurring during the transition period from childhood to adulthood. Females had the lowest concentrations in the 16- to 79-year partition, likely because of the loss of menstrual blood in premenopausal women and the influence of estrogens on increased expression of UDP-glucuronyl transferase (21, 22).

As expected, concentrations of the renal markers serum creatinine, urea, and uric acid all increased with age. The amount of creatinine produced daily from breakdown of creatine in muscles correlates with increased muscle mass. Similarly, urea and uric acid are related to protein degradation and would also be expected to increase with growth and development. This was particularly evident in childhood and adolescence, in which 5 age partitions were required for creatinine, 3 for urea, and 4 for uric acid (Fig. 3), consistent with previous CALIPER trends (1). The upper limit of uric acid for males and females >13 years old was consistent with established cutoffs for hyperuricemia (23). Unique to our study is the analysis of urine creatinine on a spot sample, which probably reflects the lower availability of serum creatinine for the kidneys to filter at an early age.

The lipid markers, total, LDL, and HDL cholesterol and triglycerides, demonstrated some noteworthy trends. Although cutoff values for these markers determined from cardiovascular outcomes studies are most clinically useful, observations from our reference intervals provide important insight into the health status of Canadians. Overall, the rising total cholesterol and LDL concentrations from early childhood to adulthood may indicate the growing demand for hormone synthesis associated with growth and development. However, given the growing burden of obesity, metabolic disease, and insulin resistance in Canada, the increasing concentrations later in life may further reflect modifiable cardiovascular risk factors such as diet and sedentary lifestyle choices within the population. In contrast to the present study, previous studies have noted higher LDL concentrations in females, particularly postmenopause, when the protective effect of estrogens is lost (24, 25). On the other hand, HDL declined with age in males but actually increased in females. These results support the role of estrogen in modulating HDL, a mechanism thought to explain, in part, the protection against cardiovascular disease in premenopausal women (26).

For glucose, reference intervals were similar to expected values in random samples from healthy individuals. The 3 age partitions within childhood highlight the regulation of glucose metabolism at an early age. However, the rising glucose concentrations later in life may provide insight into underlying insulin resistance that occurs with age (27). Indeed, this trend has been previously reported and is suggested to occur as a result of higher total percent adipose mass and visceral fat accumulation, which could contribute to insulin-signaling imbalances between liver and adipose cells (28). Interestingly, the glucose scatterplot (see online Supplemental Fig. 13) showed that concentrations increased substantially after 40 and 60 years of age for a subset of the population. Additionally, BMI frequencies showed that approximately 70% of respondents aged 40–79 were classified as overweight or obese before exclusion, whereas only approximately 25% and 50% of respondents 3–17 and 18–39 years, respectively, were overweight or obese (see online Supplemental Table 2). Hemoglobin A1c, an important marker of diabetes/prediabetes [examined in our companion manuscript (18)], mirrored the trend observed here for glucose, further indicating that the number of individuals with diabetes or components of the metabolic syndrome increased with age. Sex differences were also evident within specific age partitions in the adult population, with lower concentrations in females, likely reflecting differences in energy demands (28) and pancreatic beta-cell function (29).

The final analyte studied was urinary iodine. As expected, concentrations were highest in early childhood, coinciding with development of the thyroid gland. According to the WHO, the following guidelines are suggested for urinary iodine: moderate deficiency occurring between 0.16 and 0.38 μmol/L, mild deficiency between 0.39 and 0.78 μmol/L, adequate intake between 0.79 and 1.57 μmol/L, more than adequate intake between 1.58 and 2.36 μmol/L, and excessive intake at ≥2.37 μmol/L (30). Here, upper reference limits fell within the category of excessive intake, whereas lower limits of 0.39 μmol/L (3–5 years) and 0.09 μmol/L (6–79 years) were within the mild- and moderate-deficiency categories, respectively. However, a limitation of this study is that urinary iodine concentrations were determined by random spot sample, without normalization to creatinine.
In summary, the nationally representative data, extensive sampling procedure, and rigorous statistical analysis of the CHMS data have resulted in reference values that are representative of the Canadian population from pediatric to geriatric ages. Although decision limits may be more useful than reference values for select analytes in interpretation of test results in a clinical setting, the trends observed here have important implications when using these biochemical markers for disease monitoring and will be useful in a broad public health research context, as well as to help initiate future studies on harmonization of reference intervals.

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