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Comparative Analysis of the Effect of Sex and Age on the Hematological and Biochemical Profile of BALB/c and C57BL/6 Inbred Mice

Suresh Patel, DVM, MVSc,[1,*] Satish Patel, DVM, PhD,[1] Ashvin Kotadiya, DVM, MVSc,[1] Samir Patel, MSc,[1] Bhavesh Shrimali, MSc,[1] Mihir Tank, DVM, MVSc,[1] Tushar Patel, MSc,[2] Harshida Trivedi, MSc,[2] Samadhan Kshirsagar, Mpharm,[2] and Mukul Jain, MPharm, PhD[2]

Mice are the most commonly used models of infectious disease, and disease in mice is similar to that of humans. As a consequence, standard hematology and biochemistry reference values in mice are essential to evaluate functional changes caused by experimental treatments, although very few data in the literature provide a comparative reference range. The aim of this investigation was to establish the reference intervals for major hematology and biochemistry analytes in 2 inbred mouse strains, BALB/c and C57BL/6, at 3 different age ranges. Parameters were assessed in 600 mice (300 male and 300 female) of BALB/c and C57BL/6 strains at 6 to 8 wk, 10 to 14 wk, and 6 to 9 mo of age. Reference intervals were calculated by nonparametric or robust methods according to sample size, and statistical analyses were performed to assess the changes in relation to sex, age, and strain. The data demonstrate that strain, sex, and age have significant effects on the hematologic and biochemical profiles of mice. Hemoglobin, Hct, MCH, MCHC, neutrophils, eosinophils, and ALP were found to be significantly greater in BALB/c mice. In contrast, WBC, lymphocytes, basophils, glucose, total protein, albumin, and urea were found to be significantly greater in C57BL/6 mice in all age ranges. Lymphocytes and ALP progressively decreased with age, while neutrophils increased with age in both strains. The study successfully defined and established reference intervals for hematologic and biochemical analytes in 2 inbred mouse strains at 3 different age ranges. The reference values reported here could be useful in characterizing the phenotype of experimental mice and assessing the changes caused by investigational treatments.

Abbreviations and Acronyms: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care International; CCSEA, The committee for the control and supervision of experiments on animals; EDTA, Dipotassium ethylenediaminetetraacetic acid; IAEC, Institutional Animal Ethics Committee; IVCs, Individually Ventilated Cage System; NABL, National Accreditation Board for Testing and Calibration Laboratories; USP, United States Pharmacopeia

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Introduction

Mice (*Mus musculus*) have been very useful as preclinical models for drug discovery due to similarities of metabolic and biochemical pathways with those of humans. Indeed, the laboratory mouse is the most widely used animal model for studying the pathogenesis and treatment of human diseases.[4] Most of these models are used for pharmacology, oncology, toxicology, drug efficacy, drug safety, and genetic research.[15] Largely, this is because the mice are easy to breed; have a short life; have a low cost of husbandry; have a genotype and phenotype that can be manipulated by biologic, chemical, or genetic means; and have a physiologic similarity to humans and other higher species of interest in veterinary care.[21]

Inbred mouse strains are generated through at least 20 generations of brother and sister mating. The uniform genetic background of inbred mice improves standardization and helps researchers worldwide to compare their results with sufficient reproducibility, thereby minimizing the repetition of experiments and the need for fewer individuals per experimental group.[9,16] Varieties of mouse strains are used for specific purposes, but BALB/c and C57BL/6 are the most commonly used inbred mouse strains, and they are available worldwide from quality vendors.

The hematologic and biochemical profiles of mice used in biomedical research are related to the lineage, genotype, and sex and are influenced by age, diet, environment, sample (blood, urine, plasma, or serum) collection techniques, and many other related factors.[2,7,22] Reference intervals for BALB/c and C57BL/6 mice have been reported by researchers from different geographic regeions.[2,6,14,17,19,20,24] However, a universal range cannot be defined due to variation in many of the factors mentioned above. On the other hand, the scientific interpretation of hematologic and biochemical profile of experimental mice is crucial for the evaluation of experimental treatment effects.[1,18,23] The aim of this study was to estimate the reference intervals for hematologic and biochemical analytes of 2 inbred mouse strains (BALB/c and C57BL/6) at different age ranges: 6 to 8 wk, 10 to 14 wk, and 6 to 9 mo. In this study, age-wise reference intervals were established for each mouse strain, which would serve as a robust reference data set for the interpretation of hematologic profiles in studies involving these preclinical mice models.

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[1]Animal Research Facility, Zydus Research Centre, Zydus Lifesciences Limited, Ahmedabad, India; and [2]Department of Pharmacology and Toxicology, Zydus Research Centre, Zydus Lifesciences Limited, Ahmedabad, India
**Corresponding author. Email: suresh.g.patel@zyduslife.com*

Materials and Methods

Animals and diets. Specific pathogen-free BALB/c and C57BL/6 mice used for this study were bred and maintained at the Animal Research Facility of Zydus Research Centre, which is registered with the Committee for Control and Supervision of Experiments on Animals, India and also accredited by AAALAC, International. Breeders (BALB/cJ and C57BL/6J) from the Jackson Laboratory were used to establish inbred mouse colonies at Zydus Research Centre, and mice were bred using a sibling by sibling breeding strategy. The data for hematology and biochemistry analytes were collected over a 10-y period (2014 to 2024) from breeding colony stock as a part of a routine health monitoring program. The animals were used from a single production site, which minimized the variation due to differences in breeding and environmental parameters. A total of 1,200 mice (600 mice/strain) in the age range of 6 to 8 wk, 10 to 14 wk, and 6 to 9 mo were included in this survey. All mice were reared in individually ventilated cages (IVCs; 5 to 7 mice/cage; cage dimensions: 32.5 × 16.5 × 14 cm) filled with sterile corncob bedding, and each cage was changed once each week. The mice were housed at controlled room temperature (23 ± 2 °C) and relative humidity (50 ± 15%) with a 12/12-h light/dark cycle. Each IVC cage had a ventilation rate set at 40 to 50 air changes per hour, and the animal room had a ventilation rate set at 10 to 15 air changes per hour. The animals had free access to a standard chow diet (2018 Teklad global 18% protein rodent diets; Inotiv, Madison, WI) and reverse osmosis-treated water. Enrichment items, such as a mouse tunnel, hut, and igloo (Bio-Serv, Inc., Flemington, NJ), were provided in the cage for the well-being of the animals. All health monitoring studies were performed in compliance with the standard operating procedures of the facility and were approved by the Institutional Animal Ethics Committee. In addition, animals were weighed weekly of to 12 wk of age to establish growth curves for both mouse strains (84 mice/strain: 42 male and 42 female).

Specimen collection. Sixty mice were selected randomly from 3 different age ranges per strain in each health monitoring study. Ten male and 10 female mice were randomly allotted to each age range. All mice were fasted overnight (with water ad libitum) and were then bled by retro-orbital plexus puncture under isoflurane anesthesia. Mice were placed in a clear induction chamber and were anesthetized with isoflurane (USP; manufactured by Raman and Weil Private, Maharashtra, India) administered with a precision vaporizer (Orchid Scientific, Ambad, India). Blood samples for hematology (450 µL/mouse) were collected from 5 mice of each sex and age range and placed in a tube with anticoagulant (50 µL/vial, 2% EDTA). Approximately 700 µL/mouse blood was collected from the other 5 mice into a centrifuge tube without anticoagulant and serum was collected from from the sample after a clot had formed. Mice were humanely euthanized after completion of specimen collection by carbon dioxide (CO_2) using a gradual fill method in a transparent euthanasia chamber (30 L/min flow rate).

Laboratory analysis and quality management. All the blood samples were analyzed at the Clinical Pathology Laboratory of Zydus Research Centre, accredited by the National Accreditation Board for Testing and Calibration Laboratories. Samples collected for clinical chemistry analysis were centrifuged (2,000 × *g* for 10 min at 24 °C) after one hour of collection time for collection of serum. Calibration and quality control of the analyzer were performed to ensure accuracy and precision before analysis of samples. All samples were stored at room temperature (25 ± 1 °C) and analyzed within 4 to 5 hours of collection. Whole blood was analyzed for hematology analytes, namely red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), white blood cell count (WBC) and differential WBC counts: neutrophils (NEU; N%), lymphocytes (LYMPH; L%), eosinophils (EOS; E%), monocytes (MONO; M%), and basophils (BASO; B%). The analyses were performed on an automated blood cell analyzer ADVIA 2120i (Siemens Healthineers, Erlangen, Germany). Serum samples were processed for biochemistry analytes by the methods/techniques described as follows: glucose (GLU) by the hexokinase method; aspartate transferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) using the International Federation of Clinical Chemistry methods; total protein (TP) by the colorimetric Biuret method; albumin (ALB) by the bromocresol green method; urea (UREA) by the kinetic method; and creatinine (CREA) by the Jaffe method. The analyses were performed using a Cobas C311 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). Each activity including blood sample collection, transportation, storage, and analysis was based on good laboratory practices using standard operating procedures to ensure data quality.

Statistical analysis. The data were grouped by sex, age, and strain. Using SPSS, a boxplot for each hematology and biochemistry analyte was visually checked for outliers, and significant outliers were eliminated in accordance with the Tukey Method.[12] After removal of significant outliers, the test of normality (Shapiro-Wilk test) was applied, and mean, SD, and median for each hematology and biochemical analyte were calculated for each sex and age range. The Harris and Boyd test[11] was used to determine combined or separated reference intervals according to sex. Combined reference intervals were calculated using the nonparametric method (>120 samples) according to age range by determinations of the 2.5 and 97.5 percentiles, whereas separate reference intervals were calculated using the robust method (<120 samples). Upper and lower reference limits of each reference interval at 90% CI were also reported. All calculations were performed in accordance with the Clinical and Laboratory Standards Institute[5] and American Society for Veterinary Clinical Pathology guidelines.[10] Sex differences were compared using the Student *t* test when conditions of normality were met. When the normality test failed, the Mann-Whitney rank sum test (nonparametric) was used to compare differences between sexes. Strain differences for each analyte according to age range were compared using a nonparametric test. The differences linked to age for each mouse strain were performed by one-way ANOVA (post hoc analysis by Tukey honestly significant difference and Bonferroni test) using a statistical software program (SPSS 21.0). The body weight differences (3 to 12 wk) between strains of the same sex were compared using the Mann-Whitney rank sum test. *P* value less than 0.05 was considered statistically significant.

Results

Comparison of growth curves. The growth curves (mean ± SD) of both mouse strains up to 12 wk of age are shown in Figure 1. Males had higher body weight values compared with females of the same age. (Figure 1 a significant difference between BALB/c and C57BL/6 was found at week 7 for males ($P < 0.05$), while in the case of females, significant differences were found at weeks 3, 4, 5, and 7 ($P < 0.05$).

Sex-associated effects. Significant sex-associated differences were observed in most of the parameters assessed (Tables 1–6). The results are expressed as mean, SD, median, reference

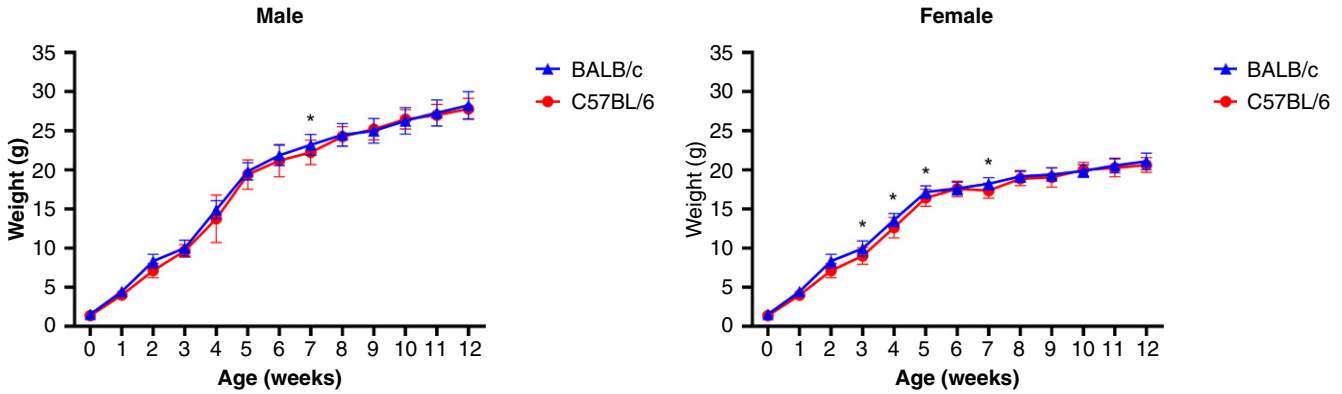

Figure 1. Growth curve of BALB/c ($n = 42$) and C57BL/6 ($n = 42$) up to 12 wk of age. Significant differences between strains of same sex: *, $P < 0.05$.

intervals, and upper and lower reference limits at 90% CI. At 6 to 8 wk of age, male BALB/c mice showed higher RBC, PLT, N%, and M% and lower WBC, MCH, LYMPH, and L% than female mice (Table 1). Male C57BL/6 mice (6 to 8 wk old) had significantly higher RBC, HGB, Hct, and PLT values than female mice, while their MCH and MCHC levels were lower. In 10- to 14-wk-old BALB/c mice (Table 2), males had significantly higher RBC, HGB, PLT, and N% and lower WBC, MCV, MCH, LYMPH, and L% than females. Male C57BL/6 mice (10 to 14 wk old) had significantly lower levels of MCH, MCHC, and N% and significantly higher levels of WBC, RBC, HGB, Hct, PLT, LYMPH, L%, and BASO than female mice. Table 3 demonstrates that male BALB/c mice (6 to 9 mo old) had significantly lower RBC, HGB, Hct, MCH, LYMPH, and L% and higher NEU, N%, MONO, and M% than female mice. Male C57BL/6 mice (6 to 9 mo old) had significantly lower levels of HGB, MCH, MCHC, PLT, and N%, while female mice had higher levels of WBC, LYMPH, L%, EOS, and BASO.

Male BALB/c mice at 6 to 8 wk of age had significantly higher levels of ALT and UREA and lower levels of AST, ALP, and ALB when compared with female mice (Table 4). Male C57BL/6 mice (6 to 8 wk old) had significantly higher TP and lower AST and ALP than female mice. Table 5 shows that in the 10- to 14-wk-old BALB/c mice age group, males had significantly higher GLU and UREA and lower ALP and ALB than females. Male C57BL/6 mice (10 to 14 wk old) had lower levels of AST, ALT, and ALP, while female mice had significantly higher levels of TP and UREA. Table 6 reveals that male BALB/c mice (6 to 9 mo old) exhibited significantly lower levels of ALP and ALB and significantly higher levels of AST, ALT, and UREA. GLU, TP, and UREA were shown to be significantly higher in male C57BL/6 mice (6 to 9 mo old), while AST, ALT, and ALP were reported to be lower in males than females.

Age-dependent effects. Age-dependent effects in hematologic parameters and biochemical analytes of both mouse strains are summarized in Table 7. In male BALB/c mice, RBC, HGB, and Hct levels were similar between 6 to 8 wk and 10 to 14 wk of age; however, there was a significant difference in 6- to 9-mo-old male mice when compared with the younger age ranges. RBC and Hct levels in female BALB/c mice were similar between age ranges; however, HGB levels in female mice aged 6 to 9 mo were lower compared to the younger age groups. MCV and MCHC concentrations in both sexes of BALB/c mice were similar among age ranges; however, MCH concentrations showed significant differences between age groups for both sexes. PLT counts were significantly impacted by age in BALB/c female mice but not in males. Age had a substantial impact on WBC counts in BALB/c mice of both sexes. Furthermore, 6- to 9-mo-old BALB/c male mice had significantly higher WBC counts compared to younger age groups, while in female BALB/c mice, it was found to be higher in 6 to 8 wk old animals. Regarding the differences in absolute leukocyte counts between 6- to 8-wk- and 10- to 14-wk-old BALB/c mice, there were no significant age differences for NEU, LYMPH, MONO, M%, EOS, E%, BASO, and B% in both sexes (with the exception of LYMPH in female mice). On the other hand, compared with younger age ranges, 6- to 9-mo-old BALB/c mice had significantly higher NEU, N%, MONO, M%, and EOS values and lower L%. Further, as age advances, N% gradually rises and L% falls for both sexes.

The levels of RBC, HGB, and Hct were significantly impacted by age in C57BL/6 mice of both sexes (Table 7). MCV and MCH concentrations were significantly impacted by age, although MCHC concentrations in C57BL/6 mice of both sexes were comparable among age ranges. PLT counts in 6- to 9-mo-old C57BL/6 mice were found to be significantly higher in both sexes when compared with younger age ranges. In C57BL/6 mice of both sexes, there were significant differences in WBC counts between age groups. In addition, WBC counts were found to be significantly greater in 6- to 9-mo-old male C57BL/6 mice, while female C57BL/6 mice aged 6 to 8 wk showed significantly higher WBC counts. When comparing NEU, LYMPH, MONO, M%, EOS, E%, and B% in C57BL/6 mice at 6 to 8 wk and 10 to 14 wk of age, there were no significant age differences (with the exception of LYMPH in female mice). Nonetheless, as compared with younger age ranges, 6- to 9-mo-old C57BL/6 mice had significantly higher NEU, N%, MONO, and M% and lower L% values in both sexes. Furthermore, LYMPH was found to be significantly higher in male C57BL/6 mice and lower in female C57BL/6 mice aged 6 to 9 mo old. EOS, E%, and BASO were found to be significantly higher only in male C57BL/6 mice, while no age differences were observed in female C57BL/6 mice.

In both sexes of BALB/c mice (Table 7), there were significant age differences for both ALT and ALP. The concentration of ALT was significantly higher in older mice (6 to 9 mo), whereas the concentration of ALP was significantly higher in younger mice (6 to 8 wk). When it came to AST, BALB/c male mice showed significant age differences, whereas BALB/c female mice did not demonstrate age differences. Comparing mice aged 6 to 8 wk and 10 to 14 wk to 6- to 9-mo-old BALB/c mice, GLU content in both sexes was significantly lower in the younger age groups. Male BALB/c mice (6 to 8 wk) had significantly higher concentrations of ALB, while female BALB/c mice (6 to 8 wk) had significantly higher concentrations of UREA compared to older mice. The concentrations of TP and CREA in BALB/c mice were comparable across age groups for both sexes.

Table 1. Reference intervals for hematologic parameters in 6- to 8-wk-old healthy mice of BALB/c and C57BL/6 strains

		BALB/c							C57BL/6						
Analytes	Sex	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL
RBC (106/µL)	M	96	8.88[a,c]	0.55	8.89	7.85–9.77	7.45–7.97	9.59–10.1	95	8.70[b,c]	0.46	8.72	7.68–9.41	7.66–7.87	9.33–9.82
	F	90	8.68	0.34	8.72				99	8.42	0.38	8.43			
HGB (g/dL)	M	94	14.1[c]	0.60	14.2	12.9–15.2	12.5–13.0	14.9–15.5	94	13.4[b]	0.68	13.5	11.8–14.4	11.5–12.0	14.2–14.6
	F	91	14.0	0.56	14.0				100	13.0	0.59	13.1			
Hct (%)	M	96	47.2[c]	3.21	47.4	40.4–53.5	39.9–41.6	51.6–54.4	97	46.4[b,c]	2.99	46.6	40.6–52.5	39.7–41.5	39.7–41.5
	F	95	46.6	3.01	46.7				100	44.5	2.32	44.7	39.9–49.2	39.3–40.6	48.6–49.8
MCV (fL)	M	98	53.2	3.43	53.7	46.7–57.7	45.9–47.1	57.4–58.0	100	53.6	2.83	54.3	47.6–57.9	47.2–48.1	57.6–58.3
	F	97	53.4	3.28	54.4				100	53.2	2.79	53.4			
MCH (pg)	M	93	15.9	0.44	15.8	15.3–17.0	15.1–15.3	16.8–17.1	97	15.4	0.50	15.4	14.5–16.4	14.2–14.5	16.2–16.5
	F	97	16.1[a]	0.47	16.1				94	15.6[a]	0.41	15.6			
MCHC (g/dL)	M	98	30.0	1.62	29.5	27.6–33.4	26.8–27.8	33.2–33.8	100	28.9	1.23	29.0	26.6–31.3	26.4–27.0	31.1–31.7
	F	97	30.2	1.71	29.8				100	29.3[a,c]	1.22	29.2			
PLT (103/µL)	M	98	935.5[b]	209.30	891.5	483–1,337	405–539	1,261–1,409	100	948.8[b]	230.38	885.5	459–1413	375–526	1,340–1,469
	F	97	774.9	160.24	720.0	414–1,090	370–471	1,025–1,144	96	771.8	157.97	746.0	442–1,081	392–490	1,031–1,129
WBC (103/µL)	M	98	3.02	1.13	3.12	1.14–6.69	0.85–1.33	6.14–7.01	99	4.41	1.51	4.20	1.85–7.87	1.72–2.11	7.17–8.09
	F	97	4.04[b,c]	1.51	4.06				99	4.36	1.65	4.22			
NEU (103/µL)	M	93	0.67	0.24	0.67	0.25–1.38	0.12–0.33	1.16–1.64	98	0.41	0.14	0.38	0.18–0.80	0.17–0.20	0.81–1.17
	F	95	0.74	0.31	0.70				96	0.44	0.17	0.41			
N%	M	96	25.5[b]	11.35	20.90	9.44–50.0	8.63–10.6	42.6–58.8	98	9.54	3.10	8.90	4.76–20.1	3.30–5.10	20.0–28.7
	F	89	18.0	3.87	17.8	10.1–25.6	8.90–11.3	24.3–26.8	93	10.5	4.37	9.7			
LYMPH (103/µL)	M	98	2.20	0.94	2.30	0.65–5.27	0.32–0.79	4.70–5.74	99	3.89	1.42	3.65	1.25–7.12	1.16–1.50	6.49–7.51
	F	97	3.16[b,c]	1.24	3.15				99	3.77	1.55	3.71			
L%	M	97	71.8	11.60	76.0	45.6–86.3	40.5–52.7	85.3–89.6	98	88.3	2.95	89.0	77.5–93.2	60.0–77.3	92.6–94.0
	F	92	78.8[b]	4.70	79.5				92	87.1	4.43	88.2			
MONO (103/µL)	M	93	0.02	0.01	0.019	0.005–0.06	0.002–0.006	0.054–0.065	92	0.02	0.01	0.018	0.002–0.09	0.001–0.004	0.087–0.13
	F	92	0.03	0.02	0.021				96	0.03	0.02	0.024			
M%	M	93	0.77[a]	0.38	0.70	0.18–1.59	0.05–0.22	1.41–1.76	97	0.52	0.29	0.50	0.09–1.70	0.05–0.13	1.7–2.1
	F	92	0.64	0.33	0.60				95	0.67	0.47	0.50			
EOS (103/µL)	M	96	0.03	0.03	0.011	0.001–0.17	0–0.001	0.14–0.18	84	0.01	0.01	0.004	0–0.02	0–0	0.017–0.025
	F	96	0.04	0.05	0.013				85	0.01	0.01	0.004			
E%	M	98	0.90	0.95	0.43	0.02–3.78	0–0.04	3.30–4.20	89	0.19	0.20	0.11	0–0.80	0–0	0.70–0.83
	F	95	1.04	1.30	0.31				91	0.21	0.22	0.11			
BASO (103/µL)	M	97	0.02	0.017	0.02	0.003–0.07	0.002–0.004	0.067–0.09	97	0.04	0.03	0.039	0.003–0.11	0–0.004	0.10–0.13
	F	97	0.03	0.026	0.03				98	0.04	0.03	0.036			
B%	M	97	0.78	0.55	0.67	0.1–2.11	0.10–0.10	1.76–2.25	99	1.04	0.68	1.03	0.10–2.50	0–0.1	2.21–2.77
	F	97	0.78	0.50	0.73				98	1.03	0.66	1.02			

F, female; LRL, lower reference limit; M, male; RI, reference intervals; URL, upper reference limit.
[a,b]Statistically significant different values between sex of mouse strain: $P < 0.05$ and $P < 0.001$, respectively; [c]statistical comparison based on parametric test.

Table 2. Reference intervals for hematologic parameters in 10- to 14-wk-old healthy mice of BALB/c and C57BL/6 strains

		BALB/c							C57BL/6						
Analytes	Sex	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL
RBC (106/μL)	M	97	8.88[b,c]	0.50	8.9	7.94–9.84	7.79–8.09	9.54–9.98	97	8.75[a,c]	0.41	8.7	7.76–9.50	7.67–7.98	9.28–9.68
	F	92	8.66	0.33	8.7				98	8.57	0.36	8.6			
HGB (g/dL)	M	89	14.1[a,c]	0.53	14.1	13.0–15.1	12.7–13.3	14.9–15.2	95	13.3[a]	0.42	13.4	12.2–14.1	12.1–12.3	14.0–14.4
	F	92	13.9	0.47	13.9				99	13.2	0.53	13.3			
Hct (%)	M	97	46.9[c]	3.18	47.3	39.9–52.8	39.2–40.8	51.1–53.5	98	45.7[a,c]	2.37	45.9	40.3–50.2	39.7–41.0	49.4–51.2
	F	99	46.1	3.30	46.4				99	44.8	2.55	44.9			
MCV (fL)	M	99	52.7	3.05	53.5	46.3–57.4	45.7–46.4	57.1–57.8	100	52.7	3.27	53.8	47.2–59.0	46.4–47.6	58.7–59.5
	F	99	53.4[a]	3.21	54.4				100	52.6	2.80	53.2			
MCH (pg)	M	96	15.7	0.39	15.7	15.0–16.7	14.9–15.2	16.5–16.8	98	15.2	0.45	15.3	14.4–16.1	14.1–14.6	16.0–16.3
	F	97	16.1[b,c]	0.35	16.1				99	15.4[a,c]	0.35	15.5			
MCHC (g/dL)	M	99	29.9	1.46	29.5	27.8–33.5	27.4–28.0	33.0–34.0	100	29.1	1.20	29.1	27.0–31.8	26.9–27.2	31.6–32.2
	F	99	30.3	1.69	29.6				100	29.5[a,c]	1.32	29.3			
PLT (103/μL)	M	96	968.6[b]	252.21	901.0	413–1,456	323–497	1,356–1,539	100	919.3[b]	178.75	880	543–1,273	490–591	1,220–1,323
	F	99	842.0	183.92	800.0	453–1,206	398–507	1,140–1,259	99	812.9	163.18	800	471–1,124	401–517	1,067–1,179
WBC (103/μL)	M	92	2.93	1.22	2.7	0.98–6.06	0.74–1.19	5.70–6.31	96	4.67[a,c]	1.60	4.4	1.37–7.64	1.21–1.95	7.28–8.35
	F	99	3.53[a,c]	1.35	3.4				100	4.08	1.56	4.0			
NEU (103/μL)	M	91	0.78	0.37	0.77	0.23–1.44	0.13–0.28	1.33–1.80	93	0.43	0.15	0.41	0.10–0.81	0.07–0.16	0.72–0.84
	F	95	0.76	0.29	0.72				99	0.44[c]	0.18	0.42			
N%	M	98	28.7[b]	10.58	26.7	6.52–49.6	4.15–8.98	45.7–52.8	93	9.1	2.84	8.7	4.27–18.0	3.8–5.1	15.5–19.4
	F	96	23.0	7.83	20.7	4.97–37.7	2.78–7.77	34.5–40.6	94	10.5[a]	3.51	10.2			
LYMPH (103/μL)	M	98	2.15	1.03	2.14	0.56–4.66	0.46–0.69	4.28–4.92	96	4.10[a]	1.46	3.78	1.27–6.90	0.90–1.51	6.42–7.43
	F	99	2.59[a]	1.13	2.30				100	3.54	1.40	3.45			
L%	M	98	67.8	11.47	69.7	47.6–85.3	44.0–49.9	84.8–86.2	93	88.6[a,c]	3.12	88.5	82.5–94.9	81.5–83.4	94.1–95.7
	F	96	73.5[a]	8.69	75.8				92	87.3	3.30	87.4			
MONO (103/μL)	M	90	0.03	0.02	0.022	0.004–0.07	0.002–0.005	0.068–0.076	95	0.03	0.02	0.019	0.004–0.07	0.002–0.005	0.07–0.08
	F	94	0.03	0.02	0.022				95	0.02	0.02	0.017			
M%	M	87	0.79	0.43	0.74	0.16–2.10	0.05–0.19	1.80–2.29	97	0.53	0.34	0.40	0.10–1.50	0.1–0.2	1.3–1.7
	F	95	0.81	0.54	0.70				97	0.61	0.39	0.50			
EOS (103/μL)	M	98	0.03	0.04	0.008	0–0.14	0–0.001	0.12–0.15	85	0.01	0.01	0.004	0–0.03	0–0	0.03–0.04
	F	92	0.03	0.04	0.010				88	0.01	0.01	0.004			
E%	M	97	0.85	0.87	0.37	0.009–5.1	0–0.030	3.90–5.33	89	0.22	0.26	0.10	0–0.80	0–0	0.6–1.1
	F	96	1.27	1.60	0.38				89	0.17	0.17	0.10			
BASO (103/μL)	M	94	0.02	0.01	0.018	0–0.07	0–0.002	0.058–0.076	97	0.06[a]	0.04	0.058	0.003–0.14	0.001–0.004	0.12–0.16
	F	97	0.03	0.02	0.022				95	0.04	0.03	0.033			
B%	M	94	0.76	0.57	0.67	0–2.02	0–0.10	1.86–2.19	100	1.22	0.79	1.20	0.10–2.90	0–0.1	2.5–3.3
	F	95	0.73	0.56	0.58				99	1.13	0.83	1.10			

F, female; LRL, lower reference limit; M, male; RI, reference intervals; URL, upper reference limit.
[a,b]Statistically significant different values between sex of mouse strain: $P < 0.05$ and $P < 0.001$, respectively; [c]statistical comparison based on parametric test.

Table 3. Reference intervals for hematologic parameters in 6- to 9-mo-old healthy mice of BALB/c and C57BL/6 strains

		BALB/c							C57BL/6						
Analytes	Sex	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL
RBC (106/µL)	M	92	8.51	0.48	8.5	7.67–9.46	7.25–7.82	9.28–9.64	97	8.53[c]	0.40	8.6	7.60–9.29	7.46–7.91	9.21–9.43
	F	94	8.67[a,c]	0.38	8.7				95	8.53	0.39	8.5			
HGB (g/dL)	M	94	13.3	0.75	13.3	12.0–14.6	11.5–12.3	14.5–15.2	98	12.6	0.60	12.7	11.2–14.2	11.0–11.5	13.8–14.6
	F	87	13.7[b,c]	0.41	13.7				98	12.9[a]	0.76	12.9			
Hct (%)	M	96	44.3	3.55	44.8	37.5–50.6	34.9–39.0	50.1–51.3	99	43.5	2.73	43.4	38.6–49.2	37.2–39.3	47.8–50.5
	F	99	45.7[a]	3.24	46.3				99	43.8[c]	2.64	43.8			
MCV (fL)	M	99	52.6	3.52	53.6	45.3–57.7	44.7–46.4	57.3–58.2	100	51.4	3.00	52.3	45.9–57.2	45.1–46.3	56.6–57.9
	F	99	52.9	3.66	54.2				100	51.7	2.98	52.0			
MCH (pg)	M	93	15.7	0.56	15.7	14.8–17.1	14.7–15.0	16.8–17.2	100	14.8	0.45	14.8	13.8–16.4	13.7–14.1	16.1–16.7
	F	98	15.9[a,c]	0.51	15.9				97	15.1[a,c]	0.72	15.1			
MCHC (g/dL)	M	99	30.0	1.30	29.6	28.0–33.5	27.6–28.2	33.2–33.9	100	29.1	1.14	28.9	27.0–31.6	26.6–27.3	31.2–32.1
	F	99	30.2	1.68	29.6				99	29.5[a,c]	1.28	29.4			
PLT (103/µL)	M	97	1,015.5	239.26	996.0	640–1,506	628–690	1,427–1,605	98	1,033.3	219.16	1,001	746–1,573	698–768	1,458–1,661
	F	98	1,019.5[c]	209.71	1,006.5				99	1,104.8[a]	212.82	1,072			
WBC (103/µL)	M	96	4.05	1.48	3.8	1.76–6.88	1.27–1.94	6.62–7.64	99	5.64[b]	2.08	5.3	1.24–9.66	0.57–1.78	8.92–10.3
	F	99	3.91	1.43	3.6				98	3.70	1.33	3.7	0.93–6.25	0.52–1.32	5.79–6.66
NEU (103/µL)	M	94	1.69[b]	0.80	1.45	0.56–4.23	0.48–0.65	3.67–4.95	88	0.59	0.22	0.56	0.22–1.25	0.18–0.26	1.17–1.38
	F	95	1.09	0.48	1.00	0.42–2.35	0.37–0.47	2.05–2.63	95	0.63	0.28	0.57			
N%	M	99	44.0[b]	16.78	42.4	9.11–76.4	5.0–13.7	71.0–81.6	92	11.7	4.40	10.7	6.1–29.9	5.1–6.9	25.8–35.8
	F	97	29.0	7.08	27.3	13.8–42.9	12.3–15.8	40.3–45.0	92	16.9[b]	6.36	15.9			
LYMPH (103/µL)	M	97	2.12	1.10	2.03	0.58–4.45	0.54–0.65	4.22–4.49	100	4.69[b]	1.98	4.29	1.83–10.24	1.62–2.06	9.13–11.4
	F	98	2.56[a]	0.92	2.46				97	2.90	1.14	2.73	0.52–5.10	0.19–0.88	4.74–5.45
L%	M	99	51.2	17.30	52.4	21.8–80.0	14.9–27.2	77.9–83.3	92	85.1[b]	4.87	85.7	75.7–95.2	74.1–77.3	93.9–96.9
	F	97	67.0[b]	7.19	67.9				92	80.4	6.91	81.8	67.4–95.5	64.9–70.1	93.2–97.8
MONO (103/µL)	M	89	0.06[a]	0.06	0.035	0.005–0.19	0.003–0.007	0.16–0.22	93	0.05	0.04	0.029	0.004–0.14	0.002–0.004	0.12–0.15
	F	93	0.04	0.02	0.030				97	0.04	0.03	0.026			
M%	M	92	1.63[a]	1.47	1.01	0.15–8.10	0.10–0.20	5.74–10.2	93	0.80	0.63	0.70	0.1–2.43	0.1–0.1	2.2–2.7
	F	93	0.93	0.58	0.82	0.19–3.60	0.16–0.24	2.80–4.27	94	0.92	0.71	0.70			
EOS (103/µL)	M	98	0.04	0.05	0.017	0.001–0.20	0–0.001	0.18–0.25	93	0.02[a]	0.03	0.008	0–0.11	0–0.001	0.08–0.12
	F	96	0.06	0.07	0.012				90	0.01	0.01	0.007			
E%	M	97	1.04	1.13	0.44	0.016–5.56	0–0.030	4.70–6.80	99	0.59	0.69	0.20	0–1.94	0–0	1.8–2.1
	F	94	1.40	1.89	0.26				92	0.23	0.19	0.20			
BASO (103/µL)	M	98	0.04	0.04	0.025	0–0.13	0–0.001	0.11–0.16	99	0.07[b]	0.05	0.063	0.002–0.16	0–0.003	0.14–0.17
	F	94	0.03	0.03	0.020				97	0.04	0.03	0.029			
B%	M	99	1.03	0.98	0.66	0–2.95	0–0.030	2.40–3.54	98	1.18	0.83	1.20	0.09–2.9	0–0.10	2.5–3.0
	F	95	0.74	0.64	0.49				98	1.05	0.79	1.00			

F, female; LRL, lower reference limit; M, male; RI, reference intervals; URL, upper reference limit.
[a,b]Statistically significant different values between sex of mouse strain: $P < 0.05$ and $P < 0.001$, respectively; [c]statistical comparison based on parametric test.

Table 4. Reference intervals for biochemistry analytes in 6- to 8-wk-old healthy mice of BALB/c and C57BL/6 strains

		BALB/c							C57BL/6						
Analytes	Sex	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL
GLU (mg/dL)	M	85	46.9	21.60	44.3	14.9–101.2	14.2–17.1	90.3–120.2	96	57.2[c]	21.43	57.3	14.4–100	8.3–21.0	93.9–105.8
	F	84	44.6	22.09	40.5				94	53.2	17.23	51.7	18.1–87.2	13.4–23.4	81.7–92.4
AST (U/L)	M	93	110.7	38.28	101.4	58.0–202.8	51.0–61.5	193.5–234.1	93	95.3	34.11	91.5	47.7–213.1	41.7–57.9	184.8–230.7
	F	94	122.8[a]	40.57	122.5				95	123.6[b]	41.51	118.5			
ALT (U/L)	M	89	41.4[a]	16.64	36.0	17.6–80.7	16.1–19.5	70.7–90.7	96	35.5	14.37	34.5	15.5–68.5	12.8–17.0	61.5–76.7
	F	91	35.1	9.47	33.7	15.1–53.4	12.5–18.0	50.0–56.4	91	37.6	11.65	33.8			
ALP (U/L)	M	97	252.9	49.11	255.7	156.0–348.7	144.4–189.2	335.7–375.8	100	222.4	53.63	220.7	143.1–326.0	95.6–160.2	310.3–342.6
	F	93	288.4[b]	39.94	290.8				96	251.6[b,c]	34.27	254.6			
TP (g/dL)	M	95	5.1[c]	0.26	5.1	4.6–5.6	4.4–4.6	5.6–5.8	98	5.5[b]	0.32	5.5	4.6–6.0	4.5–4.7	5.9–6.3
	F	95	5.0	0.29	5.0				98	5.3	0.35	5.2			
ALB (g/dL)	M	98	3.3	0.32	3.3	2.7–4.0	2.5–3.0	4.0–4.0	100	3.6	0.35	3.5	3.0–4.5	2.9–3.1	4.4–4.6
	F	86	3.5[a]	0.29	3.4				100	3.7	0.43	3.6			
UREA (mg/dL)	M	93	54.3[b]	16.58	51.5	17.6–84.8	13.4–22.8	79.1–91.2	90	81.8	14.25	77.0	59.0–110.6	52.8–62.1	108.2–116.7
	F	91	46.4	13.16	44.6	18.7–71.8	15.3–22.3	67.2–76.1	94	79.9	13.45	77.3			
CREA (mg/dL)	M	99	0.36	0.16	0.38	0.07–0.71	0.06–0.09	0.60–0.81	98	0.35	0.14	0.39	0.11–0.69	0.08–0.12	0.56–0.78
	F	95	0.34	0.18	0.36				99	0.36	0.17	0.39			

F, female; LRL, lower reference limit; M, male; RI, reference intervals; URL, upper reference limit.
[a,b]Statistically significant different values between sex of mouse strain: $P < 0.05$ and $P < 0.001$, respectively; [c]statistical comparison based on parametric test.

Table 5. Reference intervals for biochemistry analytes in 10- to 14-wk-old healthy mice of BALB/c and C57BL/6 strains

		BALB/c							C57BL/6						
Analytes	Sex	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL
GLU (mg/dL)	M	87	58.2[b]	25.87	55.3	21.9–130.3	19.2–25.5	114.5–147.0	99	66.3[a,c]	19.60	65.1	30.7–103.9	26.2–35.5	98.4–108.9
	F	81	44.0	19.34	40.2	16.3–105.9	89.2–124.9	89.2–124.9	96	60.3	16.06	61.3	29.0–93.2	24.1–34.1	88.6–97.4
AST (U/L)	M	90	110.5	35.36	105.0	53.6–202.3	42.1–60.3	191.6–211.8	92	95.5	35.69	89.3	50.5–237.9	45.3–54.70	206.3–271.3
	F	90	117.5	36.80	112.1				98	132.5[b]	51.55	128.9			
ALT (U/L)	M	91	44.4	20.33	36.9	16.4–93.0	14.8–18.7	79.5–105.6	93	36.9	12.40	35.2	19.2–82.4	16.4–21.5	80.6–102.9
	F	90	37.0	10.16	34.7	14.9–56.6	11.9–18.01	52.6–60.3	93	45.5[a]	18.96	41.7			
ALP (U/L)	M	95	157.4	36.09	157.4	92.1–247.7	77.4–105.9	238.1–270.4	94	122.9	25.27	124.2	77.9–204	59.2–89.7	200.6–226.7
	F	92	195.4[b,c]	29.54	196.9				94	166.4[b]	28.13	169.3			
TP (g/dL)	M	97	5.2[c]	0.31	5.2	4.47–5.7	4.3–4.6	5.6–5.8	93	5.6[a]	0.31	5.6	4.9–6.3	4.7–5.0	6.2–6.4
	F	91	5.1	0.29	5.1				93	5.5	0.36	5.5			
ALB (g/dL)	M	89	3.3	0.22	3.3	2.9–4.2	2.7–2.9	4.1–4.3	100	3.7	0.38	3.6	3.0–4.5	2.9–3.1	4.5–4.6
	F	92	3.5[b]	0.37	3.4				100	3.8	0.46	3.6			
UREA (mg/dL)	M	90	52.9[b]	14.05	50.8	23.3–80.2	19.6–27.5	75.1–84.6	92	80.3[a,c]	12.46	80.7	54.9–104.7	51.4–58.3	101.1–108.4
	F	93	45.6	14.38	43.3	14.0–72.1	10.6–18.1	66.9–77.8	98	73.5	18.35	75.1	37.6–111.2	32.0–43.5	106.1–116.9
CREA (mg/dL)	M	98	0.35	0.15	0.37	0.08–0.71	0.07–0.10	0.58–0.78	99	0.36	0.15	0.39	0.11–0.70	0.1–0.12	0.65–0.75
	F	94	0.34	0.17	0.35				99	0.37	0.19	0.40			

F, female; LRL, lower reference limit; M, male; RI, reference intervals; URL, upper reference limit.
[a,b]Statistically significant different values between sex of mouse strain: $P < 0.05$ and $P < 0.001$, respectively; [c]statistical comparison based on parametric test.

Table 6. Reference intervals for biochemistry analytes in 6- to 9-mo-old healthy mice of BALB/c and C57BL/6 strains

		BALB/c						C57BL/6					
Analytes	Sex	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL	*n*	Mean	SD	Median	RI	90% CI LRL	90% CI URL
GLU (mg/dL)	M	99	65.8	36.20	56.9	17.7–197.9	14.6–21.4	164.1–232.6	94	77.3[b,c]	20.69	80.3	35.6–119.2	30.3–41.9	113.4–124.7
	F	88	57.3	30.37	55.0	15.7–184.8	12.6–19.7	148.0–225.0	99	67.2	19.03	65.4	28.8–105.1	23.8–34.3	99.5–110.1
AST (U/L)	M	95	184.3[a]	138.59	116.2	32.1–505.2	25.5–39.3	369.4–655.8	91	87.0	27.66	80.0	55.7–197.4	47.5–58.9	183.7–224.1
	F	88	112.8	28.33	113.8	53.5–166.9	45.5–61.7	157.2–176.2	98	126.3[b]	39.47	119.2			
ALT (U/L)	M	90	63.3[a]	40.49	46.8	14.5–168.8	12.9–18.2	135.7–200.2	94	34.9	11.54	33.3	19.3–100.7	18.5–22.2	83.1–114.9
	F	86	39.6	9.95	37.8	18.3–58.8	14.7–21.6	54.6–62.5	97	52.8[b]	22.83	45.2			
ALP (U/L)	M	95	67.2	17.98	65.1	37.7–193.7	23.3–46.0	161.4–204.8	93	77.1	16.15	75.1	45.5–146	36.1–54.4	133.0–167.9
	F	93	125.1[b,c]	30.62	122.8				96	102.3[b]	26.94	103.2			
TP (g/dL)	M	98	5.1	0.34	5.1	4.49–5.8	4.3–4.6	5.7–5.8	92	5.7[b,c]	0.28	5.6	5.0–6.1	4.9–5.1	6.1–6.2
	F	96	5.2[c]	0.29	5.2				93	5.5	0.26	5.5			
ALB (g/dL)	M	96	3.0	0.23	3.0	2.6–4.1	2.5–2.7	4.0–4.2	100	3.6	0.45	3.5	2.7–4.4	2.6–2.9	4.3–4.5
	F	94	3.5[b]	0.36	3.4				99	3.5	0.38	3.5			
UREA (mg/dL)	M	92	49.8[b]	15.33	45.3	15.2–78.9	10.9–20.7	72.4–83.9	99	67.0[b,c]	17.12	66.2	32.4–100.8	28.0–37.0	95.5–105.6
	F	94	36.9	9.87	35.6	16.5–56.3	13.5–18.9	53.1–59.2	95	57.9	13.78	55.4	28.0–83.9	24.3–31.8	78.8–88.8
CREA (mg/dL)	M	100	0.31	0.15	0.34	0.067–0.71	0.04–0.08	0.53–0.79	100	0.35	0.17	0.38	0.09–0.75	0.07–0.09	0.64–0.77
	F	93	0.32	0.18	0.33				99	0.35	0.17	0.40			

F, female; LRL, lower reference limit; M, male; RI, reference intervals; URL, upper reference limit.
[a,b]Statistically significant different values between sex of mouse strain: $P < 0.05$ and $P < 0.001$, respectively; [c]statistical comparison based on parametric test.

In C57BL/6 mice of both sexes (Table 7), ALP and UREA showed significant age differences, with younger mice (6 to 8 wk) showing higher concentrations of these analytes than older mice (6 to 9 mo). Regarding ALT, significant age differences were observed in female C57BL/6 mice, but no such differences were observed in male mice. When compared with mice that were 6- to 8-wk and 10- to 14-wk-old, the GLU content in both sexes of C57BL/6 mice aged 6 to 9 mo was significantly higher. The CREA concentrations in both sexes of C57BL/6 mice were comparable between age ranges.

Strain-associated effects. RBC, HGB, MCH, MCHC, NEU, and EOS were significantly higher in both sexes of BALB/c mice (6 to 8 wk old) when mouse strains were compared (Figure 2), but LYMPH and BASO were significantly higher in both sexes of C57BL/6 mice. Moreover, Hct was found to be significantly higher only in female BALB/c mice, and male C57BL/6 mice showed significantly higher WBC counts. HGB, Hct, MCH, MCHC, NEU, and EOS were significantly higher in both sexes of BALB/c mice in 10- to 14-wk-old mice (Figure 2), whereas WBC, LYMPH, and BASO were significantly higher in both sexes of C57BL/6 mice. On the other hand, BALB/c females had significantly higher MCV and male BALB/c mice had significantly higher RBC in contrast to C57BL/6 mice. In the 6- to 9-mo-old mice (Figure 2), both sexes of BALB/c mice had significantly higher HGB, Hct, MCV, MCH, MCHC, NEU, and EOS compared to younger age groups, while both sexes of C57BL/6 mice had considerably higher LYMPH and BASO.

AST, ALT, and ALP were significantly higher in BALB/c male mice (6 to 8 wk old), whereas ALP was higher in BALB/c female mice, as shown in Figure 3. In contrast, GLU, TP, ALB, and UREA were significantly higher in both sexes of C57BL/6 mice. In the mice aged 10 to 14 wk (Figure 3), male BALB/c mice showed significantly higher concentrations of AST and ALP, while female BALB/c mice showed significantly higher concentrations of ALP. In contrast, both sexes of C57BL/6 mice showed significantly higher concentrations of GLU, TP, ALB, and UREA. ALT was also noticeably higher in only female C57BL/6 mice as compared with female BALB/c mice. AST and ALT were significantly higher in BALB/c male mice in the age range of 6 to 9 mo old (Figure 3), while ALP was significantly higher in only BALB/c female mice. In contrast, C57BL/6 male mice had significantly higher GLU, ALP, TP, ALB, and UREA and C57BL/6 female mice had significantly higher GLU, ALT, TP, and UREA.

Discussion

In this study, the reference intervals for numerous hematologic and biochemical parameters of 2 inbred mouse strains of BALB/c and C57BL/6 were assessed in 3 periods of a one-year span. The hematologic parameters and biochemical analytes differed according to sex and age in both mouse strains. In addition, strain differences were observed in all 3 age groups for most of the tested variables. This finding supports the use of age-specific reference intervals for hematologic parameters and biochemical analytes in studies involving mice. In fact, these reference intervals can serve as the tools for a more precise evaluation of changes after experimental intervention in mouse models.

Regarding hematologic parameters in BALB/c mice, RBC, HGB, and Hct concentrations were relatively similar up to 14 wk of age in both sexes; however, values were found to be significantly higher in 6- to 9-mo-old females compared with males (Table 3). In addition, older mice had lower values compared with younger mice (Table 7). Similar values have been obtained by others[6,19] using blood samples that were collected blood by retro-orbital plexus puncture. Further, evaluation of samples

Table 7. Age-related mean values of hematology and biochemistry analytes for BALB/c and C57BL/6 mice

	Male			Female		
Analytes/strain	6–8 wk	10–14 wk	6–9 mo	6–8 wk	10–14 wk	6–9 mo
RBC (106/μL)						
BALB/c	8.88[C]	8.88[B]	8.51	8.68	8.66	8.67
C57BL/6	8.70[c]	8.75[b]	8.53	8.42	8.57[a]	8.53
HGB (g/dL)						
BALB/c	14.1[C]	14.1[B]	13.3	14.0[c]	13.9[b]	13.7
C57BL/6	13.4[C]	13.3[B]	12.6	13.0	13.2[b]	12.9
Hct (%)						
BALB/c	47.2[C]	46.9[B]	44.3	46.6	46.1	45.7
C57BL/6	46.4[C]	45.7[B]	43.5	44.5	44.8[b]	43.8
MCV (fL)						
BALB/c	53.2	52.7	52.6	53.4	53.4	52.9
C57BL/6	53.6[C]	52.7[b]	51.4	53.2[c]	52.6	51.7
MCH (pg)						
BALB/c	15.9[a,c]	15.7	15.7	16.1[a]	16.1[b]	15.9
C57BL/6	15.4[a,C]	15.2[B]	14.8	15.6[a,C]	15.4[B]	15.1
MCHC (g/dL)						
BALB/c	30.0	29.9	30.0	30.2	30.3	30.2
C57BL/6	28.9	29.1	29.1	29.3	29.5	29.5
PLT (103/μL)						
BALB/c	935.5	968.6	1,015.5	774.9	842.0[a]	1,019.5[B,C]
C57BL/6	948.8	919.3	1,033.3[B,c]	771.8	812.9	1,104.8[B,C]
WBC (103/μL)						
BALB/c	3.02	2.93	4.05[B,C]	4.04[a]	3.53	3.91
C57BL/6	4.41	4.67	5.64[B,C]	4.36[c]	4.08	3.70
NEU (103/μL)						
BALB/c	0.67	0.78	1.69[B,C]	0.74	0.76	1.09[B,C]
C57BL/6	0.41	0.43	0.59[B,C]	0.44	0.44	0.63[B,C]
N %						
BALB/c	25.5	28.7	44.0[B,C]	18.0	23.0[A]	29.0[B,C]
C57BL/6	9.54	9.15	11.7[B,C]	10.5	10.5	16.9[B,C]
LYMPH (103/μL)						
BALB/c	2.20	2.15	2.12	3.16[a,c]	2.59	2.56
C57BL/6	3.89	4.10	4.69[b,c]	3.77[C]	3.54[b]	2.90
L%						
BALB/c	71.8[C]	67.8[B]	51.2	78.8[A,C]	73.5[B]	67.0
C57BL/6	88.3[C]	88.6[B]	85.1	87.1[C]	87.3[B]	80.4
MONO (103/μL)						
BALB/c	0.02	0.03	0.06[B,C]	0.03	0.03	0.04[b,c]
C57BL/6	0.02	0.03	0.05[B,C]	0.03	0.02	0.04[b]
M%						
BALB/c	0.77	0.79	1.63[B,C]	0.64	0.81	0.93[C]
C57BL/6	0.52	0.53	0.80[B,C]	0.67	0.61	0.92[B,c]
EOS (103/μL)						
BALB/c	0.03	0.03	0.04[c]	0.04	0.03	0.06[b]
C57BL/6	0.01	0.01	0.02[B,C]	0.01	0.01	0.01
E%						
BALB/c	0.90	0.85	1.04	1.04	1.27	1.40
C57BL/6	0.19	0.22	0.59[B,C]	0.21	0.17	0.23
BASO (103/μL)						
BALB/c	0.02	0.02	0.04[B,C]	0.03	0.03	0.03
[C]57BL/6	0.04	0.06[a]	0.07[C]	0.04	0.04	0.04
B%						
BALB/c	0.78	0.76	1.03[b]	0.78	0.73	0.74
C57BL/6	1.04	1.22	1.18	1.03	1.13	1.05

(*continued*)

Table 7. (Continued)

	Male			Female		
Analytes/strain	6–8 wk	10–14 wk	6–9 mo	6–8 wk	10–14 wk	6–9 mo
GLU (mg/dL)						
BALB/c	46.9	58.2[a]	65.8[C]	44.6	44.0	57.3[b,c]
C57BL/6	57.2	66.3[a]	77.3[B,C]	53.2	60.3[a]	67.2[b,C]
AST (U/L)						
BALB/c	110.7	110.5	184.3[B,C]	122.8	117.5	112.8
C57BL/6	95.3	95.5	87.0	123.6	132.5	126.3
ALT (U/L)						
BALB/c	41.4	44.4	63.3[B,C]	35.1	37.0	39.6[c]
C57BL/6	35.5	36.9	34.9	37.6	45.5[a]	52.8[b,C]
ALP (U/L)						
BALB/c	252.9A[C]	157.4[B]	67.2	288.4[A,C]	195.4[B]	125.1
C57BL/6	222.4[A,C]	122.9[B]	77.1	251.6[A,C]	166.4[B]	102.3
TP (g/dL)						
BALB/c	5.1	5.2	5.1	5.0	5.1	5.2
C57BL/6	5.5	5.6[a]	5.7[c]	5.3	5.5[A]	5.5[C]
ALB (g/dL)						
BALB/c	3.3[C]	3.3[B]	3.0	3.5	3.5	3.5
C57BL/6	3.6	3.7	3.6	3.7[c]	3.8[B]	3.5
UREA (mg/dL)						
BALB/c	54.3	52.9	49.8	46.4[C]	45.6[B]	36.9
C57BL/6	81.8[C]	80.3[B]	67.0	79.9[a,C]	73.5[B]	57.9
CREA (mg/dL)						
BALB/c	0.36	0.35	0.31	0.34	0.34	0.32
C57BL/6	0.35	0.36	0.35	0.36	0.37	0.35

[A]Significant difference 6 to 8 wk compared with 10 to 14 wk at $P < 0.001$; [B]significant difference 10 to 14 wk compared with 6 to 9 mo at $P < 0.001$; [C]significant difference 6 to 8 wk compared with 6 to 9 mo at $P < 0.001$.
[a]Significant difference 6 to 8 wk compared with 10 to 14 wk at $P < 0.05$; [b]significant difference 10 to 14 wk compared with 6 to 9 mo at $P < 0.05$; [c]significant difference 6 to 8 wk compared with 6–9 mo at $P < 0.05$.

obtained by intracardiac puncture yielded values similar to those described in the present study[21] . Hence, the method of blood collection may have less impact hematologic and biochemical parameters than age, sex, and genotype. For example, 10- to 14-wk-old BALB/c mice demonstrated differences in MCV values depending on sex (Table 2), but there were no age differences when compared with age ranges (Table 7). PLT counts were higher in male BALB/c mice up to 14 wk of age (Tables 1 and 2), and there were significant age differences in female BALB/c mice (Table 7). WBC counts were higher in female BALB/c mice up to 14 wk of age (Tables 1 and 2); however, there were also significant age differences (Table 7). In contrast, higher WBC counts have been reported[6] ($7.81 \pm 0.67 \times 10^9$/L in 8- to 9-wk and $8.81 \pm 0.66 \times 10^9$/L in 20- to 21-wk mice), but lower WBC counts have also been reported[19] ($2.6 \pm 0.9 \times 10^3/mm^3$; age: 2 to 3 mo). Concerning differential leukocyte count in BALB/c mice, N% were significantly higher in males and L%, and LYMPH counts were significantly higher in females in all age ranges; however, NEU counts were found to be significantly higher in only 6- to 9-mo-old males. Seemingly, N% and NEU counts tended to increase with age, while L% and LYMPH counts tended to decrease with age in BALB/c mice. E%, B%, EOS, and BASO counts were relatively similar between sexes in all age ranges of BALB/c mice. There was no significant sex difference in M% and MONO counts up to 14 wk of age; however, both values were found significantly higher in 6- to 9-mo-old male BALB/c mice. In comparison to age, M%, MONO, EOS, and BASO counts were found higher in older BALB/c mice compared with younger mice.

RBC, HGB, and Hct concentrations were found to be significantly higher in 6- to 8-wk- and 10- to 14-wk-old male C57BL/6 mice compared with females (Tables 1 and 2). RBC, HGB, and Hct concentrations differed significantly with age, and values were found to be higher in 6- to 8-wk-old males and 10- to 14-wk-old females (Table 7). Similar values have been reported by others using blood samples collected by retro-orbital plexus puncture or by cardiac puncture. Others have reported[14] that RBC, HGB, and Hct to increased with the animal's age in C57BL/6J mice; however, the same age effects were not observed in C57BL/6 mice as described here. MCH and MCHC values were found to be significantly higher in female C57BL/6 mice in all age ranges. However, no sex differences were found in MCV values. Age appeared to influence MCV and MCH values in our study; however, MCHC values were similar among age ranges (Table 7). One study[6] reported lower MCV values (42.64 ± 0.5 fL in 6- to 8-wk and 42.36 ± 0.6 fL in 20- to 21-wk mice) and higher MCHC values (MCHC: 33.42 ± 0.36 g/dL in 6- to 8-wk and 34.12 ± 0.39 g/dL in 20- to 21-wk mice) but also reported similar MCH values (MCH: 14.25 ± 0.27 pg in 6- to 8-wk and 14.46 ± 0.1 pg in 20- to 21-wk mice). Similar findings[20] have been reported for MCV, MCH, and MCHC for 60 d mice. PLT counts were higher in males up to 14 wk of age; however, they were found to be lower in 6- to 9-mo-old males. There were age effects on PLT values which were observed

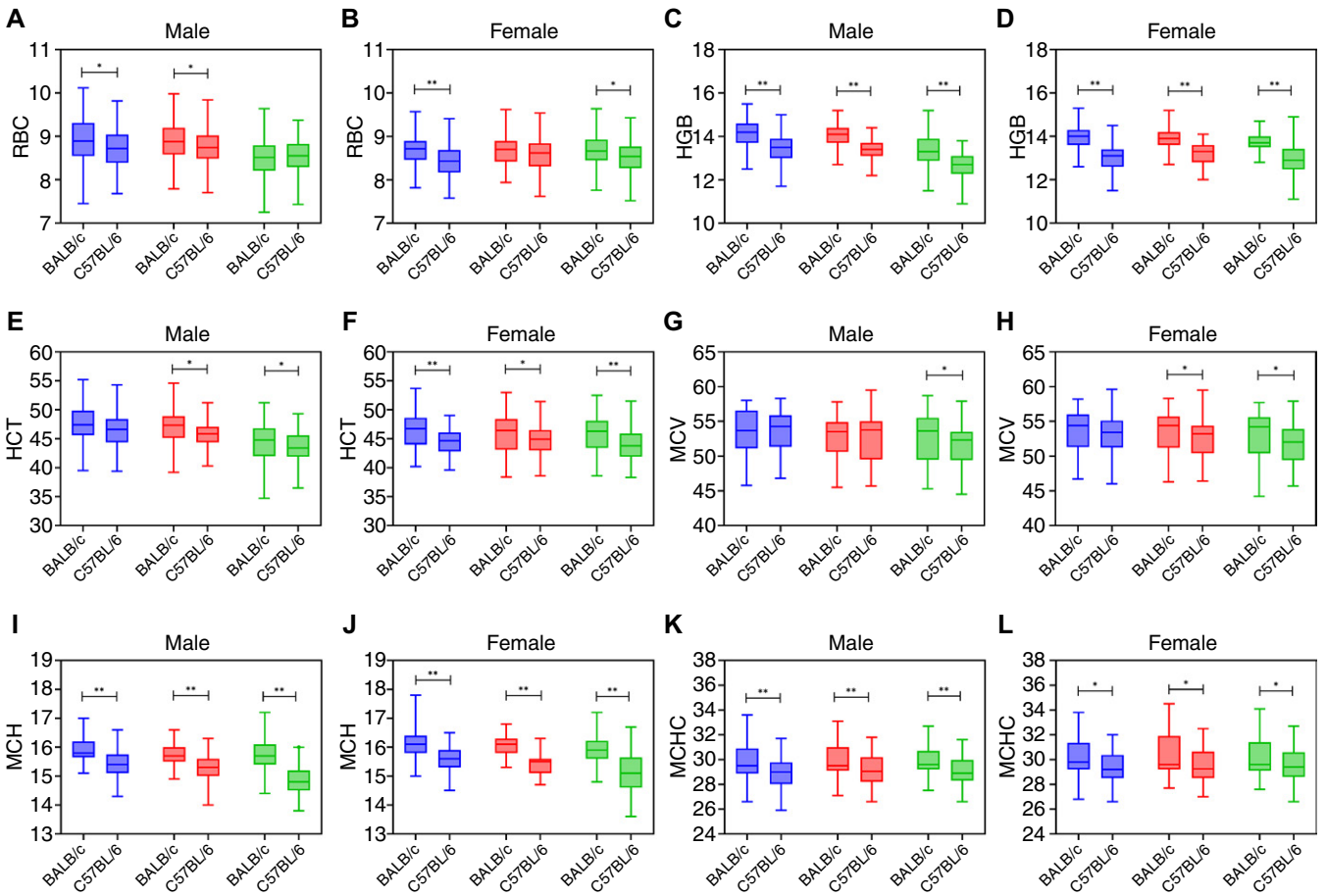

Figure 2. (A to Z) Box and Whisker plots show differences in hematologic parameters of BALB/c and C57BL/6 mice at different ages. Statistically significant different values in reported mice strain compared with another strain: *, $P < 0.05$ and **, $P < 0.001$.

to be significantly greater in older C57BL/6 mice (6 to 9 mo old), consistent with other findings.[14] Similar PLT values have been reported[6] ($895 \pm 102 \times k/\mu L$ in 8- to 9- wk and $970 \pm 126 \times k/\mu L$ in 20- to 21-wk mice), whereas lower PLT values have also been reported as $627 \pm 146 \times 10^3/mm^3$ (age: 2 to 3 mo)[21] and $170 \pm 56 \times 10^3/\mu L$ (age: 60 d).[20] We found WBC counts to be significantly higher in 10- to 14-wk- and 6- to 9-mo-old C57BL/6 males, and significant age differences were observed in both sexes. In addition, 6- to 9-mo-old males had higher WBC counts and, in contrast, higher counts were observed in 6- to 8-wk-old females. (Table 7). Similar WBC counts have been reported[6] ($3.56 \pm 0.77 \times 10^9/L$ in 8- to 9-wk and $6.62 \pm 2.59 \times 10^9/L$ in 20- to 21-wk mice), but lower WBC counts have also been reported[19] ($2.2 \pm 0.9 \times 10^3/mm^3$; age: 2 to 3 mo). Higher WBC counts have been described[14] in males than in females, and comparable sex effects have been observed in C57BL/6 mice. The other parameters of WBC have similar values in 6- to 8-wk-old mice; however, N% and BASO counts were higher in females, and L% and LYMPH counts were higher in males in 10- to 14-wk- as well as 6- to 9-mo-old mice. In relation to age, there were significant differences in N%, NEU, M%, MONO, E%, and EOS counts that were higher in older mice (6 to 9 mo) compared with younger mice (6 to 8 wk), whereas L% was significantly higher in younger mice.

Biochemical analytes exhibited significant sex- and age-related differences in both mouse strains. ALP values were significantly higher in younger mice (6 to 8 wk), and this analyte usually showed decreasing activity with increasing age in both mouse strains. In addition, ALP was found to be higher in females in all age ranges that were studied. One study[20] reported similar ALP values (209 U/L; age: 60 d) in BALB/c mice and lower ALP values (97.29 U/L; age; 60 d) in C57BL/6 mice. Another study[14] also reported similar ALP values (67 to 18 U/L; age: 4 to 8 mo) in C57BL/6J mice. We found sex differences in AST and ALT concentrations in both mouse strains. In relation to age, BALB/c male mice had significant age differences for AST and ALT concentrations; however, C57BL/6 male mice had no age differences. In addition, ALT was found significantly higher in 6- to 9-mo-old mice in BALB/c mice of both sexes and C57BL/6 female mice. Others have reported[6] lower AST values (BALB/c range: 76 to 104 U/L in 8- to 9-wk and 69 to 111 U/L in 20- to 21-wk mice; C57BL/6 range: 32 to 67 UL in 8- to 9-wk and 35 to 61 U/L in 20- to 21-wk mice) and similar ALT values (BALB/c range: 33 to 72 U/L in 8- to 9-wk and 29 to 81 U/L in 20- to 21-wk mice; C57BL/6 range: 27 to 44 U/L in 8- to 9-wk and 36 to 52 U/L in 20- to 21-wk mice). Further, another study[14] reported lower AST (55 to 91 U/L; age: 4 to 8 mo) and similar ALT values (46 to 70 U/L; age: 4 to 8 mo) in C57BL/6J mice. The results are consistent with findings[24] that older C57BL/6 mice had higher AST, ALT, and GLU concentrations than younger mice.

ALB concentrations were found to be significantly higher in BALB/c females in all age ranges, whereas no sex differences were observed in C57BL/6 mice. There were age differences observed only in BALB/c males and C57BL6 females. Lower ALB values have been reported[20] (BALB/c: 2.40 ± 0.47 g/dL; C57BL/6: 2.35 ± 0.12 g/dL; age: 60 d) in both mouse strains. ALB values similar to those we obtained have also been reported[14] (range: 2.0 to 4.7 g/dL; age: 4 to 8 mo). Male mice in both mouse

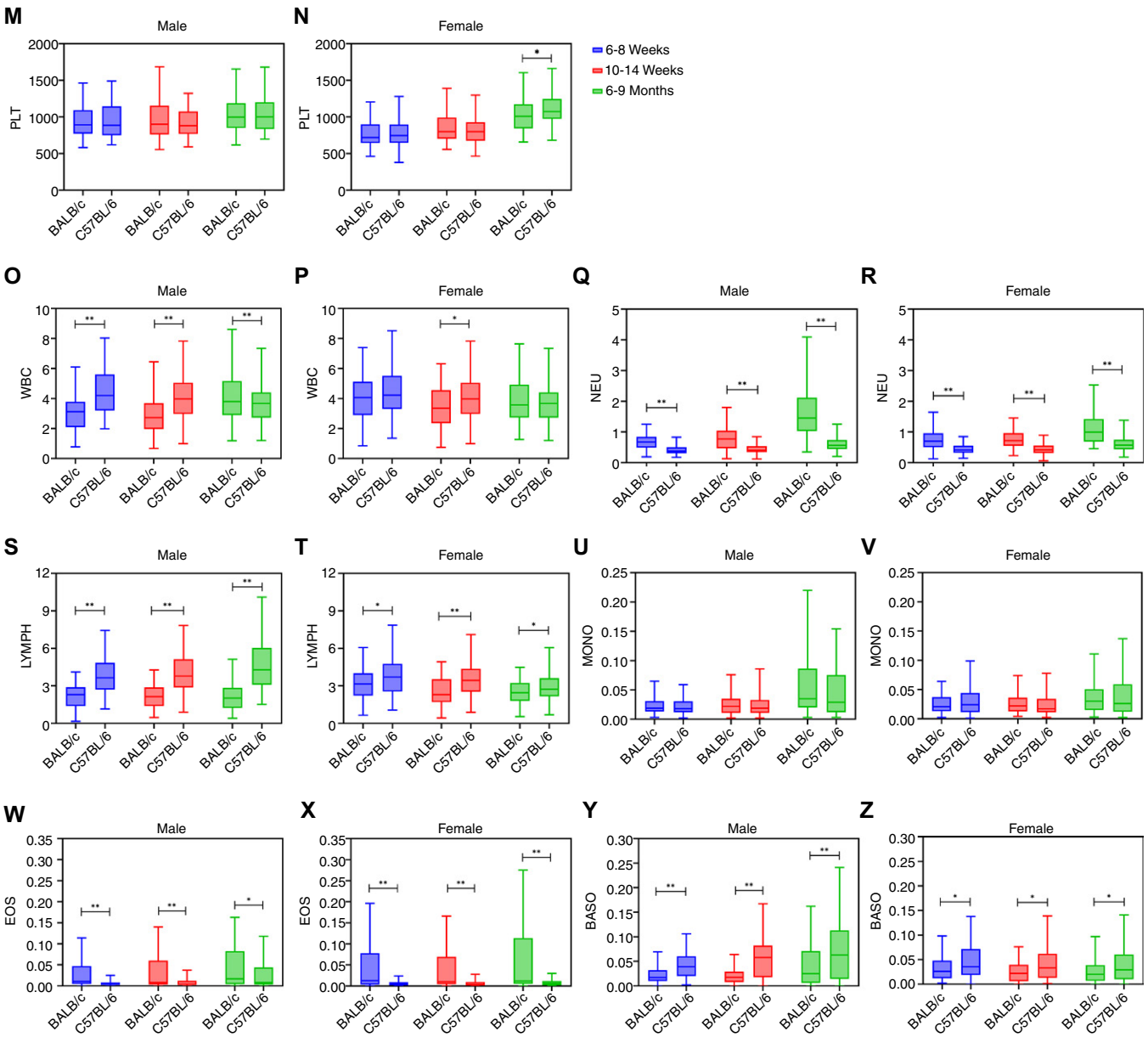

Figure 2. (Continued)

strains had significantly higher UREA concentrations and both sexes of C57BL/6 mice showed age differences, while only BALB/c females had age differences. Similar UREA values (40.48 ± 3.91 mg/dL; age: 60 d) in BALB/c mice and lower UREA values (57.66 ± 3.16 mg/dL) have been reported[20] in C57BL/6 mice. Lower UREA values have also been reported[8] in C57BL/6J mice. Further, we found that BALB/c mice had similar TP concentrations according to sex and age; however, C57BL/6 mice had sex and age differences. One study[20] reported higher TP values (BALB/c: 5.21 ± 0.46 g/dL; C57BL/6: 8.03 ± 0.34 g/dL, Age: 60 d) in both strains, and another study[14] also reported higher TP values (4.7 to 7.27 g/dL; age: 4 to 8 mo) in C57BL/6J mice. There were no age or sex differences in CREA concentrations in both of the mouse strains we evaluated. Similar CREA values have been reported in a study[8] of C57BL/6J mice that collected blood via retro-orbital method. We found age differences in GLU concentrations in both mouse strains; however, sex differences were observed only in 10- to 14-wk-old mice of both strains and 6- to 9-mo-old C57BL/6 mice.

The WBC data demonstrated interesting differences associated with age, genotype, and sex. Interestingly, BALB/c showed a tendency to have higher NEU and EOS values in contrast to C57BL/6 mice, which showed a tendency to have greater WBC, LYMPH, and BASO counts for all age ranges. This obvious difference between mouse strains may partially explain why they evoke different types of immune responses. One study[13] reported that C57BL/6 (11 to 12 wk) showed a tendency to have more LYMPH and MONO in contrast to BALB/c; however, MONO values were found to be similar between strains.

We found HGB, Hct, MCH, and MCHC were found higher in BALB/c mice compared with C57BL/6 mice in all age ranges. RBC was found to be significantly higher in BALB/c males in contrast to C57BL/6 males up to 14 wk of age; however, no significant strain differences were observed in 6- to 9-mo-old mice. In BALB/c females, RBC was found to be significantly higher in 6- to 8-wk and 6- to 9-mo-old mice compared with C57BL/6 mice. One study,[20] found that RBC

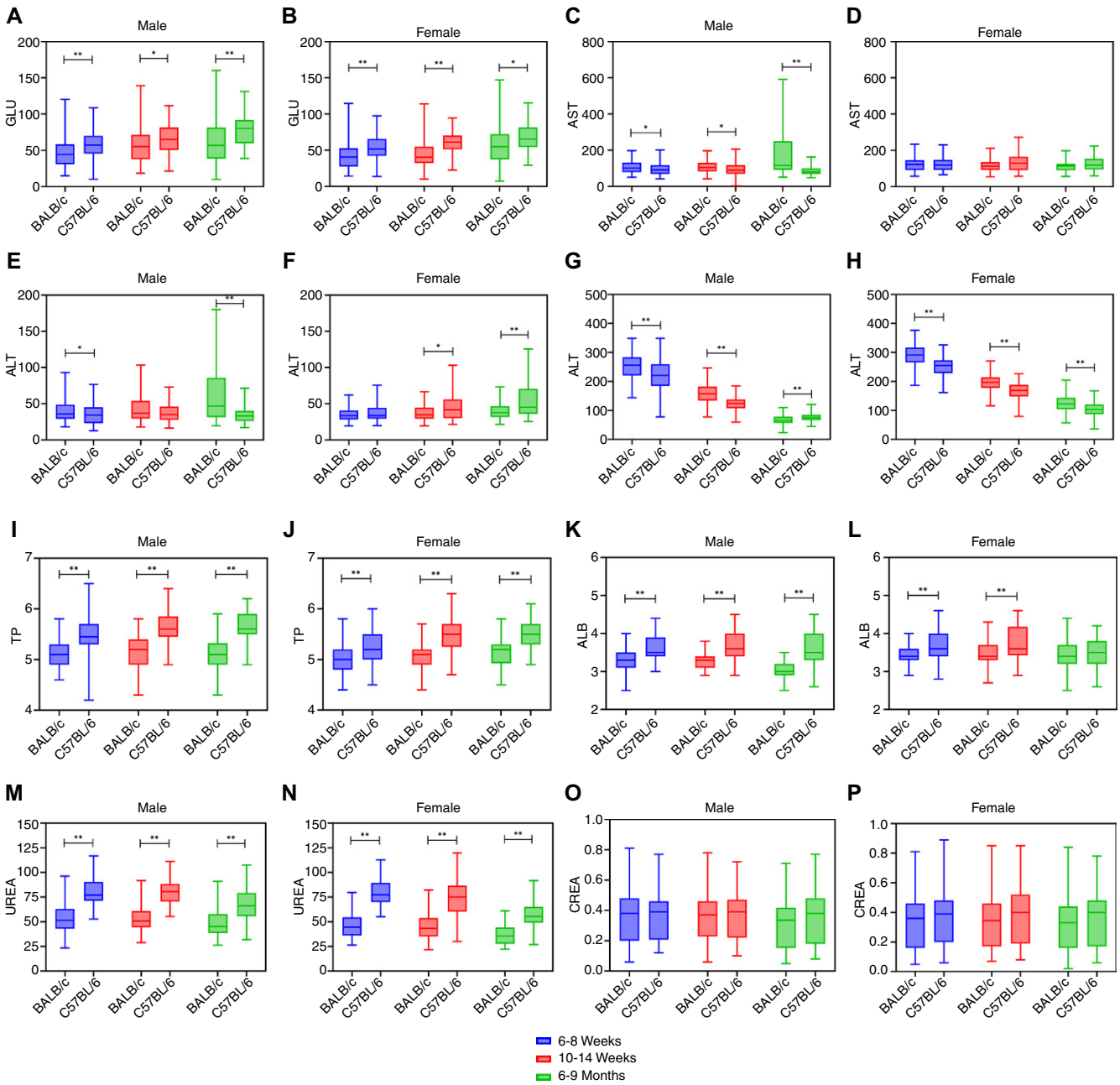

Figure 3. (A to P) Box and Whisker plots show differences in biochemistry analyte levels of BALB/c and C57BL/6 mice at different ages. Statistically significant different values in reported mice strain compared with another strain: *, $P < 0.05$ and **, $P < 0.001$.

and MCHC were highest in BALB/c females versus males. We found PLT counts to be similar between the two mouse strains in all age ranges, except 6- to 9-mo-old mice. Another study[3] reported the highest PLT counts in C57BL/6 mice in contrast to BALB/c mice.

Conclusion The reference interval for selected hematologic and biochemical parameters was established in this study by using healthy mice and standardized analysis conditions. Strain-specific, sex, or age-dependent reference intervals could be useful as reliable reference data for research, testing, and health evaluation of BALB/c and C57BL/6 mice.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Author Contributions

Animal Research Facility Team: Suresh Patel, Satish Patel, Ashvin Kotadiya, Samir Patel, Bhavesh Shrimali, and Mihir Tank were involved in these studies for acquisition and interpretation of data; Tushar Patel and Harshida Trivedi analyzed samples; and Suresh Patel contributed to compilation of data, literature review, wrote the manuscript, and conducted statistical analysis and production of tables and figures. Satish Patel, Samadhan Kshirsagar, and Mukul Jain were involved in drafting and revising it critically for important intellectual content.

References

1. **Almeida AS, Faleiros ACG, Teixeira DNS, Cota UA, Chica JEL.** 2008. Reference values for blood-based biochemical parameters in BALB/c and C57BL/6 wild-type mice. J Bras Patol Med Lab **44**:429–432.
2. **Barbosa BDS, Praxedes ÉA, Lima MA, Pimentel MML, Santos FA, Brito PD, Lelis ICNG, et al.** 2017. Haematological and biochemical profile of Balb-c mice. Acta Scientiae Vet **45**:5.
3. **Barrios M, Rodríguez-Acosta A, Gil A, Salazar AM, Taylor P, Sánchez EE, Arocha-Piñango CL, Guerrero B.** 2009. Comparative hemostatic parameters in BALB/c, C57BL/6 and C3H/He mice. Thromb Res **124**:338–343.
4. **Capecchi MR.** 2005. Gene targeting in mice: Functional analysis of the mammalian genome for the twenty-first century. Nat Rev Genet **6**:507–512.
5. **CLSI.** 2008. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline–third edition. CLSI document EP28-A3c. Wayne (PA): Clinical and Laboratory Standards Institute.
6. **Coman C, Vlase E, Dinu A.** 2018. Hematological and plasma biochemical values in four Specific pathogen-free mice strains produced in the animal facility at Cantacuzino Institute, Bucharest. Sci Works Series C Vet Med LXIV:115–122.
7. **Diniz MFFM, Medeiros IA, Santos HB, Oliveira KM, Vasconcelos THC, Aguiar FB, Toscano MG, Ribeiro EAN.** 2006. Haematological and biochemical parameter standardization of Swiss mice and Wistar rats. Rev Bras Ciênc Saúde **10**:171–176.
8. **Fernández I, Peña A, Del Teso N, Pérez V, Rodríguez-Cuesta J.** 2010. Clinical biochemistry parameters in C57BL/6J mice after blood collection from the submandibular vein and retroorbital plexus. J Am Assoc Lab Anim Sci **49**:202–206.
9. **Ferreira LM, Hochman B, Barbosa MV.** 2005. Modelos experimentais em pesquisa [Experimental models in research]. Acta Cir Bras **20** (Suppl 2):28–34.
10. **Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J, American Society for Veterinary Clinical Pathology.** 2012. ASVCP reference interval guidelines: Determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol **41**:441–453.
11. **Harris EK, Boyd JC.** 1990. On dividing reference data into subgroups to produce separate reference ranges. Clin Chem **36**:265–270.
12. **Horn PS, Pesce AJ.** 2003. Reference intervals: An update. Clin Chim Acta **334**:5–23.
13. **Kong DY, Park JH, Lee KW, Park H, Cho JA.** 2016. Comparative analysis of 3 experimental mouse models for blood hematology and chemistry. Biomed Sci Lett **22**:75–82.
14. **Mazzaccara C, Labruna G, Cito G, Scarfò M, De Felice M, Pastore L, Sacchetti L.** 2008. Age-related reference intervals of the main biochemical and hematological parameters in C57BL/6J, 129SV/EV and C3H/HeJ mouse strains. PLoS One **3**:e3772.
15. **Otto GP, Rathkolb B, Oestereicher MA, Lengger CJ, Moerth C, Micklich K, Fuchs H, et al.** 2016. Clinical chemistry reference intervals for C57BL/6J, C57BL/6N, and C3HeB/FeJ Mice (*Mus musculus*). J Am Assoc Lab Anim Sci **55**:375–386.
16. **Otto GP, Claus RA.** 2014. Criticizing reporting standards fails to improve quality in animal research. Crit Care **18**: 421.
17. **Raut S, Chopra BS, Khatri N.** 2023. Haematological profile of various inbred strains of mice maintained at IMTech Centre for Animal Resources and Experimentation (iCARE). Toxolog Int **30**:651–657.
18. **Restell TI, Porfirio LC, Souza AS, Silva IS.** 2014. Hematology of Swiss mice (*Mus musculus*) of both genders and different ages. Acta Cir Bras **29**:306–312.
19. **Santos EW, Oliveira D. C d, Hastreiter A, Silva G. B d, Beltran J. S d O, Tsujita M, Crisma AR, et al.** 2016. Hematological and biochemical reference values for C57BL/6, Swiss Webster and BALB/c mice. Braz J Vet Res Anim Sci **53**:138–145.
20. **Silva-Santana G, Bax JC, Fernandes DCS, Bacellar DTL, Hooper C, Dias AASO, Silva CB, et al.** 2020. Clinical hematological and biochemical parameters in Swiss, BALB/c, C57BL/6 and B6D2F1 *Mus musculus*. Animal Model Exp Med **3**:304–315.
21. **Silva-Santana DS, Mattos-Guaraldi AL, Lenzi-Almeida KC.** 2022. Benefits to human health of experimental models by using laboratory mice and rats: Overview and attributes in microbiology medical research. Biomed Res **33**:224–239.
22. **Spinelli MO, Cruz RJ, Godoy CMSC, Motta MC, Bortolatto J, Junqueira MS.** 2012. Hematological comparative study between sex and sanitary categories of C57BL/6 and BALB/c mouse. RESBCAL **1**:64–70.
23. **Spinelli MO, Motta MC, Cruz RJ, Godoy CM.** 2014. Reference intervals for hematological parameters of animals bred and kept at the vivarium of the Faculty of Medicine of the State University of São Paulo. Acta Sci Health Sci **36**:1–4.
24. **Zhou X, Hansson GK.** 2004. Effect of sex and age on serum biochemical reference ranges in C57BL/6J mice. Comp Med **54**:176–178.