Analysis of the Sex-specific Variability of Blood Parameters in C3H Inbred Mice by Using Data from a Long-term, High-throughput Project

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Short title: Sex-specific variability of blood parameters in C3H mice

Summary

Mice are important models for biomedical research by providing the possibility of standardizing genetic background and environmental conditions, which both affect phenotypic variability. Use of both sexes in experiments is strongly recommended because of possible differences in the outcome. However, sex-specific phenotypic variability is discussed with regard to putative consequences on the group size which is necessary for achieving valid and reproducible results. Here, we retrospectively analyzed the sex-specific variability of 25 blood parameters of C3H inbred mice in two different mouse facilities within the long-term, high-throughput Munich ENU mouse mutagenesis project. Using the 95% data range, data of 4,780-20,706 mice per parameter were analyzed and resulted in ratios of the coefficient of variation (= female CV / (female CV + male CV)) from 0.44 to 0.58 for the 25 parameters, with an overall mean of 0.51 in both facilities. Together with data analyses of three additional, smaller studies with 72-247 animals per parameter examined and various genetic backgrounds (inbred strains, F1 hybrids) included, hints for reproducible sex-specific variability were observed for particular parameters. Thus, the overall analysis comprising all 25 clinical chemical and hematological parameters of the standardized, long-term analysis of a high number of group housed, young adult, twelve-week-old C3H inbred mice showed no evidence for substantial sex-specific variability. The results may provide a basis for the examination of sex-specific variability in particular blood parameters.

Key words

animal model, clinical chemistry, hematology, sex, variability

Introduction

In biomedical research with animal models, use of both sexes is strongly recommended because of possible differences in the outcome (Clayton and Collins 2014, Sandberg *et al.* 2015). In the context of highly standardized conditions on genetic background and environment used in mouse experiments, sex-specific phenotypic variability is discussed as an experimental factor with putative consequences on the group size for achieving valid and reproducible results. Female hormone cycles are suspected to decrease the homogeneity of study populations and to confound effects of experimental manipulations. In addition, group housing especially of male animals leads to the establishment of a dominance hierarchy and to differences in the social status thereby leading to individual phenotypic variations ((Beery and Zucker 2011, Itoh and Arnold 2015, Prendergast *et al.* 2014, Varholick *et al.* 2018) and refs. therein).

For nociceptive traits, more than 8,000 individual measurements, collected from 40 different mouse strains in 3 laboratories, showed that females tested at random points in their estrous cycles were not more variable than males (Mogil and Chanda 2005). In a meta-analysis of 293 articles, behavioral, morphological, physiological, and molecular traits were monitored in male mice and females tested without regard to the estrous cycle stage. The variability was not significantly greater in females than males for any endpoint and was substantially greater in males for several traits. In addition, group housing of mice was observed to increase the variability in both males and females by 37% (Prendergast *et al.* 2014). In another study, the analysis of 293 microarray datasets measuring gene expression in various tissues of mice and humans, comprising the analysis of more than 5 million probes, showed that on average, male gene expression is slightly more variable than that of females although the difference was small (Itoh and Arnold 2015).

In biomedical research, standardized, long-term, high-throughput analyses of a high number of phenotypic parameters in both sexes of mice have been carried out in phenotype-driven ENU mouse mutagenesis projects worldwide. Random chemical mutagenesis of a

large number of animals followed by systematic screening for clinically relevant disease phenotypes was carried out with the alkylating agent *N*-ethyl-*N*-nitrosourea (ENU) which predominantly induces point mutations in premeiotic spermatogonial stem cells. This allowed the production of a large number of randomly mutagenized offspring from treated males, which were used for the establishment of novel mutant mouse lines harbouring disease-related alleles. However, by far most of the offspring showed physiological values for a given phenotype parameter. In the Munich ENU mouse mutagenesis project using C3HeB/FeJ (C3H) inbred mice as genetic background, a standardized screening profile of a high number of phenotypic parameters was established for the analysis of offspring of mutagenized mice in order to detect phenotypic variants (Hrabé de Angelis *et al.* 2000, Hrabé de Angelis *et al.* 2007).

Here, we retrospectively re-analyzed data from this project, which were formerly used to establish mutant mouse lines, for the new aim to investigate the sex-specific variability of 25 blood parameters in a high number of animals. The blood parameters were chosen for the re-analysis as the data have been collected in our own research group therefore making it possible to track the entire experimental process.

Methods

Long-term, high-throughput analysis

Blood parameters were determined in the context of the clinical chemical and hematological screen of the phenotype-driven Munich ENU mouse mutagenesis project by using standardized protocols (Rathkolb *et al.* 2000a, Rathkolb *et al.* 2000b). Data were derived over a time period of over six years (08/1998-10/2004) and comprised the analysis of almost 22,000 C3HeB/FeJ (C3H) inbred mice (The Jackson Laboratory) housed in two different facilities A and B. They were G1 and G3 male and female offspring derived from

ENU-mutagenized G0 founder males which were bred by defined breeding schemes (Hrabé de Angelis *et al.* 2000, Hrabé de Angelis *et al.* 2007). Up to five mice were housed together in groups of the same sex in Macrolon type II standard cages. Mouse husbandry was carried out under a continuously controlled specific pathogen-free (SPF) hygiene standard according to the FELASA protocols (Nicklas *et al.* 2002) (http://www.felasa.eu). Mouse husbandry and all tests were carried out under the approval of the responsible animal welfare authority (Regierung von Oberbayern, Germany). Data analysis was carried out using the software program Microsoft Excel 2016 (Microsoft Corp., Redmond, WA). The chi-squared test was used for the statistical analysis of the data.

Clinical chemistry and hematology

The analysis of the clinical chemical blood plasma parameters and the hematological parameters was carried out by standardized protocols as previously described (Gailus-Durner *et al.* 2005, Rathkolb *et al.* 2000a, Rathkolb *et al.* 2000b). Briefly, blood samples from weekly cohorts of twelve-week-old male and female mice were obtained by puncture of the retro-orbital sinus under ether anesthesia. The clinical chemical parameters were analyzed by using the Roche Hitachi 717 autoanalyzer (Roche, Mannheim, Germany) and the adapted reagents for human samples (Roche), and subsequently the Olympus AU400 autoanalyzer (Olympus, Hamburg, Germany) and the adapted reagents for human samples (Olympus) within their linear measurement ranges. Hematological parameters were measured using the ABC Animal Blood Counter (Scil, Viernheim, Germany) which was validated by the manufacturer for the analysis of mouse blood. Calibration and quality control were performed daily according to the manufacturer's protocols using the calibration samples obtained from the manufacturers.

Additional non-mutagenized inbred and F1 hybrid mice

Non-mutagenized inbred and F1 hybrid mice were also used in the context of the Munich ENU mouse mutagenesis project in the studies I, II, and III, which were carried out in three subsequent time periods of two years each in the mouse facilities A or B. The group housed mice for study I were maintained in facility B, and the group housed mice analyzed in studies II and III were maintained in facility A (Klempt *et al.* 2006). In studies I and II, the inbred strains C3HeB/FeJ (C3H) (study I: 85-132 males and 114-115 females; study II: 50-51 males and 79-80 females) and C57BL/6JIco (C57BL/6) (study I: 138-139 males and 72-74 females; study II: 64-71 males and 50 females), and the F1 hybrid mice B6C3F1 (study I: 91-120 males and 89-116 females; study II: 39 males and 37-38 females) and C3B6F1 (study I: 90-107 males and 77-94 females; study II: 44-45 males and 38 females) were used. In study III, the inbred strains C3HeB/FeJ (C3H) (51-55 males and 39-40 females) and BALB/cJ (BALB/c) (70-82 males and 90-100 females), and the F1 hybrid mice CC3F1 (58 males and 41 females) and C3CF1 (55 males and 61 females) were analyzed (Klempt *et al.* 2006).

Results

Long-term, high-throughput analysis of C3H inbred mice

For the analysis of the sex-specific variability of blood parameters in a great number of animals, data from almost 22,000 young adult, twelve-week-old C3HeB/FeJ (C3H) inbred mice derived from the long-term, high-throughput phenotype-driven Munich ENU mouse mutagenesis project were re-analyzed. The project was carried out in two different facilities A and B where G1 and G3 male and female offspring - which were derived from ENU-mutagenized G0 founder males by the use of defined breeding schemes - were examined for clinical chemical and hematological parameters in a standardized procedure (examination of group housed, twelve-week-old animals by standardized protocols) over a time period of six years. By far most of the offspring showed physiological values for a given phenotype

parameter. The data have been previously used for determining mutagenized phenotypic G1 and G3 variants for breeding ENU mutant mouse lines with interesting abnormal phenotypes (Aigner *et al.* 2009a, Aigner *et al.* 2009b, Aigner *et al.* 2011, Klempt *et al.* 2006, Rathkolb *et al.* 2015).

As the data were derived from offspring of ENU-mutagenized mice and, therefore, are expected to include a small number of animals harbouring mutations which may alter specific phenotypic parameters, in the current study the 95% data range (by excluding 2.5% each of the highest and lowest values) was chosen for each parameter separately to exclude values derived from such mutant mice as well as technical outliers.

The phenotypic variability was analyzed by determining the coefficient of variation (CV = standard deviation / mean) both for the male and female C3H mice. A CV ratio (= female CV / (female CV + male CV)) < 0.5 indicates that the female CV is lower than the male CV, whereas a CV ratio > 0.5 indicates that the female CV is higher than the male CV.

In both facilities A and B, 25 clinical chemical and hematological parameters in male (facility A: 2,630-9,099 mice per parameter analyzed; facility B: 553-3,731 mice) and female (facility A: 1,033-4,452 mice; facility B: 564-3,427 mice) mice were examined (Table 1). Some parameters were analyzed with data from relatively low numbers of animals due to a shorter time period of examination (e.g. ferritin, transferrin, lipase) or because technical procedures changed within the project (calcium, chloride, phosphorus, potassium, sodium, α-amylase). In the later case, the subgroup covering the highest number of animals was chosen for the current analysis for the respective parameter. The smaller subgroups were examined separately and mostly showed analogous results compared to the larger subgroups included in the current project (see Table 1).

The CV ratios ranged from 0.44 to 0.58 in facility A with CV ratios < 0.5 (= lower female CV) for n = 9 of 25 (36%) parameters, and ranged from 0.47 to 0.56 in facility B with CV ratios < 0.5 (= lower female CV) for n = 10 of 25 (40%) parameters. For both facilities,

the chi-squared test showed no significant difference ($p > 0.05$) of the detected counts of parameters with a CV ratio < 0.5 and a CV ratio > 0.5 compared to the hypothesis of equal numbers of parameters with a CV ratio < 0.5 and a CV ratio > 0.5. Consistent CV ratios (either CV > 0.5 or CV < 0.5) in both facilities were observed for 16 of 25 (64%) parameters (11 of 25 with CV ratios > 0.5, and 5 of 25 with CV ratios < 0.5), whereas 9 of 25 (36%) parameters showed inconsistent CV ratios. Comparing the CV ratios of a given parameter between the facilities A and B, a difference of > 5% to < 10% appeared for 9 of 25 (36%) parameters analyzed, i.e. uric acid, calcium, phosphorus, sodium, alanine aminotransferase (ALT), alkaline phosphatase (AP), lipase, mean corpuscular volume (MCV), and platelets. This includes parameters with consistent CV ratios as well as with inconsistent CV ratios. In total, the mean ± standard deviation of the CV ratios of all 25 parameters was 0.505 ± 0.028 in facility A, 0.506 ± 0.023 in facility B, and 0.506 ± 0.025 for both facilities (Table 1). Therefore, no substantial sex-specific variability was evident for the overall analysis comprising all 25 clinical chemical and hematological parameters.

To investigate if the chosen data range influenced the outcome of the analysis, the data set was additionally examined by using the 99% (excluding 0.5% each of the highest and lowest values) and 90% (excluding 5% each of the highest and lowest values) data range. In total, the mean ± standard deviation of the CV ratios of all 25 parameters for both facilities was 0.508 ± 0.024 for the 99% data range, and 0.506 ± 0.027 for the 90% data range. 32 of 50 parameters (64%) showed CV ratios > 0.5 (= higher female CV) for the 99% data range, which was 31 of 50 parameters (62%) for the 95% data range, and 30 of 50 parameters (60%) for the 90% data range (Figure 1). In addition, similar differences of the CV ratios of a given parameter between the facilities A and B were detected with all three data ranges. Thus, analogous results were derived with all three data ranges (99%, 95%, 90%). The analysis of the complete data set was judged not to be informative for the aim of the current project because of the presence of data of ENU mutant animals, and therefore, was not carried out.

Analysis of inbred strains and F1 hybrid mice

A second data set of clinical chemical and hematological parameters was generated in the context of the Munich ENU mouse mutagenesis project where three studies I, II, and III were carried out in three subsequent time periods of two years each in the mouse facilities A (studies II and III) and B (study I) by using smaller groups (n = 76-247 males and females per parameter analyzed) of group housed, native, non-mutagenized inbred (studies I and II: C3H, C57BL/6; study III: C3H, BALB/c) and F1 hybrid mice (studies I and II: B6C3F1, C3B6F1; study III: CC3F1, C3CF1) also at the age of twelve weeks.

These data sets have been previously used to investigate the phenotypic variability in the genetic backgrounds of inbred versus F1 hybrid mice. The analysis resulted in overall CV ratios (= F1 hybrid CV / (F1 hybrid CV + inbred CV)) (mean ± standard deviation) of 0.50 ± 0.06 for study I, 0.37 ± 0.09 for study II, and 0.50 ± 0.06 for study III, and therefore, clearly demonstrated the possibility of major interactions between genotype and environment regarding the variability of clinical chemical and hematological parameters (Klempt *et al.* 2006).

For the current analysis, these data sets were re-analyzed for the aim to investigate the sex-specific variability of blood parameters in additional genetic backgrounds beside C3H mice, i.e. C57BL/6, BALB/c, and F1 hybrid mice. To exclude technical outliers, the data sets were analyzed with exclusion of outliers > 3 × distance of the first and third quartiles. The CV ratio (= female CV / (female CV + male CV)) was determined for every parameter within each of the three studies I, II and III for each inbred strain 1 (IN1) and inbred strain 2 (IN2), and for each F1 hybrid mice 1 (F1A) and F1 hybrid mice 2 (F1B) (Table 2).

The CV ratios ranged from 0.41 to 0.66 in study I with CV ratios < 0.5 (= lower female CV) for n = 32 of 80 (40%) parameters, from 0.31 to 0.66 in study II with CV ratios < 0.5 for n = 38 of 83 (46%) parameters, and from 0.32 to 0.71 in study III with CV ratios < 0.5

for n = 29 of 83 (35%) parameters (Table 2). For the studies I and II, the chi-squared test showed no significant difference ($p > 0.05$) of the detected counts of parameters with a CV ratio < 0.5 and a CV ratio > 0.5 compared to the hypothesis of equal numbers of parameters with a CV ratio < 0.5 and a CV ratio > 0.5. However, a significant difference ($p < 0.01$) appeared for study III. In total, the mean ± standard deviation of the CV ratios of all parameters analyzed was 0.517 ± 0.054 in study I, 0.503 ± 0.076 in study II, and 0.528 ± 0.070 in study III (Figure 1).

To investigate if the chosen data range influenced the outcome of the analysis, the data sets were additionally examined by using the complete data sets without exclusion of outliers (which has also been used in the previous analysis presented in (Klempt *et al.* 2006)), and in the case of study I with the largest group sizes of the data set, also with exclusion of outliers > 1.5 × distance of the first and third quartiles. Using the complete data sets without exclusion of outliers, the mean ± standard deviation of the CV ratios of all parameters analyzed was 0.507 ± 0.060 in study I (with CV ratios < 0.5 for n = 31 of 80 (39%) parameters), 0.501 ± 0.080 in study II (with CV ratios < 0.5 for n = 40 of 84 (48%) parameters), and 0.516 ± 0.079 in study III (with CV ratios < 0.5 for n = 36 of 85 (42%) parameters). Using the data set of study I with exclusion of outliers > 1.5 × distance of the first and third quartiles, the mean ± standard deviation of the CV ratios of all parameters analyzed was 0.528 ± 0.072, with CV ratios < 0.5 for n = 32 of 80 (40%) parameters (Figure 1).

Thus, analogous overall results within the studies I, II and III were derived with all different data set ranges, i.e. without exclusion of outliers, with exclusion of outliers > 3 × distance of the first and third quartiles, or with exclusion of outliers > 1.5 × distance of the first and third quartiles.

Joint analysis of the results of both projects

At least higher extents of sex-specific variability in a particular parameter irrespective of genetic background and/or environmental factors are expected to result in consistent values of either CV ratios < 0.5 or CV ratios > 0.5 for a given parameter in both analyses shown in Table 1 (n = 2 separate analyses) and Table 2 (n = 12 separate analyses). In the search for particular parameters with consistent values in the long-term study with the high numbers of C3H mice both in facility A and facility B shown in Table 1, as well as with the same consistent values in most of the respective 12 analyses shown in Table 2, hints for sex-specific variability may be observed for the following parameters: cholesterol (CV ratio > 0.5 in 12 of all 14 (86%) analyses including all 5 C3H analyses), triglycerides (CV ratio > 0.5 in 12 of all 14 (86%) analyses including all 5 C3H analyses), urea (CV ratio > 0.5 in 12 of all 14 (86%) analyses including all 5 C3H analyses), and α-amylase (CV ratio > 0.5 in 12 of all 14 (86%) analyses including 4 of the 5 C3H analyses). The result for alanine aminotransferase (ALT, CV ratio < 0.5 in 12 of all 13 (92%) analyses including 4 of the 5 C3H analyses) was not supported by the data analysis using the data sets of study I, II and III without exclusion of outliers. No parameter showed consistent values of the CV ratio for all 14 separate analyses in Table 1 and Table 2.

Discussion

The retrospective analysis of the sex-specific variability of 25 blood parameters of C3H inbred mice in two different mouse facilities derived from the standardized, long-term, high-throughput Munich ENU mouse mutagenesis project resulted in an overall mean of 0.51 for the ratio of the coefficient of variation (= female CV / (female CV + male CV)) in both facilities. Both sexes were group housed, and females were tested without regard to the stage of the estrous cycle. The project was standardized to achieve comparable phenotypic results of a high number of animals over a long time period, but not especially for the analysis of sex-

specific phenotypic variability. The 95% data range was chosen for the current study to exclude values derived from ENU mutant mice as well as technical outliers, and refers to the data range defined by the mean ± two standard deviations.

In a meta-analysis of 293 articles including the analysis of behavioral, morphological, physiological, and molecular traits, group housing of mice increased the variability in both males and females by 37% (Prendergast *et al.* 2014). Therefore, no sex is expected to take advantage of this housing method in respect to the extent of the variability compared to the other sex. In the Munich ENU mouse mutagenesis project, both male and female mice were group housed in comparable group sizes after weaning until the phenotypic analysis including the measurement of the blood parameters took place. Normally, this works well also for C3H males in the given context (group housing particularly of offspring of the same litter, no previous use in breeding, time period within the first three months of age), therefore, single housing due to aggressive behavior was carried out only exceptionally. The similar increase of the variability in group housed males and females observed by (Prendergast *et al.* 2014) is also expected to cover the consequences of the Lee Boot effect which leads to the suppression of the estrous cycle in group housed female mice (Bind *et al.* 2013). In addition, this represents the housing method usually carried out when working with mice in biomedical research.

The meta-analysis did not describe particular parameters with a robust difference of the phenotypic variability between both sexes which may be used as a "positive control" to evaluate the results detected in our study. The published increase of the phenotypic variability (= CV) within each sex by 37% in group housed animals (Prendergast *et al.* 2014) would effect a CV ratio of 0.39 or 0.61 when comparing single housed mice and group housed mice. This deviation from the hypothesized CV ratio of 0.5 is much higher than the deviations of the CV ratio of 0.5 detected in our study as the overall means of all parameters analyzed.

Comparison of the overall results of the CV ratios of the C3H mice in the long-term experiment (means of 0.504 to 0.509 (minimum - maximum) with standard deviations of 0.022 to 0.030 (minimum - maximum) for all six analyses of the 90%, 95% and 99% data range in both facilities) with the smaller groups of non-mutagenized C3H mice in study I (means of 0.525 to 0.558 with standard deviations of 0.050 to 0.071 for all three analyses with all data, without extreme outliers and without outliers), study II (means of 0.504 and 0.522 with standard deviations of 0.061 and 0.076 for both analyses), and study III (a mean of 0.523 with standard deviations of 0.091 and 0.093 for both analyses) showed consistently higher standard deviations for the studies I, II and III with the small groups of animals. This is thought to be caused not by the slight difference in the panel of blood parameters which were available for the separate studies, but by the number of examined animals by itself.

Comparison of the CV ratios on the basis of the identical genetic background, i.e. of the five groups of C3H mice (long-term study in facility A, long-term study in facility B, study I, study II, study III) for a given parameter may indicate that the sex-specific variability may distinctly vary due to interacting factors. As this was observed also between facility A and facility B with high numbers of mice involved, the same effect may be expected in experiments with low group sizes which are normally used in biomedical research. This refers to the result which was previously received in the investigation of the variability of phenotypic parameters in the inbred versus F1 hybrid genetic background (Klempt *et al.* 2006).

In summary, the overall analysis comprising all 25 clinical chemical and hematological parameters of the standardized, long-term analysis of a high number of group housed, young adult, twelve-week-old C3H inbred mice showed no evidence for substantial sex-specific variability. The results may provide a basis for the examination of sex-specific variability in particular blood parameters.

Conflict of Interest

All authors have no competing financial or other interests in relation to the manuscript.

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Table 1. Coefficient of variation ratios (= female CV / (female CV + male CV)) of the blood parameters of C3H mice in the Munich ENU mouse mutagenesis project (95% data range)

Parameter	CV ratio, facility A	CV ratio, facility B	No. of mice (m/f), facility A	No. of mice (m/f), facility B
Cholesterol	0.54	0.53	6387 / 3362	3181 / 2860
Creatinine	0.51	**0.499**	8965 / 4379	3703 / 3398
Glucose	0.504	0.52	8968 / 4385	3704 / 3397
Total protein	**0.497**	0.52	9033 / 4398	3708 / 3401
Triglycerides	0.58	0.56	6373 / 3364	3179 / 2859
Urea	0.53	0.53	9003 / 4395	3704 / 3401
Uric acid	0.51	0.54	6475 / 3402	3185 / 2877
Ferritin	**0.497**	**0.48**	**3029 / 1180**	**669 / 666**
Transferrin	0.51	0.51	5325 / 2201	1351 / 1311
Calcium	0.51	***0.48***	**3515 / 2130**	*2321 / 2061*
Chloride	0.52	*0.52*	**3515 / 2134**	*2324 / 2064*
Phosphorus	0.51	***0.49***	**3515 / 2133**	*2324 / 2063*
Potassium	0.505	0.503	**3515 / 2135**	**2324 / 2065**
Sodium	**0.48**	*0.51*	**3514 / 2135**	*2324 / 2064*
ALT	**0.44**	**0.48**	9094 / 4451	3731 / 3426
AST	0.504	**0.48**	9099 / 4452	3728 / 3427
α-amylase	0.54	0.53	**5575 / 2296**	**2324 / 2060**
AP	0.52	**0.47**	7195 / 3719	3494 / 3184
CK	**0.49**	**0.47**	**6432 / 3414**	**3199 / 2878**
Lipase	**0.45**	**0.49**	2630 / 1033	553 / 564
Hemoglobin	0.51	0.504	6173 / 3263	3337 / 3040
MCV	**0.48**	0.51	6175 / 3263	3337 / 3040
RBC	0.51	0.51	6174 / 3257	3338 / 3041
WBC	**0.49**	**0.49**	6147 / 3256	3334 / 3039
Platelets	**0.497**	0.54	6176 / 3266	3338 / 3040
Mean ± SD	0.505 ± 0.028	0.506 ± 0.023		

For the 9 parameters indicated in italics (uric acid, calcium, phosphorus, sodium, ALT, AP, lipase, MCV, and platelets), comparison of the CV ratios between the facilities A and B revealed a difference of > 5% to < 10% of the values to each other.

CV, coefficient of variation = standard deviation / mean. The values of the CV ratios are indicated in bold for the parameters where the female CV is lower than the male CV (= CV ratio < 0.5) (n = 9 of 25 (36%) in facility A, and n = 10 of 25 (40%) in facility B). For facility B, the values and the respective numbers of mice of the 4 parameters calcium, chloride, phosphorus and sodium are indicated in italics, because the separate analysis of an additional smaller subgroup resulted in an inconsistent CV ratio, i.e. a CV ratio < 0.5 for the larger subgroup shown in the table, and a CV ratio > 0.5 for the smaller subgroup (not shown), or vice versa. Both CV ratios showed a difference of > 5% to < 10% of the values to each other. For the CV ratios with their animal numbers of facility A/B indicated in bold, the separate analysis of an additional smaller subgroup resulted in a consistent CV ratio, i.e. a CV ratio either < 0.5 or > 0.5 both for the larger subgroup shown in the table and for the smaller subgroup (not shown). Both CV ratios showed a difference of < 5% of the values to each other.

ALT, alanine aminotransferase (EC 2.6.1.2); AST, aspartate aminotransferase (EC 2.6.1.1); α-amylase (EC 3.2.1.1); AP, alkaline phosphatase (EC 3.1.3.1); CK, creatine kinase (EC 2.7.3.2); lipase (EC 3.1.1.3); MCV, mean corpuscular volume; RBC, red blood cell count, WBC, white blood cell count. The parameters hematocrit, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were not included in the study as they were subsequently calculated by using parameters directly measured.

Table 2. Coefficient of variation ratios (= female CV / (female CV + male CV)) of the blood parameters of inbred strains and F1 hybrid mice (data sets without outliers > 3 × distance of the first and third quartiles)

Parameter	Study I (C3H, C57BL/6)				Study II (C3H, C57BL/6)				Study III (C3H, BALB/c)			
	IN1	IN2	F1A	F1B	IN1	IN2	F1A	F1B	IN1	IN2	F1A	F1B
Cholesterol	0.57	0.52	0.58	**0.48**	0.54	0.60	0.58	**0.45**	0.53	0.59	0.61	0.66
Glucose	**0.48**	0.53	0.59	**0.45**	0.53	**0.48**	**0.46**	**0.45**	0.62	0.58	0.63	**0.48**
Total protein	0.58	**0.47**	0.51	0.503	**0.46**	0.58	0.61	**0.48**	**0.48**	0.58	**0.44**	***0.47***
Triglycerides	0.59	0.52	0.53	**0.48**	0.62	0.66	0.54	**0.47**	0.59	0.53	0.57	0.61
Urea	0.53	**0.48**	**0.47**	0.60	0.55	0.55	0.59	0.52	0.59	0.54	0.52	0.58
Uric acid	0.55	**0.49**	0.63	0.66	0.52	**0.31**	0.53	**0.35**	**0.48**	**0.45**	0.51	**0.47**
Ferritin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	**0.38**	***0.48***	**0.39**	0.52
Transferrin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.51	0.63	0.501	0.52
Calcium	*0.51*	0.60	**0.42**	0.53	*0.53*	**0.48**	0.53	**0.48**	**0.47**	n.d.	n.d.	0.64
Chloride	0.66	**0.49**	0.51	**0.496**	*0.58*	0.57	**0.38**	0.54	0.68	n.d.	**0.43**	0.56
Phosphorus	0.53	0.54	**0.49**	**0.48**	0.51	**0.45**	**0.42**	**0.47**	**0.49**	**0.43**	0.61	**0.48**
Potassium	0.53	0.56	**0.43**	**0.43**	0.57	**0.39**	0.66	**0.45**	**0.49**	n.d.	0.5002	**0.499**
Sodium	0.59	**0.41**	**0.496**	0.60	*0.55*	0.60	**0.43**	**0.44**	0.71	n.d.	*0.52*	0.62
ALT	0.53	**0.49**	***0.44***	**0.48**	**0.43**	***0.49***	***0.46***	n.d.	**0.48**	**0.46**	**0.39**	***0.49***
AST	**0.48**	**0.43**	*0.52*	0.52	*0.59*	***0.48***	*0.54*	**0.33**	**0.46**	0.57	**0.47**	*0.57*

α-amylase	***0.48***	0.53	0.56	***0.47***	0.56	0.60	0.52	0.53	0.62	0.55	*0.55*	0.68
AP	0.52	0.55	**0.41**	0.53	0.59	**0.34**	**0.42**	0.52	0.63	**0.48**	0.52	0.54
CK	0.51	**0.45**	*0.54*	**0.47**	0.55	0.57	*0.62*	**0.44**	0.53	*0.57*	0.502	0.53
Hemoglobin	0.59	0.56	0.52	0.52	**0.47**	**0.35**	**0.48**	***0.49***	**0.49**	0.52	*0.51*	*0.54*
MCV	n.d.	n.d.	n.d.	n.d.	***0.42***	0.504	**0.44**	**0.43**	n.d.	n.d.	n.d.	n.d.
RBC	***0.47***	0.54	**0.48**	0.5001	*0.54*	0.503	0.51	0.55	**0.32**	0.56	**0.495**	*0.54*
WBC	**0.46**	**0.46**	**0.48**	0.57	**0.49**	**0.44**	0.53	0.61	**0.47**	0.51	0.54	0.53
Platelets	0.55	0.55	0.59	0.52	**0.38**	**0.45**	0.51	0.58	*0.52*	*0.55*	0.53	**0.495**
Mean ± SD	0.54 ± 0.05	0.51 ± 0.05	0.51 ± 0.06	0.51 ± 0.06	0.52 ± 0.06	**0.496 ± 0.09**	0.51 ± 0.07	**0.48 ± 0.07**	0.52 ± 0.09	0.53 ± 0.05	0.51 ± 0.06	0.55 ± 0.06
Outliers: % (m/f)		0.9% / 0.5%				0.9% / 1.1%				1.3% / 0.9%		
Outliers: % affected parameters (m/f)		36% / 24%				27% / 20%				39% / 30%		

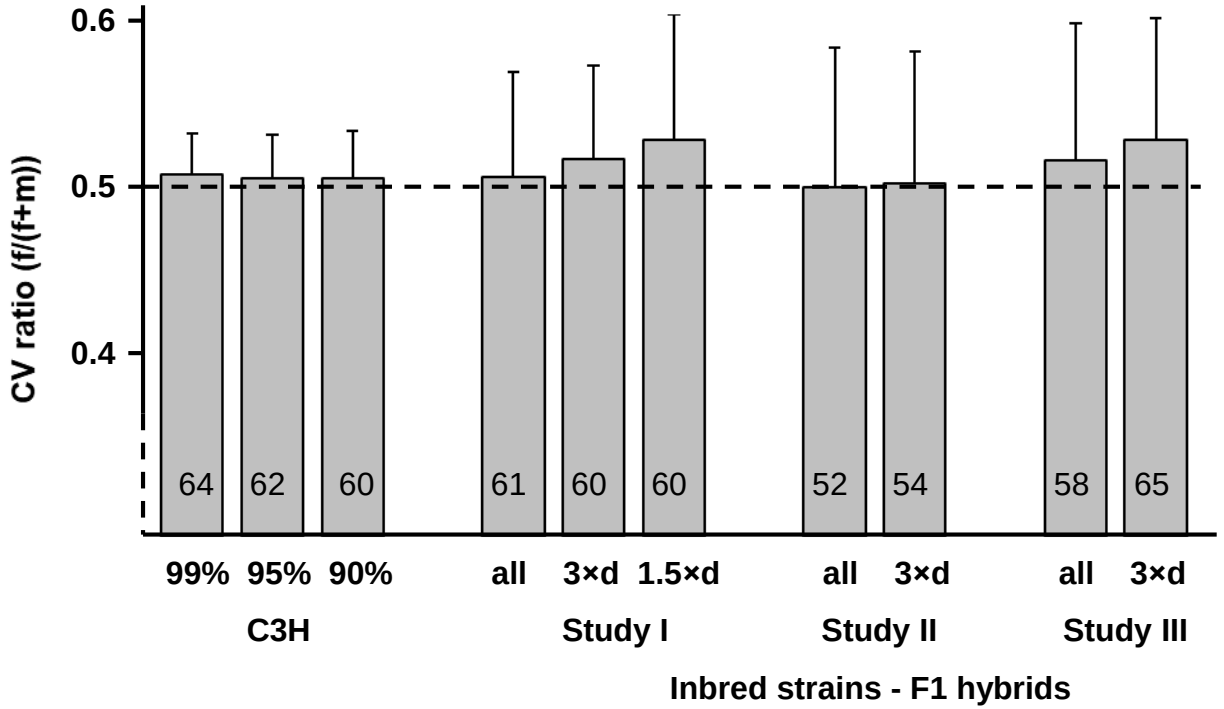
Study I and II: Inbred strain IN1: C3H; inbred strain IN2: C57BL/6; F1 hybrids F1A: B6C3F1; F1 hybrids F1B: C3B6F1. Study III: IN1: C3H; IN2: BALB/c; F1A: CC3F1; F1B: C3CF1. The number of mice (males and females) included in the analysis of the data sets without outliers > 3 × distance of the first and third quartiles is (minimum-maximum (mean ± standard deviation)) 159-247 (196 ± 22) in study I, 72-131 (101 ± 23) in study II, and 90-182 (117 ± 30) in study III per parameter examined. The number of outliers is indicated separately for males and females in % of all values used in the study.

CV, coefficient of variation = standard deviation / mean. n.d., not determined. The values of the CV ratios are indicated in bold for the parameters where the female CV is lower than the male CV (= CV ratio < 0.5) (n = 32 of 80 (40%) in study I, 38 of 83 (46%) in study II, and 29 of 83 (35%) in study III). In addition, values of the CV ratios are indicated in italics where the analysis of the data set without exclusion of outliers resulted in an inconsistent CV ratio, i.e. a CV ratio < 0.5 for the analysis shown in the table, and a CV ratio > 0.5 for the analysis of the data set without exclusion of outliers (not shown), or vice versa (with the 5 parameters of enzyme activities mostly affected).

ALT, alanine aminotransferase (EC 2.6.1.2); AST, aspartate aminotransferase (EC 2.6.1.1); α-amylase (EC 3.2.1.1); AP, alkaline phosphatase (EC 3.1.3.1); CK, creatine kinase (EC 2.7.3.2); MCV, mean corpuscular volume; RBC, red blood cell count; WBC, white blood cell count.

Figure 1. Mean coefficient of variation ratios (= female CV / (female CV + male CV)) ± standard deviations of the overall analyses comprising all blood parameters.

The blood parameters of C3H inbred mice of the Munich ENU mouse mutagenesis project in both facilities A and B including 5,032-21,794 animals (males and females) per parameter examined were analyzed using the 99%, 95% or 90% data range, separately for each parameter. The analysis of the blood parameters of inbred strains and the F1 hybrid mice produced thereof (study I and II: C3H, C57BL/6; study III: C3H, BALB/c) included 76-247 animals (males and females) per parameter examined. They were analyzed without exclusion of outliers ("all"), with exclusion of outliers > 3 × distance of the first and third quartiles ("3×d"), or with exclusion of outliers > 1.5 × distance of the first and third quartiles ("1.5×d", only study I). The coefficient of variation ratios are depicted as mean ± standard deviation for all blood parameters analyzed (see Table 1 for the C3H inbred mice of the Munich ENU mouse mutagenesis project, and Table 2 for the three studies I, II and III of inbred strains and F1 hybrid mice). The number in the columns indicates the count of the parameters in % where the female CV is higher than the male CV for the respective analysis (= CV ratio > 0.5).


0.6
0.5
0.4
CV ratio (f/(f+m))
64
62
60
61
60
60
52
54
58
65
99%
95%
90%
all
3×d
1.5×d
all
3×d
all
3×d
C3H
Study I
Study II
Study III
Inbred strains - F1 hybrids