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Age-dependent change trends of clinicopathological parameters in F344 rats

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Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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Ethics Approval
The study protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Inhalation Toxicity Research Center (IACUC approval numbers: IACUC-1911, -1913, -1915, -1916, -1917, -1918, -1812, -1813, -1815, -1817, -1818, -1612, -1601).

Abstract

Clinical pathology, including hematology and serum chemistry, is an important indicator of biological changes. Animals for inhalation studies are kept in specific chambers and require historical data for accuracy. Age-related characteristics are essential for interpreting experimental results. This study aimed to provide historical clinical pathology data and analyze age-related trends in these parameters. We collected hematological and biochemical parameters from control groups of male and female F344 rats in the 4-, 13-, 26-, and 52-week repeated inhalation toxicity tests. The number of F344 rats from collected control groups were 24, 60, 50, and 25 males and 25, 60, 50, and 25 females in the 4-, 13-, 26-, and 52-week studies, respectively. Mean comparison, correlation analysis and simple linear regression analysis was conducted to reveal age-related trends. Neutrophil count, eosinophil count, neutrophil percentage, monocyte percentage, total protein, albumin, triglyceride, total cholesterol (TCHO) showed increasing trends, whereas lymphocyte count, lymphocyte percent, platelet count, alkaline phosphatase, albumin/globulin ratio, and inorganic phosphate showed decreasing trends in both the mean comparison and regression analyses. TCHO was considered the most affected parameter by aging in both sexes based on statistical results. In this study, we presented clinicopathological data from F344 rats for inhalation toxicity studies. We confirmed aging trends in clinicopathological parameters and identified TCHO as the parameter most affected by aging in F344 rats. These results would be helpful for inhalation research using F344 rats.

Keywords: Fischer 344 rats; inhalation; age-dependent trend; pathology, clinical; statistics

INTRODUCTION

The inhalation repeated toxicity study evaluates systemic toxicity induced by repeatedly inhaled test substances using experimental animals. During the experimental period, animals are kept in specific chambers equipped for spraying test materials and performing analyses. Control group animals are also kept in chambers supplied with fresh air to maintain the same environmental conditions as the test groups. This specific closed environment could induce stress [1] and the resulting biological changes [2, 3], making it is essential to establish historical data for inhalation toxicity tests.

Clinical pathology including hematology and serum chemistry, reflects pathological or func-

tional changes in various organs [4–6] and is essential for distinguishing between normal and abnormal conditions and evaluating whether it is a toxic change [7, 8]. It is a crucial parameter in several test guidelines for evaluating the toxicity of chemical substances, including test guidelines of the Organization for Economic Cooperation and Development [9–11]. Clinical pathology is also valuable for extrapolating the potential impact on humans.

Historical data provide a basis for understanding the biological characteristics and range of variance in animal species and strains. They help determine whether observed alterations in the toxicity assessment of chemicals, including drugs, are actual or biological changes caused by individual differences [12–15]. Understanding age-related biological characteristics is also essential for interpreting and understanding experimental results.

Recent studies on historical data have focused on neoplastic lesions of 104-week-old rats [16–18]. However, more studies for clinicopathological background data are required to evaluate toxicity for animals in diverse environment. To date, clinicopathological background data for inhalation studies have not been reported, and statistical approaches have been limited in age-related trend analysis. Therefore, this study aimes to obtain background clinicopathological data for F344 rats of different week-ages used in inhalation toxicity studies. In addition, we statistically analyzed age-dependent trends in the data collected to determine aging changes in clinical pathology.

MATERIALS AND METHODS

Animals

Six-week old male and female F344/NSlc rats from a specific pathogen-free colony were purchased from Japan SLC (Hamamatsu, Japan) via Joongang Experimental Animal (Seoul, Korea) for use as the control group in 4-, 13-, 26-, and 52-week repeated inhalation toxicity studies. The rats were used after 1 week of quarantine and acclimatization. They rats were housed in a room maintained at 22°C ± 3°C with 50% ± 20% relative humidity, artificial lighting from 08:00 to 20:00 hr, and 12–15 air changes/hr. The rats were housed individually in wire-bottomed stainless-steel mesh cages placed in exposure chambers and provided sterilized tap water and commercial rodent chow (Teklad Certified Irradiated Global 18% Protein Rodent Diet; Envigo, Indianapolis, IN, USA) *ad libitum*. Rats were exposed to clean dry air for 6 hr/d, 5 d per week for 4, 13, 26, or 52 weeks in whole-body chambers. The study protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Inhalation Toxicity Research Center (IACUC approval numbers: IACUC-1911, -1913, -1915, -1916, -1917, -1918, -1812, -1813, -1815, -1817, -1818, -1612, -1601).

Euthanasia and sample collection

All rats were fasted overnight before blood sample collection and anesthesia with isoflurane preceded euthanasia. Euthanasia was peformed by cutting the abdominal aorta and caudal vena cava after blood collection. Blood samples (7–8 mL) were collected from the abdominal aorta. Approximately 3 mL of each blood sample was placed in EDTA-containing vacutainers

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for hematological measurements. Subsequently, approximately 0.5–1 mL of blood mixed with 3.2% sodium citrate was centrifuged at 3,000 rpm for 10 min at 4°C to measure prothrombin time (PT) and activated partial thromboplastin time (APTT). Blood samples for blood chemistry analysis were also centrifuged at 3,000 rpm for 10 min at 4°C to obtain serum within 1 hr of sample collection.

Hematology

Measured hematology parameters included erythrocytes s(red blood cell, RBC), hemoglobin (HGB) concentration, hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet (PLT), leucocytes (white blood cell, WBC), differential WBC count (neutrophils [NEUA], lymphocytes [LYMA], monocytes [MONA], eosinophils [EOSA], and basophils [BASA]), and each cell-to-WBC ratio expressed as neutrophil percentage (NEU%), lymphocyte percentage (LYM%), monocyte percentage (MON%), eosinophil percentage (EOS%), basophil percentage (BAS%), respectively. Reticulocyte count (RETA) and the reticulocyte-to-RBC ratio, expressed as the reticulocyte percentage (RET%), were also measured, along with PT and APTT. Hematological parameters, excluding PT and APTT, were analyzed using ADVIA 2120i (Siemens, Munich, Germany), whereas PT and APTT were analyzed using ACL ELITE systems (Instrumentation Laboratory, Bedford, MA, USA).

Biochemistry

The biochemical parameters measured included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine (CREA), total bilirubin (TBIL), total protein (TP), albumin (ALB), albumin/globulin (A/G) ratio, total cholesterol (TCHO), triglyceride (TG), glucose (GLU), potassium (K), calcium (Ca), chloride (Cl), inorganic phosphorus (IP), and sodium (Na). These biochemical parameters were analyzed using a TBA-120FR automated clinical analyzer (Toshiba, Tokyo, Japan).

Data analysis

Data are presented as means ± S.D. Statistical analyse were performed using the SPSS Statistics version 28 software (IBM, Armonk, NY, USA). Differences between data were statistically evaluated. The homogeneity of variance was determined using Levene's test. Homogeneous and heterogeneous data were compared using one-way analysis of variance (ANOVA) and non-parametric Kruskal–Wallis test, respectively. If statistical significance ($p<0.05$) was observed, Dunnett's test (for ANOVA) or Dunn's test (for Kruskal–Wallis) was used to compare 4-week data with those of 13-, 26-, and 52-week. In addition, correlation and simple linear regression analyses were performed to determine the relationship between age and each parameter. A simple linear regression analysis was performed on parameters that showed a significant relationship with the experimental week. Regression analysis was performed on the parameters that significant in the correlation analysis.

RESULTS

Change trends of hematological parameters at different ages in male rats

Statistically significant differences were observed in NEUA, EOSA, LYM%, NEU%, MON%, and PLT at 13, 26, and 52 weeks; HCT and MCHC at 26 weeks; LYMA and BAS% at 26 and 52 weeks; EOS% at 13 and 52 weeks; RBC, MCV, MCH, and WBC at 13 and 26 weeks; MONA and PT at 26 weeks; and RETA, RET%, and BASA at 13 weeks compared those at 4 weeks (Table 1). Their *p*-values are presented in Table 1. The correlation coefficients between experimental week and MCH ($p<0.01$), WBC ($p<0.05$), LYMA ($p<0.01$), NEUA ($p<0.01$), EOSA ($p<0.05$), BASA ($p<0.01$), LYM% ($p<0.01$), NEU% ($p<0.01$), EOS% ($p<0.01$), BAS% ($p<0.01$), MON% ($p<0.01$), and PLT ($p<0.01$) were significant (Table 2). All parameters analyzed using regression tests, except for WBC count, showed significant *F* values (MCH, LYMA, NEUA, BASA, LYM%, NEU%, EOS%, BAS%, MON%, and PLT:

Table 1. Hematology data for F344 rats exposed to fresh air in whole-body chamber

Experimental week	4	13	26	52
No. of animals[1)]	24	60	50	25
Males				
RBC (× 10^6/μL)	8.78 ± 0.26	9.04 ± 0.20**	9.02 ± 0.36**	8.85 ± 0.38
HGB (g/dL)	14.9 ± 0.5	14.8 ± 0.3	14.9 ± 1.9	15.0 ± 0.6
HCT (%)	43.5 ± 1.1	43.3 ± 0.8	42.8 ± 1.6*	43.9 ± 1.7
MCV (fL)	49.6 ± 0.5	47.9 ± 0.7**	47.4 ± 0.8**	49.7 ± 0.7
MCH (pg)	17.0 ± 0.4	16.4 ± 0.4**	16.9 ± 0.3*	17.0 ± 0.3
MCHC (g/dL)	34.3 ± 0.7	34.2 ± 0.7	35.6 ± 0.7**	34.3 ± 0.4
RETA (× 10^3/μL)	204.2 ± 27.3	226.1 ± 24.9**	207.2 ± 24.6	214.7 ± 25.5
RET% (%)	2.3 ± 0.3	2.5 ± 0.3*	2.3 ± 0.3	2.4 ± 0.3
WBC (× 10^3/μL)	3.97 ± 0.90	4.47 ± 0.86*	4.91 ± 0.88**	3.64 ± 0.65
LYMA (× 10^3/μL)	2.93 ± 0.77	2.96 ± 0.64	2.67 ± 0.57*	1.88 ± 0.33**
NEUA (× 10^3/μL)	0.89 ± 0.31	1.29 ± 0.33**	1.99 ± 0.63**	1.56 ± 0.41**
EOSA (× 10^3/μL)	0.05 ± 0.02	0.08 ± 0.02**	0.07 ± 0.02**	0.08 ± 0.02**
BASA (× 10^3/μL)	0.01 ± 0.01	0.01 ± 0.01**	0.01 ± 0.00	0.00 ± 0.00
MONA (× 10^3/μL)	0.07 ± 0.02	0.10 ± 0.03	0.13 ± 0.04**	0.10 ± 0.03
LYM% (%)	73.5 ± 6.7	66.2 ± 5.6*	54.6 ± 8.3**	51.9 ± 5.6**
NEU% (%)	22.8 ± 6.8	28.9 ± 5.3*	40.2 ± 8.8**	42.5 ± 5.7**
EOS% (%)	1.4 ± 0.7	1.8 ± 0.5*	1.5 ± 0.4	2.2 ± 0.5**
BAS% (%)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1**	0.1 ± 0.1**
MON% (%)	1.7 ± 0.4	2.3 ± 0.6**	2.7 ± 0.7**	2.7 ± 0.5**
PLT (× 10^3/μL)	748 ± 42	706 ± 42*	676 ± 99**	617 ± 119**
APTT (sec)	18.0 ± 1.8	18.5 ± 2.1	16.7 ± 1.9	19.4 ± 3.3
PT (sec)	10.5 ± 0.5	10.7 ± 0.4	12.6 ± 1.4**	10.6 ± 0.3

Differences among ages are expressed as * $p<0.05$, ** $p<0.01$ based on the result of Dunnett's or Dunn's test.

[1)] APTT and PT were examined in 23 males in the 52-week study.

RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RETA, reticulocyte count; RET%, reticulocyte percentage; WBC, white blood cell; LYMA, lymphocyte count; NEUA, neutrophil count; EOSA, eosinophil count; BASA, basophil count; MONA, monocyte count; LYM%, lymphocyte percentage; NEU%, neutrophil percentage; EOS%, eosinophil percentage; BAS%, basophil percentage; MON%, monocyte percentage; PLT, platelet; APTT, activated partial thromboplastin time; PT, prothrombin time.

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Table 2. Correlation coefficients in hematology

Sex	Male	Female
Parameter	Week	Week
Week	1	1
RBC	–0.066	–0.348**
HGB	0.061	–0.089
HCT	0.057	0.07
MCV	0.151	0.716**
MCH	0.279**	0.527**
MCHC	0.138	–0.018
RETA	–0.046	–0.151
RET%	–0.026	–0.101
WBC	–0.182*	–0.570**
LYMA	–0.537**	–0.270**
NEUA	0.362**	0.012
EOSA	0.167*	–0.260**
BASA	–0.376**	–0.298**
MONA	0.065	–0.102
LYM%	–0.651**	–0.533**
NEU%	0.629**	0.548**
EOS%	0.309**	0.248**
BAS%	–0.394**	–0.274**
MON%	0.438**	0.177*
PLT	–0.297**	–0.464**
APTT	0.054	0.324**
PT	0.129	–0.323**

Week indicates the experimental week.

Statistical significance is expressed as * $p<0.05$, ** $p<0.01$.

RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RETA, reticulocyte count; RET%, reticulocyte percentage; WBC, white blood cell; LYMA, lymphocyte count; NEUA, neutrophil count; EOSA, eosinophil count; BASA, basophil count; MONA, monocyte count; LYM%, lymphocyte percentage; NEU%, neutrophil percentage; EOS%, eosinophil percentage; BAS%, basophil percentage; MON%, monocyte percentage; PLT, platelet; APTT, activated partial thromboplastin time; PT, prothrombin time.

$p<0.01$, EOSA: $p<0.05$; Table 3). All parameters analyzed using regression test, except for MCH and WBC count, showed significant t value (MCH, LYMA, NEUA, BASA, LYM%, NEU%, EOS%, BAS%, MON%, and PLT: $p<0.01$, EOSA: $p<0.05$; Table 3). R^2 value is presented in Table 3.

Change trends of hematological parameters at different ages in female rats

Statistically significant differences were observed in MCV, MCH, LYMA, LYM%, NEU%, BAS%, and PLT at 13, 26, and 52 weeks; RBC, RETA, WBC, and BASA at 26 and 52 weeks; EOS% at 52 weeks; MCHC, RET%, PT, and APTT at 26 weeks; and HGB, HCT, and NEUA at 13 weeks compared with those at 4 weeks (Table 4). Their p-values are presented in Table 4. The correlation coefficients between experimental week and RBC count ($p<0.01$), MCV ($p<0.01$), MCH ($p<0.01$), WBC count ($p<0.01$), LYMA ($p<0.01$), EOSA ($p<0.01$), BASA ($p<0.01$), LYM% ($p<0.01$), NEU% ($p<0.01$), EOS% ($p<0.01$), BAS% ($p<0.01$), MON%

Table 3. Results of simple linear regression analysis for males in hematology

Parameter	F	t	Unstandardized Coefficients		Standardized Coefficients	R^2
			B	S.E	β	
MCH						
Constant	11.343**	278.178	16.528	0.059		0.070
Week		3.368**	0.008	0.002	0.265	
WBC						
Constant	3.184	35.142	4.542	0.129		0.021
Week		–1.784	–0.009	0.005	–0.144	
LYMA						
Constant	55.612**	36.646	3.178	0.087		0.270
Week		–7.457**	–0.025	0.003	–0.52	
NEUA						
Constant	26.361**	15.038	1.166	0.078		0.149
Week		5.134**	0.015	0.003	0.387	
EOSA						
Constant	5.38*	21.056	0.066	0.003		0.035
Week		2.32*	0.000	0.000	0.186	
BASA						
Constant	22.473**	12.237	0.009	0.001		0.130
Week		–4.741**	0.000	0.000	–0.361	
LYM%						
Constant	113.622**	61.624	71.009	1.152		0.431
Week		–10.659**	–0.472	0.044	–0.657	
NEU%						
Constant	100.506**	21.555	24.698	1.146		0.401
Week		10.025**	0.441	0.044	0.633	
EOS%						
Constant	14.928**	19.599	1.464	0.075		0.091
Week		3.864**	0.011	0.003	0.301	
BAS%						
Constant	25.182**	17.158	0.217	0.013		0.144
Week		–5.018**	–0.002	0	–0.379	
MON%						
Constant	35.285**	23.233	1.998	0.086		0.190
Week		5.94**	0.02	0.003	0.436	
PLT						
Constant	17.176**	51.644	732.191	14.178		0.103
Week		–4.144**	–2.256	0.544	–0.321	

Week indicates experimental week.

Statistical significance is expressed as * $p<0.05$, ** $p<0.01$.

MCH, mean cell hemoglobin; WBC, white blood cell; LYMA, lymphocyte count; NEUA, neutrophil count; EOSA, eosinophil count; BASA, basophil count; LYM%, lymphocyte percentage; NEU%, neutrophil percentage; EOS%, eosinophil percentage; BAS%, basophil percentage; MON%, monocyte percentage; PLT, platelet.

($p<0.05$), PLT ($p<0.01$), APTT ($p<0.01$), and PT ($p<0.01$) were significant (Table 2). All parameters analyzed using the regression test showed significant *F*- (RBC, MCV, MCH, WBC, LYMA, EOSA, BASA, LYM%, NEU%, BAS%, PLT, and APTT: $p<0.01$, EOS%, MON%,

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Table 4. Hematology data for F344 rats exposed to fresh air in whole-body chamber

Experimental week	4	13	26	52
No. of animals[1)]	25	60	50	25
Females				
RBC (× 10^6/μL)	8.54 ± 0.37	8.46 ± 0.22	8.29 ± 0.32**	8.16 ± 0.25**
HGB (g/dL)	15.0 ± 0.6	14.6 ± 0.4**	15.3 ± 0.5	14.8 ± 0.4
HCT (%)	43.2 ± 1.3	42.1 ± 0.9**	42.6 ± 1.9	43.1 ± 1.1
MCV (fL)	50.6 ± 1.1	49.8 ± 0.6**	51.4 ± 0.5**	52.8 ± 1.0**
MCH (pg)	17.6 ± 0.4	17.3 ± 0.4**	18.4 ± 0.3**	18.1 ± 0.4**
MCHC (g/dL)	34.8 ± 0.9	34.7 ± 0.7	35.8 ± 0.6**	34.4 ± 0.5
RETA (× 10^3/μL)	232.2 ± 158.3	184.7 ± 25.7	171.0 ± 18.9**	182.4 ± 51.3**
RET% (%)	2.8 ± 2.3	2.2 ± 0.3	2.1 ± 0.3*	2.2 ± 0.7
WBC (× 10^3/μL)	3.13 ± 0.87	2.84 ± 0.80	2.77 ± 0.73*	1.42 ± 0.36**
LYMA (× 10^3/μL)	2.37 ± 0.73	1.97 ± 0.61**	1.76 ± 0.54*	0.80 ± 0.28**
NEUA (× 10^3/μL)	0.62 ± 0.16	0.73 ± 0.24**	0.87 ± 0.34	0.51 ± 0.18
EOSA (× 10^3/μL)	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.02
BASA (× 10^3/μL)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00**	0.00 ± 0.00**
MONA (× 10^3/μL)	0.06 ± 0.03	0.07 ± 0.07	0.07 ± 0.02	0.05 ± 0.02
LYM% (%)	75.5 ± 4.1	69.2 ± 8.7*	63.1 ± 8.0**	56.1 ± 14.2**
NEU% (%)	20.4 ± 4.0	25.8 ± 6.0**	31.9 ± 8.2**	36.9 ± 12.6**
EOS% (%)	1.5 ± 0.4	1.8 ± 0.6	1.5 ± 0.8	2.4 ± 1.9*
BAS% (%)	0.2 ± 0.1	0.2 ± 0.1*	0.1 ± 0.1**	0.1 ± 0.1**
MON% (%)	1.9 ± 0.6	2.5 ± 3.7	2.5 ± 0.5	3.5 ± 0.8
PLT (× 10^3/μL)	808 ± 99	742 ± 51*	649 ± 85**	534 ± 169**
APTT (sec)	17.5 ± 2.1	18.7 ± 2.0	19.3 ± 4.1	21.0 ± 1.8**
PT (sec)	10.5 ± 0.7	10.4 ± 0.5	10.0 ± 0.7	9.9 ± 0.4**

Differences among ages are expressed as * p<0.05, ** p<0.01 based on the result of Dunnett's or Dunn's test.

[1)] APTT and PT were examined in 21 females in the 52-week study.

RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RETA, reticulocyte count; RET%, reticulocyte percentage; WBC, white blood cell; LYMA, lymphocyte count; NEUA, neutrophil count; EOSA, eosinophil count; BASA, basophil count; MONA, monocyte count; LYM%, lymphocyte percentage; NEU%, neutrophil percentage; EOS%, eosinophil percentage; BAS%, basophil percentage; MON%, monocyte percentage; PLT, platelet; APTT, activated partial thromboplastin time; PT, prothrombin time.

and PT: p<0.05) and t-values (RBC, MCV, MCH, WBC, LYMA, EOSA, BASA, LYM%, NEU%, BAS%, PLT, APTT, and PT: p<0.05, EOS% and MON%: p<0.05; Table 5). R^2 value are presented in Table 5.

Change trends of biochemistry parameters at different ages in male rats

Statistically significant differences were observed in ALP, TP, ALB, A/G ratio, TG, TCHO, and IP at 13, 26, and 52 weeks; ALT, AST, and Cl at 26 and 52 weeks; GLU at 13 and 26 weeks; CREA at 52 weeks; and Na and Ca at 26 weeks compared with those at 4 weeks (Table 6). Their p-values are presented in Table 6. The correlation coefficients between experimental week and ALT (p<0.01), AST (p<0.01), ALP (p<0.01), TP (p<0.01), ALB (p<0.01), A/G ratio (p<0.01), TG (p<0.01), TCHO (p<0.01), GLU (p<0.01), BUN (p<0.05), CREA (p<0.05), Na (p<0.01), Cl (p<0.01), and IP (p<0.01) levels were significant (Table 7). All parameters analyzed using the regression test showed significant F- and t-values (ALT, AST, ALP, TP, ALB,

Table 5. Results of simple linear regression analysis for females in hematology

Parameter	F	t	Unstandardized Coefficients		Standardized Coefficients	R^2
			B	S.E.	β	
RBC						
Constant	20.967**	189.257	8.545	0.045		0.128
Week		–4.579**	–0.008	0.002	–0.358	
MCV						
Constant	144.754**	377.207	49.554	0.131		0.503
Week		12.031**	0.062	0.005	0.709	
MCH						
Constant	56.467**	244.87	17.35	0.071		0.283
Week		7.514**	0.021	0.003	0.532	
WBC						
Constant	59.757**	30.075	3.301	0.11		0.295
Week		–7.73**	–0.033	0.004	–0.543	
LYMA						
Constant	8.95**	14.304	1.533	0.107		0.059
Week		–2.992**	–0.013	0.004	–0.243	
EOSA						
Constant	12.167**	18.471	0.051	0.003		0.078
Week		–3.488**	0.000	0.000	–0.28	
BASA						
Constant	12.121**	7.265	0.006	0.001		0.078
Week		–3.481**	0.000	0.000	–0.28	
LYM%						
Constant	53.658**	60.243	74.226	1.232		0.273
Week		–7.325**	–0.355	0.048	–0.522	
NEU%						
Constant	60.074**	21.88	21.604	0.987		0.296
Week		7.751**	0.301	0.039	0.544	
EOS%						
Constant	5.724*	12.083	1.483	0.123		0.038
Week		2.393*	0.012	0.005	0.196	
BAS%						
Constant	12.153**	14.676	0.216	0.015		0.078
Week		–3.486**	–0.002	0.001	–0.28	
MON%						
Constant	4.712*	5.706	1.94	0.34		0.032
Week		2.171*	0.029	0.013	0.179	
PLT						
Constant	65.99**	47.366	803.03	16.954		0.316
Week		–8.123**	–5.418	0.667	–0.562	
APTT						
Constant	16.997**	40.523	17.723	0.437		0.106
Week		4.123**	0.071	0.017	0.326	
PT						
Constant	19.31*	127.187	10.582	0.083		0.119
Week		–4.394**	–0.014	0.003	–0.345	

Week indicates the experimental week.

Statistical significance is expressed as * $p<0.05$, ** $p<0.01$.

RBC, red blood cell; MCV, mean cell volume; MCH, mean cell hemoglobin; WBC, white blood cell; LYMA, lymphocyte count; EOSA, eosinophil count; BASA, basophil count; LYM%, lymphocyte percentage; NEU%, neutrophil percentage; EOS%, eosinophil percentage; BAS%, basophil percentage; MON%, monocyte percentage; PLT, platelet; APTT, activated partial thromboplastin time; PT, prothrombin time.

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Table 6. Serum chemistry data for F344 rats exposed to fresh air in whole-body chamber

Experimental week	4	13	26	52
No. of animals	25	60	50	25
Males				
ALT (IU/L)	42.6 ± 13.8	46.8 ± 13.6	111.5 ± 45.2**	117.8 ± 38.8**
AST (IU/L)	79.5 ± 11.6	93.2 ± 20.5	162.7 ± 42.2**	167.4 ± 39.4**
ALP (IU/L)	697 ± 39	397 ± 49**	317 ± 32**	323 ± 30**
TBIL (mg/dL)	0.18 ± 0.04	0.17 ± 0.03	0.20 ± 0.03	0.20 ± 0.00
TP (g/dL)	5.6 ± 0.4	6.1 ± 0.5**	6.8 ± 0.3**	6.5 ± 0.2**
ALB (g/dL)	3.9 ± 0.2	4.1 ± 0.3**	4.3 ± 0.2**	4.2 ± 0.1**
A/G ratio	2.24 ± 0.11	2.04 ± 0.13**	1.79 ± 0.08**	1.77 ± 0.06**
TG (mg/dL)	53.9 ± 15.5	96.3 ± 46.6**	100.4 ± 34.4**	135.1 ± 47.6**
TCHO (mg/dL)	62.2 ± 5.7	77.3 ± 12.2**	76.2 ± 7.0**	127.3 ± 15.0**
GLU (mg/dL)	161 ± 18	145 ± 29*	131 ± 10**	179 ± 26
BUN (mg/dL)	18.6 ± 2.6	17.4 ± 2.9	19.9 ± 2.0	18.7 ± 1.6
CREA (mg/dL)	0.41 ± 0.05	0.44 ± 0.05	0.42 ± 0.03	0.45 ± 0.03**
Na (mmol/L)	137.2 ± 9.9	135.6 ± 11.0	143.8 ± 5.2**	143.1 ± 0.7
Cl (mmol/L)	97.3 ± 7.0	95.9 ± 7.9	103.3 ± 3.8**	103.7 ± 0.9**
K (mmol/L)	4.31 ± 0.60	4.19 ± 0.43	4.43 ± 0.28	4.33 ± 0.25
Ca (mg/dL)	9.5 ± 0.8	9.8 ± 1.1	9.9 ± 0.4**	10.0 ± 0.2
IP (mg/dL)	6.6 ± 1.0	5.4 ± 0.8**	6.2 ± 0.5*	4.5 ± 0.8**

Differences among ages are expressed as * p<0.05, ** p<0.01, based on Dunnett's or Dunn's test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; TP, total protein; ALB, albumin; A/G, albumin/globulin; TG, triglyceride; TCHO, total cholesterol; GLU, glucose; BUN, blood urea nitrogen; CREA, creatinine; Na, sodium; Cl, chloride; K, potassium; Ca, calcium; IP, inorganic phosphorus.

A/G ratio, TG, TCHO, GLU, Na, Cl, and IP: p<0.01, BUN and CREA: p<0.01; Table 8 and Fig. 1). R^2 value was presented in Table 8.

Change trends of biochemistry parameters at different ages in female rats

Statistically significant differences were observed in ALP, TP, ALB, A/G ratio, TCHO, and IP at 13, 26, and 52 weeks; ALT, TG, CREA, and Ca at 26 and 52 weeks; AST at 13 and 52 weeks; GLU at 52 weeks; and TBIL, Na, Cl, and K at 26 weeks compared with those at 4 weeks (Table 9). Their p-values are presented in Table 9. The correlation coefficients between experimental week and ALT (p<0.01), AST (p<0.01), ALP (p<0.01), TBIL (p<0.01), TP (p<0.01), ALB (p<0.01), A/G ratio (p<0.01), TG (p<0.01), TCHO (p<0.01), GLU (p<0.01), BUN (p<0.01), CREA (p<0.01), Na (p<0.01), Cl (p<0.01), Ca (p<0.01), and IP (p<0.01) were significant (Table 7). All parameters analyzed using the regression test showed significant F- and t-values (F- and t-values for ALT, AST, ALP, TBIL, TP, ALB, A/G ratio, TG, TCHO, GLU, BUN, CREA, Na, Cl, Ca, and IP: p<0.01; Table 10 and Fig. 1). R^2 value are presented in Table 10.

DISCUSSION

We collected background clinicopathology data for an inhalation study and described trends and alterations in hematological and biochemical parameters with aging in F344 rats.

Table 7. Correlation coefficients in serum chemistry

Sex	Male	Female
Parameter	Week	week
Week	1	1
ALT	0.476**	0.350**
AST	0.650**	0.494**
ALP	–0.630**	–0.685**
TBIL	0.064	–0.227**
TP	0.495**	0.753**
ALB	0.315**	0.705**
A/G	–0.703**	–0.667**
TG	0.442**	0.529**
TCHO	0.807**	0.853**
GLU	0.237**	0.604**
BUN	0.187*	0.298**
CREA	0.199*	0.426**
Na	0.301**	0.292**
K	0.1	0.053
Cl	0.408**	0.290**
Ca	0.153	0.290**
IP	–0.407**	–0.412**

Week indicates the experimental week.

Statistical significance is expressed as * $p<0.05$, ** $p<0.01$.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; TP, total protein; ALB, albumin; A/G, albumin/globulin; TG, triglyceride; TCHO, total cholesterol; GLU, glucose; BUN, blood urea nitrogen; CREA, creatinine; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; IP, inorganic phosphorus.

Statistical significance in correlation analysis with age was noted in 27 parameters in males and 31 parameters in females for hematology and clinical chemistry. Regression analysis was performed on parameters showing the statistical significance in correlation analysis. Positive correlation coefficients indicate an increasing tendency and a direct linear relationship, whereas negative values indicate a decreasing tendency and an inverse linear relationship [19, 20]. Correlation and linear regression analyses revealed a direct linear relationship with age in both sexes for MCH, NEU%, EOS%, MON%, ALT, AST, TP, ALB, TG, TCHO, GLU, BUN, CREA, Na, Cl and an inverse linear relationship for PLT, LYMA, BASA, LYM%, BAS%, ALP, A/G ratio, and IP. Females additionally showed a direct linear relationship with MCV, APTT, and Ca and an inverse linear relationship with RBC, PT, WBC, EOSA, and TBIL with increasing weeks. Males showed a direct linear relationship with NEUA and EOSA, with an increase in weeks. However, most parameters, except for TCHO (R^2 = 0.652 in males, R^2 = 0.728 in females) and MCV (R^2 = 0.503) and TP (R^2 = 0.567) in females, had R^2 values below 0.5. In addition, these results indicated that although aging is one of the factors affecting values, there are other more affectable factors to the values. Several factors, including aging pathobiology [21, 22], spontaneous changes [23, 24], and sample size, could affect data on clinical pathological parameters.

The intersection of results for mean comparison and regression analysis was examined to

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Table 8. Results of simple linear regression analysis for males in serum chemistry

Parameter	F	t	Unstandardized Coefficients		Standardized Coefficients	R^2
			B	S.E.	β	
ALT						
Constant	46.207**	5.4	48.521	8.986		0.226
Week		6.798**	2.309	0.340	0.476	
AST						
Constant	115.713**	15.28	69.860	4.572		0.423
Week		10.757**	1.859	0.173	0.650	
ALP						
Constant	104.05**	36.193	531.345	14.681		0.397
Week		–10.201**	–5.662	0.555	–0.630	
TP						
Constant	51.327**	83.692	5.872	0.070		0.245
Week		7.164**	0.019	0.003	0.495	
ALB						
Constant	17.463**	109.613	4.002	0.037		0.100
Week		4.179**	0.006	0.001	0.315	
A/G ratio						
Constant	154.073**	104.666	2.152	0.021		0.494
Week		–12.413**	–0.010	0.001	–0.703	
TG						
Constant	38.429**	11.837	66.352	5.606		0.196
Week		6.199**	1.314	0.212	0.442	
TCHO						
Constant	295.616**	29.711	55.768	1.877		0.652
Week		17.193**	1.220	0.071	0.807	
GLU						
Constant	9.426**	37.185	139.237	3.744		0.056
Week		3.07**	0.435	0.142	0.237	
BUN						
Constant	5.732*	8.851	15.979	1.805		0.035
Week		2.394*	0.163	0.068	0.187	
CREA						
Constant	6.521*	67.522	0.418	0.006		0.040
Week		2.554*	0.001	0.000	0.199	
Na						
Constant	15.727**	112.172	135.439	1.207		0.091
Week		3.966**	0.181	0.046	0.301	
Cl						
Constant	31.592**	108.464	95.423	0.880		0.167
Week		5.621**	0.187	0.033	0.408	
IP						
Constant	31.303**	46.397	6.266	0.135		0.165
Week		–5.595**	–0.029	0.005	–0.407	

Week indicates the experimental week.

Statistical significance is expressed as * $p<0.05$, ** $p<0.01$.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TP, total protein; ALB, albumin; A/G, albumin/globulin; TG, triglyceride; TCHO, total cholesterol; GLU, glucose; BUN, blood urea nitrogen; CREA, creatinine; Na, sodium; Cl, chloride; IP, inorganic phosphorus.

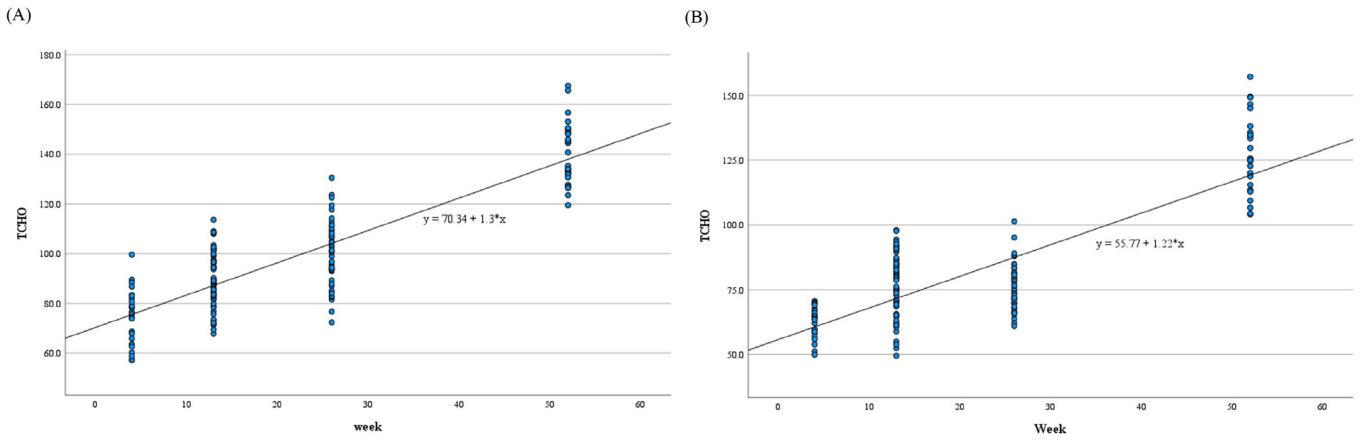

Fig. 1. Age-dependent TCHO change trend in F344 rats. Regression analysis for TCHO shows a direct linear relationship between TCHO and age in both sexes. The R^2 values for TCHO were 0.652 in males (A) and 0.728 in females (B). TCHO, total cholesterol.

Table 9. Serum chemistry data for F344 rats exposed to fresh air in whole body chamber

Experimental week	4	13	26	52
No. of animals	25	60	50	25
Females				
ALT (IU/L)	40.3 ± 11.7	41.8 ± 9.5	85.2 ± 40.5**	73.1 ± 22.7**
AST (IU/L)	81.5 ± 10.5	96.2 ± 15.3*	136.8 ± 38.7	153.1 ± 60.2**
ALP (IU/L)	537 ± 53	295 ± 44**	279 ± 37**	203 ± 33**
TBIL (mg/dL)	0.16 ± 0.03	0.15 ± 0.02	0.14 ± 0.03*	0.14 ± 0.04
TP (g/dL)	5.7 ± 0.5	6.3 ± 0.4**	7.0 ± 0.4**	7.5 ± 0.4**
ALB (g/dL)	4.0 ± 0.3	4.2 ± 0.2**	4.5 ± 0.2**	4.8 ± 0.2**
A/G ratio	2.25 ± 0.16	2.00 ± 0.12**	1.78 ± 0.11**	1.77 ± 0.08**
TG (mg/dL)	24.5 ± 20.0	21.8 ± 8.6	33.7 ± 16.0*	49.4 ± 15.8**
TCHO (mg/dL)	74.8 ± 11.1	89.6 ± 11.0**	99.2 ± 12.8**	140.2 ± 12.7**
GLU (mg/dL)	127 ± 14	115 ± 18	120 ± 12	161 ± 10**
BUN (mg/dL)	20.2 ± 3.7	19.1 ± 2.5	20.6 ± 2.2	21.9 ± 2.3
CREA (mg/dL)	0.40 ± 0.03	0.42 ± 0.04	0.43 ± 0.04**	0.47 ± 0.04**
Na (mmol/L)	137.8 ± 10.2	136.4 ± 10.9	148.9 ± 1.8**	142.5 ± 1.1
Cl (mmol/L)	100.4 ± 8.4	99.3 ± 8.0	107.8 ± 2.1**	104.1 ± 1.4
K (mmol/L)	4.14 ± 0.43	4.00 ± 0.41	4.64 ± 0.71**	4.00 ± 0.36
Ca (mg/dL)	9.6 ± 0.8	9.7 ± 0.9	10.8 ± 1.1**	10.3 ± 0.3**
IP (mg/dL)	6.7 ± 1.0	4.9 ± 0.8**	5.8 ± 1.2*	4.1 ± 1.0**

Differences among ages are expressed as * $p<0.05$, ** $p<0.01$ based on the result of Dunnett's or Dunn's test.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; TP, total protein; ALB, albumin; A/G, albumin/globulin; TG, triglyceride; TCHO, total cholesterol; GLU, glucose; BUN, blood urea nitrogen; CREA, creatinine; Na, sodium; Cl, chloride; K, potassium; Ca, calcium; IP, inorganic phosphorus.

confirm change trends with increasing age. Mean comparison results identified parameters that showed significant differences from the 4-week mark across all weeks. LYM%, NEU%, PLT, ALP, TP, ALB, A/G ratio, TCHO, and IP in both sexes, LYMA in females, and NEUA, EOSA, MON%, and TG in males exhibited similar change patterns between mean comparison and regression analyses. NEUA, EOSA, NEU%, MON%, TP, ALB, TG, and TCHO increased, whereas LYMA, LYM%, PLT, ALP, A/G ratio, and IP decreased in both analyses. Parameters

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Table 10. Results of simple linear regression analysis for females in serum chemistry

Parameter	F	t	Unstandardized Coefficients		Standardized Coefficients	R^2
			B	S.E.	β	
ALT						
Constant	21.756**	7.053	50.563	7.169		0.122
Week		4.664**	1.264	0.271	0.35	
AST						
Constant	50.314**	14.53	71.615	4.929		0.244
Week		7.093**	1.321	0.186	0.494	
ALP						
Constant	137.72**	37.961	422.898	11.14		0.469
Week		–11.735**	–4.942	0.421	–0.685	
TBIL						
Constant	8.499**	28.1	0.158	0.006		0.052
Week		–2.915**	–0.001	0	–0.227	
TP						
Constant	204.488**	88.11	5.868	0.067		0.567
Week		14.3**	0.036	0.003	0.753	
ALB						
Constant	154.496**	111.834	3.994	0.036		0.498
Week		12.43**	0.017	0.001	0.705	
A/G ratio						
Constant	125.315**	98.625	2.131	0.022		0.445
Week		–11.194**	–0.009	0.001	–0.667	
TG						
Constant	60.471**	8.447	16.868	1.997		0.279
Week		7.776**	0.587	0.075	0.529	
TCHO						
Constant	417.612**	41.8	70.335	1.683		0.728
Week		20.436**	1.3	0.064	0.853	
GLU						
Constant	89.591**	44.926	107.725	2.398		0.365
Week		9.465**	0.858	0.091	0.604	
BUN						
Constant	15.154**	50.778	19.088	0.376		0.089
Week		3.893**	0.055	0.014	0.298	
CREA						
Constant	34.58**	67.77	0.4	0.006		0.181
Week		5.88**	0.001	0	0.426	
Na						
Constant	14.504**	108.489	137.393	1.266		0.085
Week		3.808**	0.182	0.048	0.292	
Cl						
Constant	14.305**	105.67	99.889	0.945		0.084
Week		3.782**	0.135	0.036	0.29	
Ca						
Constant	14.28**	72.581	9.709	0.134		0.084
Week		3.779**	0.019	0.005	0.290	

Table 10. Continued

Parameter	*F*-value	*t*-value	Unstandardized coefficients		Standardized coefficients	R^2
			B	S.E.	β	
IP						
Constant	31.945**	36.35	6.011	0.165		0.170
Week		–5.652**	–0.035	0.006	–0.412	

Week indicates the experimental week.

Statistical significance is expressed as * $p<0.05$, ** $p<0.01$.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TBIL, total bilirubin; TP, total protein; ALB, albumin; A/G, albumin/globulin; TG, triglyceride; TCHO, total cholesterol; GLU, glucose; BUN, blood urea nitrogen; CREA, creatinine; Na, sodium; Cl, chloride; Ca, calcium; IP, inorganic phosphorus.

with R^2 values greater than 0.5 in regression analysis among the common significant parameters in both mean comparison and regression analysis were TCHO in both sexes and TP in females. Therefore, we considered that TCHO is the parameter most affected by aging in both sexes. Age-related changes in TCHO have also been reported in humans, with suggested causes being decreased low-density lipoprotein receptor and the conversion of cholesterol to bile acid with aging [25, 26].

Until now, most studies on trends in historical data have not used statistical analysis or only performed mean comparisons [27–29]. We conducted a mean comparison and regression analysis to clarify the relationships with aging. The results showed that mean difference comparison and trend analysis did not yield exactly the same results; however, they showed similar pattern and could be complementary. Therefore, we propose applying both mean comparison and regression analyses together for trend analysis.

In this study, the clinicopathological background data of F344 rats used in inhalation toxicity studies were presented. To our knowledge, we are the first to present the clinicopathological historical data for inhalation study and statistically analyze the age-related change trends. We confirmed aging trends in clinicopathological parameters and concluded that TCHO is most affected by aging under condition of this study. Further research is required to understand how age affects clinicopathological parameters. These results will be helpful for study design and data interpretation in the field of research using rats.

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