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Effect of Sex and Age on Serum Biochemical Reference Ranges in C57BL/6J Mice

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The C57BL/6J mouse strain is widely used as a common genomic background for many gene-modified murine models. However, few data on its clinical biochemistry are available. Therefore, we conducted a study to provide new protocols for serum biochemical screening and developed the reference range for a set of 13 analytes that pertain to lipoprotein metabolism, electrolyte balance, and data reflecting function of the heart, liver, kidneys, and pancreas. Male and female mice were studied, and blood samples were obtained at six and 20 weeks of age. Of 13 parameters studied, 12 were affected by age and sex. Briefly, male mice had higher triglycerides, cholesterol, high-density lipoprotein cholesterol, glucose, and amylase values. With age, mice of both sexes developed higher triglycerides and glucose concentrations, as well as aspartate and alanine transaminase activities. A significant difference between mice and humans was noted for amylase activity, which is extremely high in this healthy mouse strain. Therefore, we suggest that caution should be taken when data are interpreted to indicate gastrointestinal disease in murine models. The reference values for murine clinical biochemical analytes obtained during the study reported here should be useful for characterizing the biochemical phenotype of experimental mice.

The laboratory mouse is the most prevalent experimental system for study of human diseases. This is due to: easy breeding, short generation time, inbred strains available, refined genetic map of the mouse genome, and detailed understanding of its immunologic properties. Advances in transgenic and gene-targeting approaches in the murine system over the past decades have made it possible for researchers to dissect in depth the mechanisms of the many human diseases and to alter gene expression in vivo in many diverse ways to explore new possibilities for treatment of diseases. Considering the huge numbers of experiments on this tiny animal, it is surprising that little is known of its biochemical phenotype. Limited clinical biochemistry information is available through a few studies (4, 5, 7, 8, 11, 13, 14). Therefore, we conducted a study to provide new protocols for serum biochemical screening of mice and to determine the reference range for a set of analytes by applying methods currently used in human medicine. The C57BL/6J mouse was chosen for study due to the fact that its common genomic background is widely used for many gene-targeted murine models. Our reference range covers lipoprotein metabolism, electrolyte balance, and data reflecting function of the heart, liver, kidneys, and pancreas.

Materials and Methods

Animals and samples. Clinically normal male and female C57BL/6J mice were obtained from Taconic M&B Breeding and Research Center (Taconic M&B, Ry, Denmark) and were housed under pathogen-free conditions in Micro-Isolator™ cages (Lab Products Inc, Seaford, Del.). containing Lignocel 3/4 bedding (Taconic M&B). The mice were serologically screened for known rodent viruses, bacteria, fungi, and parasites according to recommendations of the Federation of European Laboratory Animal Science Associations (FELASA), and positive results were not obtained. All mice were fed a standard diet, Altromin 1314 containing 22.5% protein and 0.5% fat, with total energy of 12.5 MJ/kg (Chr.petersen, ringsted, Denmark). Room conditions were maintained by use of a 12/12-h light/dark cycle at 20 to 22°C and 30 to 53% humidity.

Mice were randomly allotted to two groups, from which blood samples were collected at six or 20 weeks of age. Each group had 20 male and 20 female mice. Blood was obtained by cardiac puncture, in conjunction with euthanasia of mice by use of carbon dioxide, and the serum was stored at –20°C until assay. All experiments were approved by the local ethics committee.

Serum analysis. All serologic parameters (except high-density lipoprotein cholesterol [HDLC] concentration) were determined by depositing 10 µl of serum from individual mice on Vitros II slides, followed by analysis using a Vitros DT60II Chemistry System (Ortho-Clinical Diagnostics, Johnson-Johnson Co., Rochester, N.Y.). For HDLC concentration, 50 µl of serum was pipetted into a VitrosDTMicro HDL Tube (Ortho-Clinical Diagnostics), mixed thoroughly, then kept standing for at least five minutes. The tube was then centrifuged, and 10 µl of cleared supernatant from the tube was transferred onto a Vitros II slide and analyzed by use of the Vitros DT60II Chemistry System.

The Vitros II slides used in this study included those for alanine transaminase (ALT; catalog No. 1859685), albumin (catalog No. 8105959), amylase (catalog No. 1560945), aspartate transaminase (AST; catalog No. 1825579), cholesterol (cat No. 1532175), creatine kinase (CK) MB isoenzyme (catalog No. 1855105), creatinine (catalog No. 8327462), glucose (catalog No. 1532316), total cholesterol (Micro; catalog No. 1335504), potassium (catalog No. 1532258), sodium

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Table 1. Serum biochemical parameters in mice at six weeks of age

	Lipid metabolism			Heart	Kidneys	Electrolytes		Liver				Pancreas	
	TG (m*M*)	Chol (m*M*)	HDLC (m*M*)	CK MB (U/L)	Creatinine (m*M*)	Na^+ (m*M*)	K^+ (m*M*)	AST (U/L)	ALT (U/L)	ALB (g/L)	TP (g/L)	GLU (m*M*)	AMYL (U/L)
						Mouse (female; n = 20)							
Mean	0.78	2.03	1.36	119.00	16.80	156.35*	5.84	43.20*	26.53	29.55	46.90	5.88	2,595
SD	± 0.17	± 0.31	± 0.14	± 22.9	± 2.14	± 10.00	± 0.51	± 9.47	± 4.68	± 2.09	± 3.24	± 1.22	± 212.85
CI min	0.70	1.88	1.30	107.61	15.80	151.66	5.59	38.77	24.27	28.57	45.38	5.31	2,496
CI max	0.86	2.17	1.43	130.39	17.80	161.04	6.08	47.63	28.78	30.53	48.42	6.45	2,694
						Mouse (male; n = 20)							
Mean	0.94*	2.91*	2.15*	106.95	17.80	150.95	6.35*	32.16	27.21	30.11	49.05*	8.69*	3,035*
SD	± 0.23	± 0.31	± 0.32	± 32.51	± 2.23	± 2.46	± 0.33	± 8.01	± 4.95	± 1.24	± 2.68	±1.34	± 678.70
CI min	0.83	2.75	1.99	91.74	16.72	149.76	6.20	28.30	24.82	29.51	47.76	8.05	2,708
CI max	1.04	3.05	2.30	122.16	18.86	152.13	6.51	36.02	29.60	30.70	50.34	9.34	3,362
						Humans							
Male	< 2.26†	< 5.16†	> 0.91†	1-300	44-106	135-145	3.6-5	8-39	3-42	35-50	63-82	3.6-5.8	30-110
Female	< 2.26†	< 5.16†	> 0.91†	1-300	62-124	135-145	3.6-5	14-50	13-61	35-50	63-82	4.2-6.1	30-110

*$P < 0.05$: six-week-old male mice vs. females of the same age.
†Age-dependent and/or skewed variable.
TG = triglycerides, Chol = cholesterol, HDLC = high-density lipoprotein cholesterol, CK MB = creatine kinase MB isoenzyme, AST = aspartate transaminase, ALT = alanine transaminase, ALB = albumin, TP = total protein, GLU = glucose, AMYL = amylase, CI = 95% confidence interval: min = minimum, max = maximum.

Table 2. Serum biochemical parameters in mice at 20 weeks of age

	Lipid metabolism			Heart	Kidneys	Electrolytes		Liver				Pancreas	
	TG (m*M*)	Chol (m*M*)	HDLC (m*M*)	CK MB (U/L)	Creatinine (m*M*)	Na^+ (m*M*)	K^+ (m*M*)	AST (U/L)	ALT (U/L)	ALB (g/L)	TP (g/L)	GLU (m*M*)	AMYL (U/L)
						Mouse (female; n = 20)							
Mean	1.00	1.99	1.49	126.25	19.40*	150.45	5.56	61.65	41.75	28.90*	52.85*	9.19	2,539
SD	± 0.22	± 0.13	± 0.17	± 110.69	± 1.73	± 2.04	± 0.45	± 22.80	± 10.2	± 2.47	± 1.88	± 0.68	± 252.35
CI min	0.91	1.93	1.41	74.45	18.59	149.50	5.35	50.97	36.97	27.74	51.97	8.88	2,421
CI max	1.11	2.05	1.56	178.05	20.21	151.40	5.77	72.33	46.53	30.06	53.73	9.51	2,657
						Mouse (male; n = 20)							
Mean	1.31*	2.48*	1.77*	152.75	17.70	151.75*	5.66	67.95	51.05	25.75	49.75	11.18*	3,254*
SD	± 0.19	±0.20	±0.21	±162.88	±1.63	± 1.55	± 0.32	± 31.70	± 26.7	± 5.92	± 2.29	± 1.38	± 557.60
CI min	1.22	2.39	1.67	76.52	16.94	151.02	5.50	55.11	38.58	22.98	48.68	10.53	2,993
CI max	1.39	2.58	1.87	228.98	18.46	152.48	5.81	84.79	63.52	28.52	50.82	11.82	3,514
						Humans							
Male	< 2.26†	< 5.16†	> 0.91†	1-300	44-106	135-145	3.6-5	8-39	3-42	35-50	63-82	3.6-5.8	30-110
Female	< 2.26†	< 5.16†	> 0.91†	1-300	62-124	135-145	3.6-5	14-50	13-61	35-50	63-82	4.2-6.1	30-110

*$P < 0.05$: 20-week-old male mice vs. females of the same age.
†Age-dependent and/or skewed variable.
See Table 1 for key.

(catalog No. 1532290), triglycerides (catalog No. 1532159), and total protein (catalog No. 1425800).

Statistical analysis. Since the distribution of the data was unknown, variables were analyzed by use of the Kruskal-Wallis nonparametric analysis of variance and the nonparametric Mann-Whitney test. Statistical significance was set at $P < 0.05$.

Results

The biochemical and metabolic values derived from mouse sera are listed in Tables 1 and 2, and provide ranges broken down by sex and age. The ranges for healthy humans are offered as a reference (Operator's Manual, Ortho-Clinical Diagnostics).

At six weeks of age. Compared with female mice, male mice at this age had higher triglycerides, total cholesterol, HDLC, potassium, glucose, amylase, and total protein values, whereas female mice had higher sodium and AST values.

At 20 weeks of age. In comparison with female mice, male mice had significantly higher triglycerides, cholesterol, HDLC, sodium, glucose, and amylase values, whereas female mice had higher creatinine, total protein, and albumin concentrations.

Age factor (20 weeks versus six weeks). Overall, older mice had higher triglycerides, AST, ALT, and glucose values. Female mice had significantly higher creatinine and total protein concentrations, whereas male mice had higher amylase activity. In contrast, HDLC, total cholesterol, potassium, and albumin concentrations decreased with age in males, whereas females had only decreased sodium concentration.

A significant difference between mice and humans was noted for amylase activity, which was extremely high in C57BL/6J mice, compared with the reference value for humans. In mice, lower concentrations were observed for creatinine, albumin, and total protein, whereas sodium and potassium concentrations were higher.

Discussion

In the study reported here, C57BL/6J mice were subjected to clinical biochemical phenotyping, which involved determination of analytes that reflect lipoprotein metabolism, electrolyte bal-

ance, and function of certain organs, including the heart, liver, kidneys, and pancreas. Age and sex had a substantial effect on 12 of 13 serum biochemical parameters. This effect has been proved in rats and hamsters: age and sex are of importance in classification of serum biochemical data (2, 3, 10).

Two time points (six and 20 weeks of age) were chosen on the basis that most experiments on mice are started at six to seven weeks of age and are ended at 20 to 24 weeks of age. For instance, in the apoE knockout mouse, a common murine model used in atherosclerosis studies, the disease begins at six to seven weeks of age by attachment of mononuclear cells to the vessel walls. These mice then develop advanced lesions at around 20 weeks of age (12). The atherosclerotic lesions were larger and more advanced in young female apoE knockout mice than in males, whereas the difference was diminished in aged female mice (1).

It has been proposed that sex plays a role in mediating immune reactions and disease progression. It may be explained by the effects of estrogens on the cellular immune response because addition of 17b-estradiol induced a proliferative T-cell response to oxidized low-density lipoprotein (LDL) concentration in spleen cells from young female apoE knockout mice; such effect was not seen in male mice (1).

Although some of the serum biochemical parameters in C57BL/6 mice have been reported by others (8, 11), our data differ from theirs by focusing on the aforementioned special time points and because we have adapted new methods that permit use of small volumes of blood, require less calibration effects, and generate results more rapidly than would otherwise be possible. Since populations used for clinical measurements often do not show a normal Gaussian distribution (6), use of standard deviation may lead to serious mistakes in the evaluation of what is "normal" for a given population. We, therefore, also provide percentiles for our data, which reflect the empiric distribution of the data rather than a theoretical one. From the provided percentiles, the readers can easily distinguish the outlying parameters in large data sets.

Although 12 of 13 serum biochemical parameters indicated significant difference due to age and/or sex, all data were obtained from healthy mice and most of the parameters were indeed within the normal range, compared with those for humans. The CK MB value, an index of cardiomyocyte damage, had large variation among individuals, which was probably due to cardiac puncture when blood samples were collected.

Surprisingly, the amylase value ranged between 2,500 and 3,200 U/L in the healthy mice, and is 30 times higher than average value in humans. Amylase activity in mice of this study was comparable to the data of another study (13), in which amylase activity ranged from 1,554 to 2,184 U/L. The blood samples in that study were collected via the vena cava of mice at 13 to 15 weeks of age. It should be noted that most of the mouse serum amylase activity is of salivary gland origin, since pancreatic amylase activity is rapidly cleared through the urine (9). Therefore, we suggest that caution should be taken when biochemical data for analytes such as amylase activity, are used to evaluate gastrointestinal diseases in murine models. Thus, pancreatic amylase activity could be assessed by analyzing this enzyme in urine (8, 9).

The modestly increased electrolyte and creatinine values may reflect higher steady-state concentrations of these analytes in the extracellular space. Alternatively, they could be due to hemoconcentration at blood sample collection, or in the case of potassium, to hemolysis. However, hemoconcentration is an unlikely explanation since serum protein concentration was actually lower in these mice. Similar tendencies for sodium and potassium concentrations argue against hemolysis as an important factor behind the observed values.

In conclusion, the clinical biochemical reference values for the C57BL/6J mouse presented here should be useful for investigators when designing a study, performing biochemical phenotype analysis on a new strain, or comparing the data obtained for their own murine models with those published by others.

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