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Selected blood serum biochemical indicators in twelve inbred strains of laboratory mice

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A total of 215 animals of twelve inbred strains of mice (A.CA/W, AKR/W, BALB/cW, BN/aW, C57BL/6W, C57Bl/10W, CBA/W, CBAT6/W, C3H/W, DBA/2W, 129Sl/SvW and HLB/219J) bred at the Cancer Centre and Institute of Oncology, Warsaw (Oncology Centre), were characterized in terms of five blood serum biochemical indicators related to their health, reproduction and genetic background. Glucose, total protein, creatinine, triglycerides (triacylglycerols) and total cholesterol were determined in mice aged four months. Significant interstrain differences were found among nearly all parameters considered, with the exception of total protein. The level of triglycerides showed wide interstrain differences and revealed a similar variation trend as the total cholesterol. For cholesterol and triglycerides, significant differences between sexes for the majority of strains were found. The results obtained may be important in the selection of experimental animals, for analysis of changes associated with various diseases, and for the explanation of their genetic background.

KEY WORDS: biochemical blood indicators / blood / inbred strains / mice

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Laboratory mouse is an animal most commonly used in mammalian biological studies and in the human disease modeling, due to the following factors: easy breeding, availability of inbred strains, short generation time, refined map of the genome and an extensive knowledge of biological and immunological properties. With the introduction of transgenic, knockout and mutant animals generated by N-ethyl-N-nitrosourea (ENU), the mechanisms of numerous human diseases may be analysed.

In spite of numerous experiments conducted on laboratory animals, their biochemical phenotype still remains not fully discovered. Only limited data on the clinical biochemistry of mice are available from studies of Frith *et al*. [1980], Ishida *et al*. [1991], Alberts *et al*. [1999], Rathcolb *et al*. [2000], Svenson *et al*. [2003], Zhou and Hansson [2004] and Klempt *et al*. [2006]. Some of the studies, however, deal with selected strains only. These publications provide information about the differences in biochemical parameters among the inbred strains of mice, *i.e* total cholesterol (TC), triglyceride (TG), total protein (TP), glucose (GLU), α-amylase (Amyl, AMS), creatinine, aspartate aminotransferase (AST), and alanine aminotransaminase (ALT). It is nevertheless essential to carry out a comparative analysis of relations between biochemical indicators in commonly used inbred mice strains to understand some biochemical pathways and changes associated with various diseases. The aim of this study is to present the results of determination of selected serum biochemical parameters in the commonly used 12 inbred strains of mice, bred at the Oncology Centre, Warsaw.

Material and methods

Animals

Twelve inbred strains of healthy mice (A.CA/W, AKR/W, BALB/cW, BN/aW, C57BL/6W, C57Bl/10W, CBA/W, CBAT6/W, C3H/W, DBA/2W, 129Sl/SvW and HLB/219J) were considered. The animals were serologically screened for known rodent viruses, bacteria and parasites according to the recommendations of the Federation of European Laboratory Animal Science Associations (FELASA), and none of the foregoing was identified in the study mice. The animals were bred under SPF (Specific Pathogen Free) conditions (in particular 12h/12h light/dark cycle, temperature 22±1°C and approximately 50% relative humidity) and fed standard diet (Labofeed H manufactured by MORAWSKI Co., Poland) containing 22% protein and 4.4% fat with the total energy value of 13.4 MJ/kg.

Blood analysis

Four-month old mice were used in the experiment (the numbers of animals are shown in Tables 1 and 2). Blood samples were collected under anaesthesia by retro-orbital bleeding into tubes and serum was separated by centrifugation at 5000 rpm, for 10 min at 4°C and stored at -20°C.

Glucose, total protein, creatinine, triglycerides and cholesterol were determined in blood serum of each specimen. Biochemical determinations were made using an automatic chemistry analyser Dimenson Max (DADE BEHRING) following the manufacturer's prescriptions and using the reagents routinely applied in that type of apparatus.

The study was approved by the Local Ethics Committee.

Statistical

ANOVA was used to identify significant differences between strains and the Mann-Whitney U test was applied to show significant differences between sexes. Means and their standard deviations of variables between inbred strains and between sexes were shown. The maximum and the minimum values for females and males of each strain are also presented.

Results and discussion

In order to determine the levels of glucose, total protein, creatinine, trigliceride and cholesterol in serum of blood, 215 animals from 12 inbred strains were used. Means and standard deviations for each trait analysed in females and males in each inbred strain as well as minimum and maximum values are presented (Tab. 1 and 2).

Significant differences between strains were revealed within glucose ($F_{11.196}$; 8.89; $P \leq 0.001$), creatinine ($F_{11,190}$; 7.45; $P \leq 0.001$), triglycerides ($F_{11,193}$; 4,15; $P \leq 0.000$), and cholesterol ($F_{11,195}$; 2,02; $P \leq 0.000$). Significant differences were found between sexes in cholesterol and creatinine in the majority of strains (U test, $P \leq 0.05$). For indicators of carbohydrate metabolism similar differences between sexes were observed only in a few strains (Tab. 3). Significant differences among strains were shown for triglycerides and cholesterol. There were two groups of strains, which revealed the minimum and maximum values of these traits (mice from strains A.CA/W, AKR/W, C57BL/6W, C57BL/10W, and 129S1/SvW were shown to have about 100 mg/dL TG and 60 mg/dL TC. However, strains CBA/W, CBAT6/W and C3H/W were shown to have 300 mg/dL and 100-120 mg/dL TG and TC, respectively). Coefficient of correlation between triglycerides and cholesterol level was as high as 0.69 for all strains tested. The total protein was the only trait showing no significant variation related to strain or to sex.

Biochemical parameters of peripheral blood presented a natural variation existing within the strains studied. It is advisable for researchers to know the natural level of phenotypic data of the commonly used strains, especially when the studies they perform refer to the genetic variation of biological functions and dysfunctions.

Glucose metabolism is important in medicine as the diabetes (type 1 or type 2) creates more and more problems in human population. Blood glucose level considerably depends on nutrition and fasting time prior to the analysis. In this study significant differences were observed between C57Bl/6W, DBA/2W and 129S1/SvW,

Table 1. Means and their standard deviations (SD) for biochemical indicators of blood plasma of female mice from 12 inbred strains

Strain	n		Compound				
			glucose (mg/dL)	total protein (g/L)	creatinine (mg/dL)	triglyceride (mg/dL)	cholesterol (mg/dL)
A.CA/W	8	**mean**	**121.9**	**51.1**	**0.29**	**107.9**	**46.4**
		SD	17.6	2.4	0.11	33.3	4.8
		min.	89	47.4	0.15	57	40
		max.	143	53.8	0.41	162	55
AKR/W	15	mean	**120.4**	**51.2**	**0.19**	**95.3**	**55.5**
		SD	26.4	3.8	0.05	29.3	5.3
		min.	91	41.4	0.11	65	48
		max.	163	54.7	0.26	142	62
BALB/cW	12	**mean**	**113.4**	**51.2**	**0.27**	**145.5**	**83.3**
		SD	12.2	1.9	0.05	25.5	4.3
		min.	93	48.3	0.20	102	76
		max.	132	54.3	0.36	188	91
BN/aW	8	**mean**	**117.6**	**50.4**	**0.22**	**108.9**	**68.3**
		SD	9.0	1.9	0.03	44.8	10.2
		min.	104	47.8	0.18	50	56
		max.	129	52.5	0.27	187	86
C57BL/6W	9	**mean**	**112.9**	**51.8**	**0.16**	**105.7**	**57.6**
		SD	17.1	2.3	0.05	40.6	9.6
		min.	90	48.3	0.10	65	43
		max.	143	56.3	0.26	185	69
C57BL/10W	7	**mean**	**139.0**	**53.0**	**0.21**	**82.2**	**63.5**
		SD	13.9	7.2	0.08	20.3	11.7
		min.	125	44.0	0.16	52	54
		max.	162	57.3	0.28	107	74
CBA/W	4	**mean**	**137.5**	**56.0**	**0.31**	**260.2**	**107.7**
		SD	24.7	3.7	0.01	125.2	29.8
		min.	106	53.3	0.30	147	72
		max.	164	60.2	0.31	396	143
CBAT6/W	8	**mean**	**125.6**	**53.5**	**0.30**	**230.7**	**99.4**
		SD	21.2	1.1	0.04	46.3	18.9
		min.	112	52.1	0.25	179	79
		max.	163	54.5	0.35	313	127
C3H/W	12	**mean**	**126.5**	**56.5**	**0.26**	**202.7**	**97.0**
		SD	19.9	3.1	0.06	56.1	7.4
		min.	91	48.8	0.16	119	89
		max.	155	61.9	0.37	311	108
DBA/2W	9	**mean**	**118.0**	**51.8**	**0.17**	**200.0**	**66.0**
		SD	12.0	1.7	0.01	50.4	4.0
		min.	96	49.2	0.15	140	62
		max.	136	55.0	0.19	308	69
129S1/SvW	12	**mean**	**116.7**	**52.9**	**0.23**	**93.83**	**63.2**
		SD	8.5	3.5	0.05	19.6	10.0
		min.	106	46.3	0.15	65	51
		max.	140	59.1	0.30	127	80
HLB/219J	1	**mean**	**225.0**	**51.8**	**0.30**	**115.0**	**64.0**
		SD	-	-	-	-	-
		min.	-	-	-	-	-
		max.	-	-	-	-	-
Grand mean	105	**mean**	**121.0**	**53.1**	**0.23**	**143**	**73.3**
		SD	17.8	0.17	0.07	68.0	20.3

Table 2. Means and their standard deviations (SD) for biochemical indicators of blood plasma of male mice from 12 inbred strains

Strain	n		Compound				
			glucose (mg/dL)	total protein (g/L)	creatinine (mg/dL)	triglyceride (mg/dL)	cholesterol (mg/dL)
A.CA/W	11	**mean**	**133.1**	**50.8**	**0.28**	**163.1**	**56.9**
		SD	15.5	2.4	0.13	34.5	6.4
		min.	117	38.4	0.11	124	43
		max.	166	54.5	0.58	186	65
AKR/W	7	**mean**	**132.4**	**46.9**	**0.20**	**155.3**	**62.6**
		SD	15.4	10.5	0.07	20.2	9.3
		min.	121	38.0	0.15	82	49
		max.	149	52.6	0.24	216	75
BALB/cW	15	**mean**	**139.1**	**51.2**	**0.29**	**184.2**	**106.4**
		SD	17.3	2.8	0.09	20.7	10.5
		min.	115	45.4	0.16	142	89
		max.	187	55.0	0.44	252	119
BN/aW	10	**mean**	**145.9**	**51.9**	**0.23**	**168.5**	**81.6**
		SD	10.5	2.4	0.05	42.3	8.8
		min.	128	47.7	0.14	117	73
		max.	155	53.8	0.29	216	102
C57BL/6W	11	**mean**	**127.8**	**55.1**	**0.24**	**98.4**	**68.2**
		SD	21.3	1.8	0.04	31.4	3.7
		min.	104	51.9	0.17	54.0	65
		max.	164	57.9	0.30	144	77
C57BL/10W	11	**mean**	**153.8**	**51.4**	**0.24**	**150.9**	**80.7**
		SD	13.5	3.9	0.09	41.4	9.5
		min.	130	42.7	0.14	120	67
		max.	172	56.5	0.36	196	97
CBA/W	8	**mean**	**155.1**	**59.2**	**0.34**	**305.9**	**152.6**
		SD	26.7	3.2	0.03	104.9	17.5
		min.	96	53.3	0.28	191	120
		max.	173	63.9	0.38	434	171
CBAT6/W	10	**mean**	**144.3**	**55.0**	**0.28**	**403.6**	**117.2**
		SD	19.9	2.7	0.07	85.5	17.9
		min.	104	52.1	0.17	290	96
		max.	170	58.9	0.39	613	141
C3H/W	8	**mean**	**137.4**	**57.1**	**0.27**	**333.5**	**125.4**
		SD	13.8	1.2	0.03	23.1	9.9
		min.	116	54.7	0.24	252	111
		max.	160	61.9	0.33	449	136
DBA/2W	8	**mean**	**118.1**	**53.2**	**0.23**	**240.1**	**86.0**
		SD	14.8	3.2	0.08	65.3	11.3
		min.	93	50.0	0.13	162	71
		max.	137	59.3	0.34	340	101
129S1/SvW	9	**mean**	**132.2**	**53.2**	**0.22**	**121.0**	**82.1**
		SD	24.8	3.0	0.06	19.7	10.2
		min.	114	48.8	0.13	97	71
		max.	173	56.7	0.30	155	102
HLB/219J	2	**mean**	**195**	**49.8**	**0.27**	**83.0**	**85.5**
		SD	-	-	-	-	-
		min.	-	-	-	-	-
		max.	-	-	-	-	-
Grand mean	110	**mean**	**139.1**	**53.4**	**0.26**	**208.3**	**93.0**
		SD	19.6	8.3	0.14	110.8	23.4

and C57BL10W, CBA/W and CBAT6/W strains. The differences between sexes occurred significant between BN/a/W and CBAT6/W (P≤0.001); females had a lower glucose level of serum as compared to males. In this study the majority of parameters were also lower in females than in males. The same trend was observed by Hough *et al.* [2002], who examined C3H and BALB/c mice and their F1 progeny.

The level of total protein differed significantly only between mice of CBA/W and C3H/W strain. The mean level was 56.33 and 58.29 g/L for C3H/W and CBA/W, respectively, *vs* about 51 g/L found in other strains. However, these results are very similar to those reported by Hough *et al.* [2002].

Major differences in blood creatinine level among and within the strains were observed. The most significant intrastrain difference was shown in A.CA/W, and the smallest in AKR/W mice. The elevated serum creatinine concentration may be attributed to the *pre*-renal, renal or *post*-renal state of the animal. Hough *et al*. [2002] found the creatinine level in two inbred strains of mice to be much higher – 0.51 mg/dL in C3H and 0.56 mg/dL in BALB/c strain – as compared to the strains examined in this study, the mean varying from 0.26 to 0.28 mg/dL in C3H/W and BALB/cW strains.

Total serum cholesterol and triglyceride levels were diagnosed in mice much more often [Welbust 1973, Ishida *et al.* 1991, Alberts *et al*. 1999, Hough *et al.* 2002, Svenson *et al*. 2003]. The disturbances in the level of these compounds are markers for many diseases, such as arteriosclerosis, hypothyroidism, nephrotic syndrome, multiple myeloma and hepatic disorders [Ratkolb *et al*. 2000].

In the current study the total cholesterol levels of serum were found similar to those obtained by others in investigated strains of mice, although differences among the strains were clearly marked. The lowest values were found in A.CA/W and the highest in CBA/W strain. Highly significant differences occurred between sexes in CBA/W, C3H/W, BN/aW and DBA/W strains (P≤0.001) and significant in CBAT6/W, 129S1/SvW and C57Bl6/W strains (P≤0.05).

Svenson *et al.* [2003] presented dramatically different data for males and females

Table 3. Significant intersex differences in blood serum biochemical parameters within inbred mice strains (test U, P<0.05)

Trait	Glucose	Creatinine	Triglyceride	Total cholesterol
A.CA/W			M>F*	
C57BL/10W			M>F*	
BN/aW	M>F**		M>F*	M>F**
DBA/2W				M>F**
CBAT6/W	M>F**		M>F**	M>F*
CBA/W		M>F**		M>F**
C3H/W			M>F**	M>F**
129SI/SvW				M>F*
C57BI/6W				M>F*

*P>0.05; **P>0.01.

Table 4. Means and their standard deviations (SD) for biochemical indicators of blood serum of mice from 12 inbred strains (sexes and strains pooled)

Statistic	n	Indicator				
		glucose (mg/dL)	total protein (g/L)	creatinine (mg/dL)	triglyceride (mg/dL)	cholesterol (mg/dL)
Mean	215	129.49	53.11	0.24	174.87	82.37
SD		20.83	4.34	0.08	97.79	26.94

of 24 strains of mice kept on a high-fat diet. They revealed a disproportionate increase of the total cholesterol level as compared to HDL-C levels. Some strains were affected to a small degree (DBA/1J and DBA/2J), but other showed significant changes. Extensive differences were found in cholesterol level between sexes in a specific response to the diet. Simultaneously, the differences between the strains kept on standard diets did not exceed 150 mg/dL.

The level of triglycerides varied significantly between the strains of mice and revealed similar tendency in variation as that of the TC. The mice of the three closely related strains: CBA/W, CBA-T6/W and C3H/W had two to four times higher level of triglycerides than mice of other strains. The triglyceride level varied from 83 mg/dL (C57BL/10W females) to 424g/dL (CBA-T6/W males). The highest values for triglycerides were found in the strains with the highest cholesterol level. Solberg *et al.* [2006] conducted similar analyses of the same inbred strains of mice, and found no such differences to occur. In their investigation the measurements were taken after 18h of fasting, and thus the results obtained are not comparable with the values presented in this study, where the animals had free access to food.

In the majority of the analysed serum biochemical traits the current study revealed significant differences between the inbred strains of mice. These differences were significant, particularly for triglycerides and cholesterol, and were also found to be significant between the sexes. Furthermore, it is very important to know the basic levels of selected biochemical indicators of blood while investigating the biochemical changes associated with various mammalian diseases and their genetic bases. Contrary to the present paper, the majority of publications associated with biochemical serum parameters supply only limited information about these traits in inbred strains. As stated by Hough *et al*. [2002] "Knowledge of biochemical fluctuations associated with human diseases provides markers for identification of mice that may be useful in modeling similar inherited disorders. Such mouse models can then be used to investigate therapeutic strategies"….

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Biochemiczne wskaźniki surowicy krwi myszy dwunastu szczepów wsobnych

S t r e s z c z e n i e

Wsobne szczepy myszy hodowane w Centrum Onkologii dostarczane są do wielu instytucji naukowych w kraju. Zwierzęta te scharakteryzowane są pod względem zdrowotnym i reprodukcyjnym oraz mają opracowany profil genetyczny. Obecna praca charakteryzuje poziom glukozy, cholesterolu, kreatyniny, trójglicerydów i białka całkowitego w surowicy krwi. Wykazano istotne różnice pod względem rozpatrywanych parametrów (z wyjątkiem białka całkowitego) między myszami poszczególnych szczepów. Największe różnice stwierdzono w poziomie cholesterolu i trójglicerydów między szczepami oraz między płciami w obrębie szczepów.