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LSI Medience Corporation, Nonclinical Research Center

カニクイザルの体重，血液学的検査，血液生化学的検査の項目間相関について

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Relationship between Body Weight and Hematological and Biochemical Parameters in Cynomolgus Monkeys

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Objective

Little has been reported on the relationship between body weight and clinical pathology parameters in toxicity studies using cynomolgus monkeys. We have previously reported on the incidence of animals showing body weight loss in toxicity studies in spite of being control animals and the features of their results in hematology, biochemistry, and pathology [1]. In this study, we analyzed the relationship among body weight, rate of change in body weight, hematology, and biochemistry in control animals including animals showing body weight loss.

[1] A Decrease in Body Weight without a Decrease in Food Consumption Observed in Cynomolgus Monkeys and Its Improvement. The 46th Annual Meeting of the Japanese Society of Toxicology 2019.

Material and Methods

Animals used: Cynomolgus monkeys in a control group (95 males and 95 females, 3 to 6 years old) in a 4-week toxicity study

Body weight: At the end of the 4-week dosing period

Rate of change in body weight: It was calculated according to the following formula.

Rate of change in body weight = {(body weight at the end of the study) – (body weight at the start of the study)} / (body weight at the start of the study) × 100

Hematology: Items listed in Table 1 were analyzed using CA-510 or XT-2000iV (Sysmex Corporation). PT and APTT were examined using plasma samples, and the other items were examined using blood samples anticoagulated with EDTA-2K.

Biochemistry: Items listed in Table 1 were analyzed using TBA-200FR (Canon Medical Systems Corporation) or Epalyzer 2 (Helena Laboratories).

Statistical analysis: The Shapiro–Wilk's test was used to check for normal distribution (Table 1) using statistical analysis system, EXSUS version 8.1.0 (CAC Croit Corporation, statistical analysis software: SAS 9.4, SAS Institute Japan Ltd.). Furthermore, data were analyzed by the Pearson correlation test for parametric tests or Spearman correlation tests for non-parametric analysis at a significance level of p<0.05, 0.01, or 0.001. The correlations were classified according to the r value (Table 2a).

Results

Table 1 Summary statistics

Items (Abbreviation; Unit)	Male: Number of animals	Male: Mean	Male: Standard deviation	Male: Statistical distribution	Female: Number of animals	Female: Mean	Female: Standard deviation	Female: Statistical distribution
Rate of change in body weight (%)	95	2.9316	5.2604	Non-parametric	95	3.7053	5.1944	Parametric
Body weight (kg)	95	3.8474	0.6354	Parametric	95	3.0495	0.3175	Non-parametric
Hematology								
Red blood cell count (RBC; $\times 10^6/\mu L$)	95	5.5402	0.3961	Parametric	95	5.3333	0.4476	Non-parametric
Hemoglobin concentration (HGB; g/dL)	95	13.3042	0.7839	Parametric	95	12.9137	0.9317	Non-parametric
Hematocrit (HCT; %)	95	43.1274	2.7166	Non-parametric	95	42.1411	2.8324	Non-parametric
Mean corpuscular volume (MCV; fL)	95	78.0242	4.7347	Parametric	95	79.2032	3.8819	Non-parametric
Mean corpuscular hemoglobin (MCH; pg)	95	24.0747	1.3633	Parametric	95	24.2600	1.1526	Parametric
Mean corpuscular hemoglobin concentration (MCHC; g/dL)	95	30.8842	1.2529	Non-parametric	95	30.7568	1.3355	Non-parametric
Reticulocyte ratio (%)	86	0.9135	0.5021	Non-parametric	86	1.0678	0.4126	Non-parametric
Reticulocyte count ($\times 10^9/L$)	54	51.3815	24.9188	Non-parametric	54	57.8074	22.1574	Non-parametric
Platelet count (PLT; $\times 10^3/\mu L$)	95	390.3789	85.6238	Parametric	95	421.6211	83.4748	Non-parametric
Prothrombin time (PT; sec)	95	9.0653	0.7236	Non-parametric	95	8.5663	0.4741	Parametric
Activated partial thromboplastin time (APTT; sec)	95	22.9295	1.8607	Parametric	95	22.3621	1.8649	Parametric
White blood cell count (WBC; $\times 10^3/\mu L$)	95	9.3411	3.4228	Non-parametric	95	8.3998	2.5239	Non-parametric
Lymphocyte (Lym; $\times 10^3/\mu L$)	95	4.7188	2.2748	Non-parametric	95	3.9560	1.2879	Non-parametric
Neutrophil (Neu; $\times 10^3/\mu L$)	95	4.0248	2.2747	Non-parametric	95	3.9545	1.9949	Non-parametric
Eosinophil (Eos; $\times 10^3/\mu L$)	95	0.1754	0.2576	Non-parametric	95	0.1398	0.1106	Non-parametric
Basophil (Baso; $\times 10^3/\mu L$)	95	0.0048	0.0071	Non-parametric	95	0.0038	0.0057	Non-parametric
Monocyte (Mono; $\times 10^3/\mu L$)	95	0.4172	0.2686	Non-parametric	95	0.3467	0.1444	Non-parametric
Biochemistry								
Aspartate aminotransferase (ASAT; U/L)	95	40.8947	26.5464	Non-parametric	95	33.9368	13.0586	Non-parametric
Alanine aminotransferase (ALAT; U/L)	95	57.3579	67.2403	Non-parametric	95	52.0000	30.4976	Non-parametric
Lactate dehydrogenase (LDH; U/L)	68	280.4559	73.0104	Non-parametric	68	266.4706	69.0491	Non-parametric
Glutamate dehydrogenase (GLDH; U/L)	15	28.8000	18.9480	Parametric	15	27.6000	19.9241	Parametric
Alkaline phosphatase (ALP; U/L)	95	1699.0105	768.2146	Non-parametric	95	790.3263	304.1855	Non-parametric
γ-Glutamyl transpeptidase (γGT; U/L)	60	77.65	38.934	Non-parametric	60	57.1	17.1886	Non-parametric
Creatine kinase (CK; U/L)	89	80.0449	89.0468	Non-parametric	89	96.5056	156.6775	Non-parametric
Total bilirubin (mg/dL)	95	0.1095	0.0670	Non-parametric	95	0.1442	0.0725	Non-parametric
Urea nitrogen (mg/dL)	95	21.5674	6.5991	Non-parametric	95	18.3495	3.7907	Non-parametric
Creatinine (mg/dL)	95	0.8411	0.1519	Non-parametric	95	0.7074	0.1034	Non-parametric
Glucose (mg/dL)	95	85.0947	12.9353	Parametric	95	80.7263	13.4001	Parametric
Total cholesterol (mg/dL)	95	107.2421	25.1425	Parametric	95	117.1158	24.0619	Non-parametric
Phospholipid (mg/dL)	70	154.1857	35.1701	Parametric	70	165.7429	29.4548	Parametric
Triglyceride (mg/dL)	95	36.8421	18.9587	Non-parametric	95	34.8737	15.4440	Non-parametric
Calcium (Ca; mg/dL)	95	10.3568	0.4780	Parametric	95	10.0221	0.4532	Parametric
Inorganic phosphorus (IP; mg/dL)	84	4.8714	1.0802	Parametric	84	4.6655	1.0382	Parametric
Sodium (Na; mmol/L)	95	150.8526	4.3929	Non-parametric	95	149.4737	3.1752	Non-parametric
Potassium (K; mmol/L)	95	5.0537	0.5506	Non-parametric	95	4.7505	0.5080	Non-parametric
Chlorine (Cl; mmol/L)	95	108.8105	3.6094	Non-parametric	95	109.8421	2.3307	Non-parametric
Total protein (g/dL)	95	8.0211	0.5562	Parametric	95	7.8358	0.4948	Non-parametric

Table 2 Correlation matrix of biochemical parameters

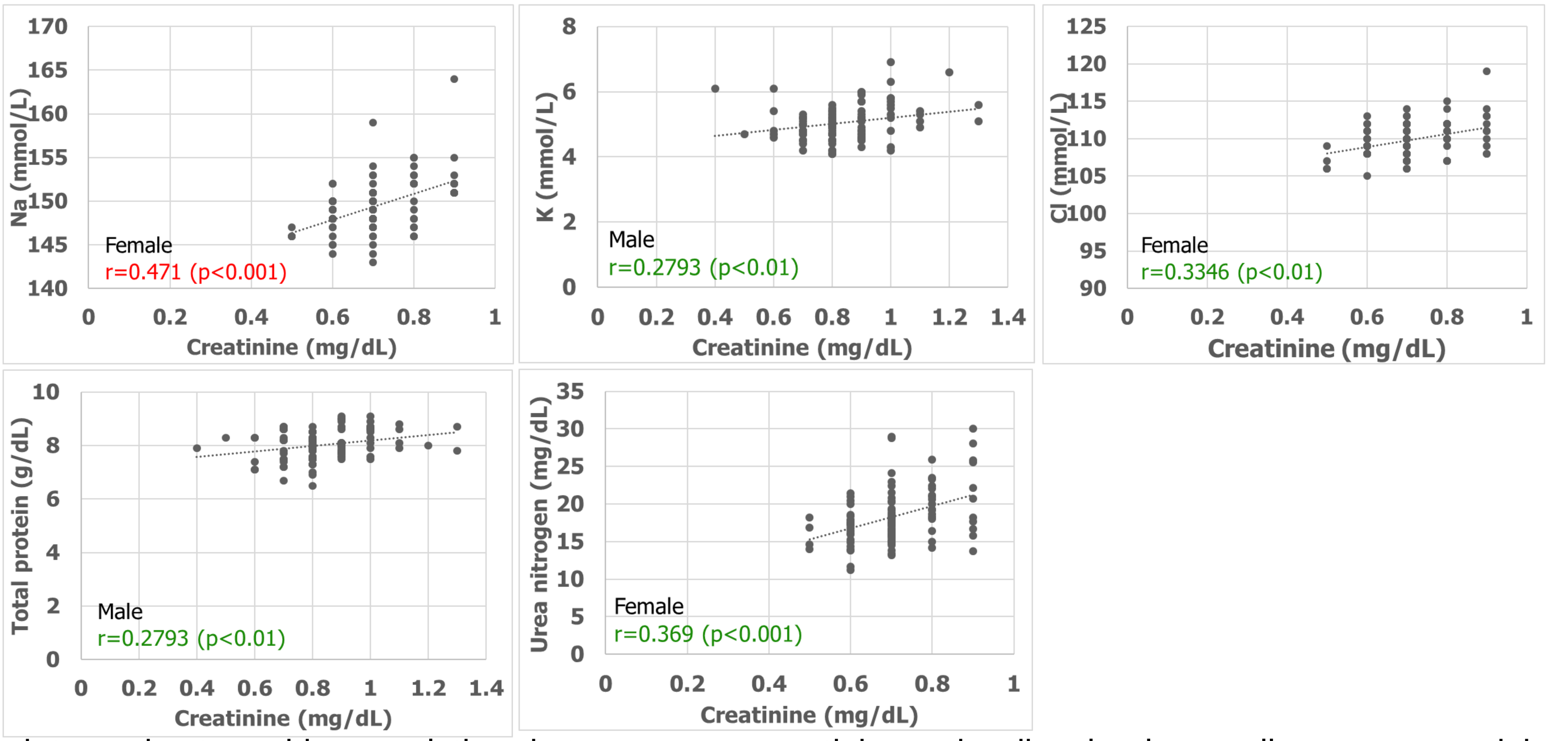
	ALAT	LDH	GLDH	ALP	γGT	CK	Total bilirubin	Urea nitrogen	Creatinine	Glucose	Total cholesterol	Phospholipid	Triglyceride	Ca	IP	Na	K	Cl	Total protein
ASAT	0.6198*** 0.7158***	0.6077*** 0.5757***	0.5526* 0.5403*	-0.0772 -0.0222	0.0298 0.0154	0.4408*** 0.3202**	0.1647 0.1367	0.2441* 0.2256*	-0.1274 0.1116	0.0777 -0.046	-0.1245 -0.2427*	-0.1011 -0.1844	0.0732 0.1459	-0.0479 -0.0497	-0.2256* -0.068	0.1316 0.0944	0.1365 0.1856	0.0642 0.0569	0.0562 -0.0447
ALAT	–	0.5147*** 0.547***	0.8120*** 0.4637	-0.0712 -0.0411	0.1442 0.0368	0.2347* 0.0997	0.0694 0.0302	0.2255* 0.1345	-0.0352 0.0279	-0.0602 -0.033	-0.1213 -0.1883	-0.208 -0.1967	0.0795 0.1547	-0.0097 -0.0949	-0.1815 -0.0882	0.153 0.066	0.224* 0.0493	-0.0769 0.0808	0.1071 -0.07
LDH		–	0.1255 0.75*	-0.0494 0.039	0.0304 0.171	0.3273** 0.2427*	0.1218 0.081	0.226 0.1668	0.0563 0.0836	0.2564* 0.1667	-0.0979 -0.0472	-0.1138 -0.0183	0.2575* 0.2059	-0.1635 -0.0601	-0.0765 -0.0541	0.1406 0.1319	0.1216 0.1539	0.0978 0.0726	0.1317 0.1221
GLDH			–	0.0125 -0.0519	0.2437 -0.0084	0.3264 0.2667	0.0828 -0.0726	0.2709 0.4114	-0.0953 0.2019	-0.1165 0.4472	0.3996 -0.1326	0.7423 -0.3802	0.1629 0.1991	0.2865 0.0262	0.2149 -0.292	-0.053 -0.1096	-0.117 -0.22	-0.3822 -0.0046	0.3373 -0.1886
ALP				–	0.135 0.118	0.0319 0.0952	-0.1115 -0.1244	-0.0523 0.0403	0.1048 -0.0373	0.1961 -0.0821	0.0653 -0.0165	0.0998 -0.037	-0.3116** -0.0908	0.0537 0.088	-0.101 0.1019	0.2087* 0.0122	-0.041 0.0303	0.2369* -0.1297	-0.0237 0.0986
γGT					–	-0.0384 -0.1326	-0.0927 0.1676	-0.1677 -0.1416	-0.0908 0.0514	-0.2221 -0.0439	-0.0196 0.1858	-0.087 0.0035	-0.3497** -0.008	0.065 -0.0178	0.0205 0.035	0.1193 -0.1281	-0.09 -0.1828	-0.0891 0.1304	-0.0372 0.0334
CK						–	0.0835 0.0304	-0.1237 0.0023	-0.2883** 0.016	0.0864 0.1653	0.0141 -0.1289	-0.1225 -0.1043	-0.1226 0.1221	0.0019 0.0221	0.0618 0.1134	0.1321 0.0321	-0.2043 0.1403	0.059 -0.1035	0.0821 -0.0469
Total bilirubin							–	-0.0796 -0.2727**	0.1203 0.0464	-0.0639 -0.2354*	-0.0535 0.0252	0.1122 -0.0124	0.0295 -0.2222*	0.0681 0.01	0.0455 0.3557***	0.1883 0.0459	-0.0761 0.0007	0.1073 -0.0948	0.1975 0.1077
Urea nitrogen								–	0.2436* 0.369***	0.1356 0.123	-0.1901 -0.267**	-0.0734 -0.1559	0.3838*** 0.2384*	-0.1304 0.1177	-0.3484** -0.4473***	0.0688 0.0936	0.133 0.0391	0.0514 0.0928	-0.148 -0.1274
Creatinine									–	0.0251 0.1009	-0.1804 -0.0141	-0.0595 0.1721	0.0768 0.0785	0.2066* 0.0798	0.0328 -0.2001	0.3319** 0.4721***	0.2793** 0.1183	0.2165* 0.3346***	0.2785** 0.2882**
Glucose										–	0.0899 0.1076	0.0063 0.2186	-0.052 0.1962	0.0929 0.0974	0.0141 -0.1516	0.1814 0.0743	0.0093 0.2024*	0.0841 -0.0832	0.0478 0.1167
Total cholesterol											–	0.8965*** 0.8169***	0.2247* 0.0905	0.0978 0.1215	0.0768 0.1808	-0.0832 -0.0003	-0.126 -0.1502	0 -0.154	-0.0129 0.1953
Phospholipid												–	0.4475*** 0.2123	0.0874 0.1895	0.1715 0.2739*	0.0629 0.1737	-0.0966 -0.0216	0.2218 -0.0389	-0.0298 0.4159***
Triglyceride													–	-0.0387 0.0312	-0.0597 -0.1171	0.0506 0.0412	0.1679 0.1065	0.1186 0.0454	-0.1105 0.0405
Ca														–	0.2407* 0.0429	0.4514*** 0.3083**	0.365*** 0.353***	0.0284 -0.0845	0.6049*** 0.4039***
IP															–	0.0837 -0.0823	0.1005 -0.0407	-0.1565 -0.2178*	0.214 0.185
Na																–	0.277** 0.3928***	0.4511*** 0.5083***	0.3137** 0.4572***
K																	–	0.1485 0.131	0.1859 0.2368*
Cl																		–	-0.0328 0.0696

Each cell shows correlation coefficient (upper: male, lower: female). *: p< 0.05 **: p< 0.01 ***: p< 0.001

Table 2a Meaning of the Correlation Coefficient (r)

r=0	No relationship
0< | r | ≤0.2	No or negligible relationship
0.2< | r | ≤0.4	Weak relationship
0.4< | r | ≤0.7	Moderate relationship
0.7< | r | ≤1.0	Strong relationship

1

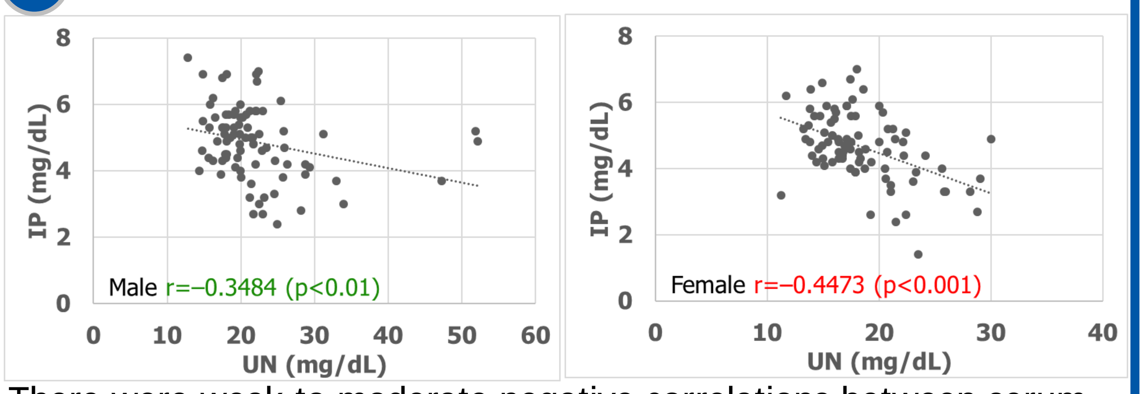


There were weak to moderate positive correlations between serum creatinine and sodium levels as well as serum creatinine and chloride levels in males and females, and serum creatinine and potassium levels in males. There were weak positive correlations between serum creatinine and total protein or urea nitrogen levels in males and females.

Serum creatinine levels are widely known as an indicator of renal function, and serum concentrations increase when glomerular filtration rate is reduced [2]. In both males and females, serum creatinine levels did not deviate widely from the background data of the test facility (3 to 6 years old, mean ± 2SD: 0.56-1.14 mg/dL for males, 0.49-0.97 mg/dL for females) and were considered to be within the range of physiological variability. These results suggest that serum creatinine levels reflect renal function even within the range of physiological variability, and animals with high serum creatinine levels tended to have higher serum electrolyte, total protein, and urea nitrogen levels because of relatively low glomerular filtration volume.

[2] Andrew S. Levey, M.D., Ronald D. Perrone, M.D., andNicolaos E. Madias, M.D. SERUM CREATININE AND RENAL FUNCTION. Annual Review of Medicine 1988; 39: 466-490.

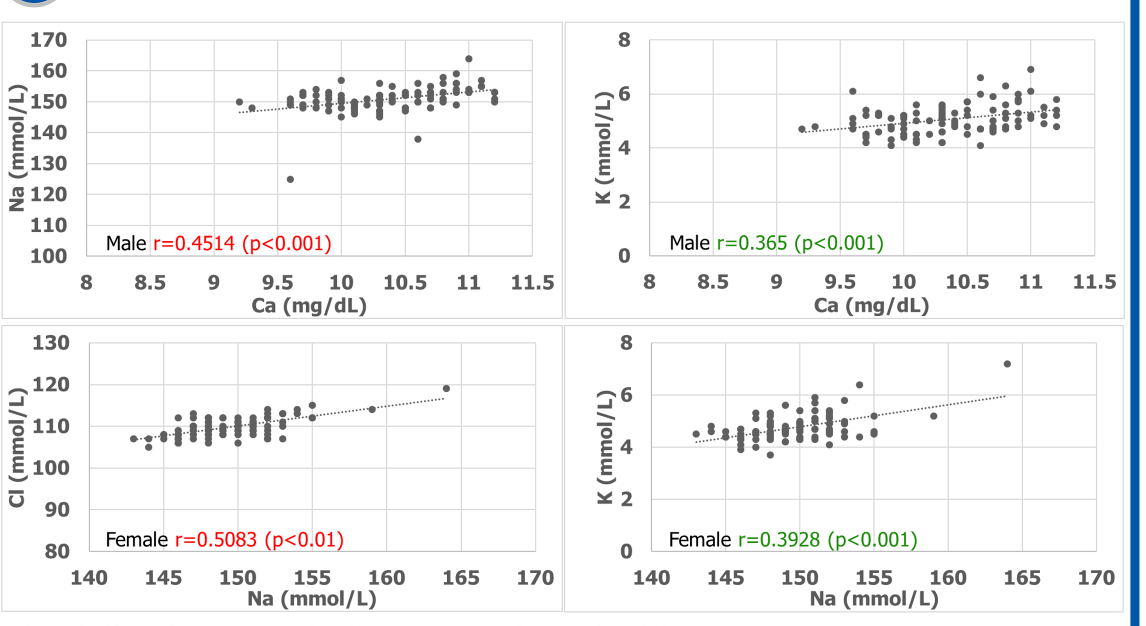
2



There were weak to moderate negative correlations between serum urea nitrogen and inorganic phosphorus levels in males and females. The serum urea nitrogen level reflected protein intake. These results indicated that the intracellular glycolytic system is promoted and phosphorus is utilized in relatively well-nourished animals with the result that inorganic phosphorus in the blood is taken up into the cells [3].

[3] Hayashi M. Recent findings in phosphate metabolism and etiology of hypophosphatemia. J Jpn Soc Intensive Care Med. 2009;16:5-6.

3



The following correlations were found with respect to serum electrolyte levels.

Statistical significances between IP and Ca (abbr.: IP-Ca) and IP-Cl were judged to be incidental and "non-correlated" because they were unisexual and the degree of correlation was weak.

Correlated: Na-Ca, Ca-K, Na-K, and Na-Cl

Non-correlated: Ca-Cl, K-Cl, IP-Na, IP-K, IP-Cl, and IP-Ca

Since Na^+ and Cl^- are ingested as dietary salt and lost in the form of Na^+ Cl^-, it was considered that a correlation was observed between Na-Cl. It is difficult to determine the mechanism of the difference between the correlated and non-correlated parameters other than Na-Cl in this study alone, and further investigation is necessary.

The others

Ca-Total protein: Because much of the calcium is bound to proteins in the blood.

Na-Total protein, Urea nitrogen-Triglyceride, and Phospholipid-Total cholesterol: Reflect nutritional status.

Urea nitrogen-ASAT: It was considered to be incidental since correlation was weak and it was not clearly related in the scatter plot.

Correlation between ASAT, ALAT, LDH, GLDH, and CK: Because they are enzymes that are distributed in many organs of the body, there is a correlation among them.

Table 3 Correlation matrix of the rate of change in body weight, body weight at the end of the 4-week dosing period, hematological, and biochemical parameters

	Body weight	RBC	HGB	HCT	MCV	MCH	MCHC	Retiulocyte ratio	Reticulocyte count	PLT	PT	APTT	WBC	Lym	Neu	Eos	Baso	Mono
Rate of change in body weight	0.0149 0.1489	0.0825 0.0602	0.008 0.2548*	0.1819 0.2684**	0.1228 0.1252	-0.0846 0.1816	-0.2964** 0.0194	0.1718 0.0531	0.1848 0.0864	-0.1402 0.0029	-0.0353 -0.2129*	0.0349 -0.1285	0.2512* -0.073	0.3901*** 0.111	0.001 -0.1972	0.2102* -0.0184	-0.0994 0.0603	0.2157* 0.1002
Body weight	–	0.0816 -0.0959	0.2335* 0.0093	0.2003 0.0315	0.088 0.1354	0.144 0.1013	0.0923 -0.0524	0.1219 0.0009	0.0074 -0.0826	-0.2223 -0.0683	0.1317 -0.0889	-0.1306 -0.0513	-0.1243 0.1554	-0.0362 0.1462	-0.1998 0.118	0.1649 0.0903	0.1658 0.0514	0.04 0.14

	ASAT	ALAT	LDH	GLDH	ALP	γGT	CK	Total bilirubin	Urea nitrogen	Creatinine	Glucose	Total cholesterol	Phospholipid	Triglyceride	Ca	IP	Na	K	Cl	Total protein
Rate of change in body weight	-0.3013** 0.2093*	-0.3106** 0.1929	-0.0144 0.0177	-0.1395 0.1793	0.2177* 0.0781	0.1836 0.0082	0.0878 -0.1594	-0.1254 0.1249	-0.142 -0.0433	-0.1021 -0.1087	0.1805 -0.0329	0.112 0.0862	0.0719 0.1352	-0.1104 0.2318*	-0.0924 0.0724	0.2953** 0.1532	-0.0892 0.0121	-0.2548* -0.0353	0.0288 -0.1199	-0.2158* -0.0682
Body weight	-0.3299** 0.1328	-0.0617 0.125	-0.238 -0.0902	-0.004 0.0360	-0.0449 -0.2679**	0.0758 0.0566	-0.2874** -0.1593	0.0416 -0.0137	-0.1674 -0.0554	0.5287*** 0.2331*	-0.2509* 0.0772	-0.2377* 0.1281	-0.2256 0.061	-0.1804 -0.0323	0.2534* 0.0442	0.1536 -0.1521	0.0559 0.2312*	0.1427 0.0703	-0.0712 0.1121	0.3544*** 0.0298

Each cell shows correlation coefficient (upper: male, lower: female). *: p< 0.05 **: p< 0.01 ***: p< 0.001

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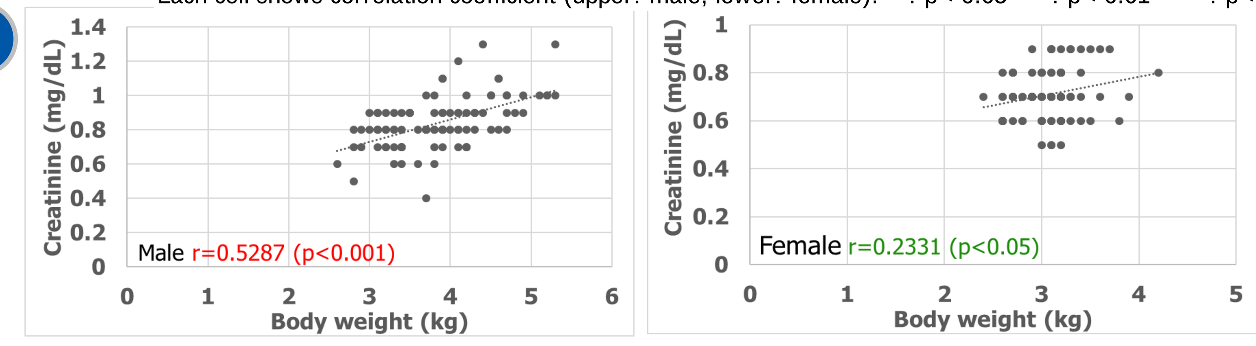

There were moderate positive correlations in males and weak positive correlations in females between body weight and serum creatinine levels. Creatinine is the final metabolite of creatine phosphate, and in humans, about 94% of the creatine in the body is distributed in muscles, suggesting that it may be a biomarker for the progression of amyotrophic lateral sclerosis [4]. These results suggest that the serum creatinine level was also proportional to skeletal muscle mass in cynomolgus monkeys and may be a biomarker of skeletal muscle mass. The degree of correlation was stronger in males than in females, possibly because males had more skeletal muscle mass than females.

[4] Mitsumoto H and Saito T. A prognostic biomarker in amyotrophic lateral sclerosis. Clinical Neurology 2018;58:729-736.

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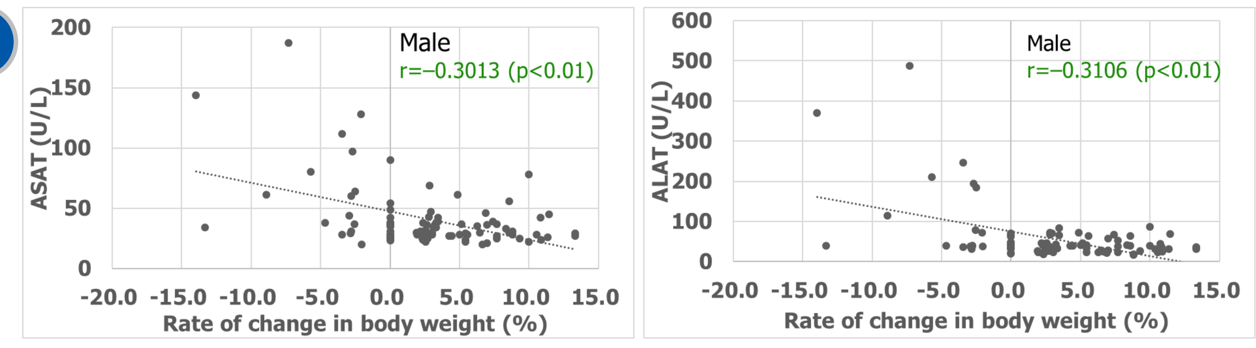

There were weak negative correlations in males between the rate of change in body weight and ASAT or ALAT.

We found that animals with a rate of change in body weight of less than 0% and ASAT or ALAT of 100 U/L or more had lost weight in spite of their feeding status (in-house data), suggesting that catabolism of skeletal muscle proteins is promoted to compensate for the deficit energy [5] and ASAT and ALAT distributed in the skeletal muscle leaked into the blood. The absence of a similar trend in females may be due to their originally low skeletal muscle mass.

[5] Ishibashi I. Enteral Nutrition and Optimal Dosage in the Early Invasive Period. The Japanese Journal of SURGICAL METABOLISM and NUTRITION 2016; 50-2: 105-109.

Conclusion

- This presentation clarifies the degree of the correlation between each test item in cynomolgus monkeys. The correlation between the basic test items will serve as a reference when abnormal values are found during the measurement.
- Males with larger body weight have higher basal creatinine levels in their blood, which should be kept in mind when assessing toxicity.
- In this presentation, since the population of animals was a control group, it is necessary to examine the relevance of each test item after administration of compounds such as nephrotoxicants and hepatotoxicants in order to better understand the degree of the correlation between each test item.