LITTER EFFECT IN MOUSE PHENOTYPIC STUDIES

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Abstract: The laboratory mouse is the most common mammalian model organism for research of the human body functions and disorders. For experimental purposes mice selected from inbred strains, developed by many generations of brother-sister crosses, are usually used. Individual mice of a given inbred strain are therefore considered genetically identical. However, our preliminary observations suggest that for a number of phenotypic traits mice originating from the same litter are significantly more similar than mice coming from different litters of the same inbred strain. We estimated the size of this litter effect for a number of traits in several phenotypic studies. By means of simulation we showed that ignoring the litter effect may result in several fold higher false positive rate and severe underestimation of minimal sample size.

1 INTRODUCTION

Starting with the work of Gregor Mendel, genetics has always been one of more mathematically oriented areas of biology. As time goes by, the geneticists adopted various statistical tools: from Student's T-test through Wright's path analysis and Fisher's work on Mendelian inheritance to modern robust and Bayesian methods for processing the microarrays.

Statistical methods, even the simplest ones, are always based on a number of assumptions. It is important to know about them and to know about consequences of their infringement. In real life variances are often heterogeneous, measurements not independent and distributions far from the ideal Gaussian bell shaped curve. Dealing with these issues is crucial and there is a vast amount of literature how ignoring the unstated assumptions can lead to false conclusions, eg. (Glass et al., 1972).

This paper is focused on a very concrete issue in the field of mouse genetics – a litter effect (LE) in phenotyping studies, particularly in large scale phenotyping studies. For genetic analyses we usually use mouse inbred strains, developed by many generations of brother-sister crosses (Silver, 1995, p. 32). Individual mice of the same inbred strain are therefore considered genetically identical.

It seems natural to assume that if we are interested in some phenotypic traits for a given inbred strain, a mode of selection of mice should not influence the measurements. Using the language of mathematical statistics – we suppose that our measurements are independent, identically distributed (iid) random variables.

The best common practice is to control for possible sources of bias and so all animals usually come from the same animal facility, year of the birth or even similar size of the litter. But what about the effect of sharing the same litter? Is it possible that mice differ across litters, e.g. two mice from the same litter are more similar than two mice from the same experimental group but a different litter? The question is not entirely new, eg. (Haseman and Kupper, 1979), but it is still ignored by the main stream of research. We want to demonstrate here that the answer is positive for a number of phenotypic traits.

In this paper we are giving an evidence of a LE in three large scale phenotyping studies in Mouse Phenome Database (Grubb, Maddatu et al., 2009) and discuss the consequences on the results of statistical tests.

2 RESULTS

In our lab the weights of sacrificed mice are routinely recorded. LE was first observed when we analyzed these records. See Sections 2.1 and 4.1 for details.

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To confirm this phenomenon we have chosen three phenotypic datasets collected at The Jackson Laboratory in Bar Habor, Maine, and publicly available at Mouse Phenome Database (MPD). See Section 2.2.

2.1 Laboratory Notebook

The size of LE was such that we were even able to observe it just by reading the protocols without any formal statistical test.

Applying methodology described in Section 4.2, LE was found highly significant ($p<0.001$). It was estimated to account for 43% (SE=6%) of variability of body weight.

2.2 The Litter Effect Observed in Three MPD Datasets

Mouse Phenome Database records contain only IDs of mice, not litters. We were able to recover litter IDs from mouse IDs in three selected large studies: Lake1, Svenson2 and Tordoff3. Only experimental groups / strains with at least two litters were considered (see Materials and Methods part for details).

LE has been found significant (likelihood-ratio test's unadjusted p-value < 0.05) in 106 out of 129 tested traits, the average proportion of residual variability attributed to the LE is 25% (standard deviation = 16%). The highest proportion of residual variability was explained by hemoglobin concentration distribution width (HDW) both for Lake1 and Svenson2 studies (not contained in Tordoff3) where LE was accounted for 74% (SE=5%) and 57% (SE=8%) of variability, respectively. See Tables 1 and 2 (at the end of the paper) for other litter effect estimates.

2.3 Simulation Study

We performed a simulation study to quantify the influence of LE to type-I-error (proportion of false positives) of T-test (on 5% level). Three scenarios were considered, each considering 12 mice per group:

a) Four litters per group, 3 mice per litter (3+3+3+3 vs. 3+3+3+3)
b) Three litters per group, 4 mice per litter (4+4+4 vs. 4+4+4)
c) Two litters per group, 6 mice per litter (6+6 vs. 6+6).

The results are visualized on Figure 1. In case of (average) 25% of residual variability attributed to LE we get 2.3, 2.9 and 4.2 times as many false positives as expected, respectively.

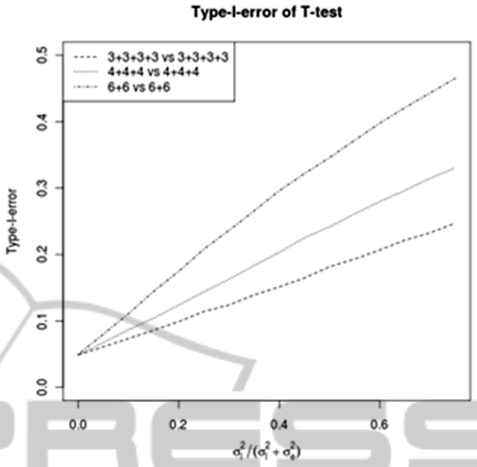

Figure 1: Type-I-error of T-test in dependence on percentage of variability attributed to LE.

The second question is how many mice we need to get a reasonable chance to significant result of the test in ANOVA model with random litter effect (described in Section 4.2). We set the parameters as follows: 4 mice per litter (e.g. 9 mice are divided into three litters as 4+4+1), 5% type-I-error (proportion of false positives), 80% power (proportion of true positives), and difference between groups equals two within-litter standard deviations.

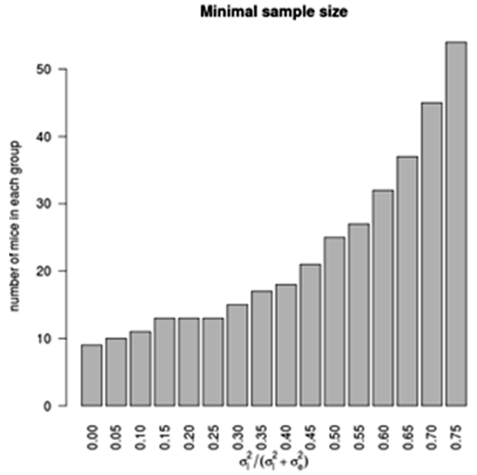

Figure 2: Minimal sample size in dependence on percentage of variability attributed to LE.

The results are visualized on Figure 2. In case of (average) 25% of residual variability attributed to

LE, 13 mice per group are needed (minimal sample size for analogical T-test is 6 mice per group).

3 DISCUSSION

In this paper we have demonstrated presence of LE in several phenotyping studies.

The consequences are particularly important for large-scale phenotyping studies (such as databases of gene knockouts) comparing many traits for a high number of experimental groups where we predict higher occurrence of false positive results than expected.

For illustration let us consider a study of 20 chromosome substitution strains (Nadeau, 2000). Comparing these strains to control parental strain result on average in 2-4 false positives (if the design would be as in Section 2.3) while only 1 false positive is expected on 5% level.

It is fair to admit that at the moment we do not know what is behind this effect since mice in the litter share many common characteristics: not only mother and father, but also exactly the same condition during prenatal development, the same cage, the same day to be born etc. Separation of these factors will be statistically challenging.

Last but not least, the assumption of independent observations is not violated only by T-test discussed in this paper but also by many other methods commonly used in mouse genetics from QTL mapping (Broman and Sen, 2009) to microarray gene expression analysis (Gentleman et al., 2005).

4 MATERIALS AND METHODS

4.1 Datasets

The first data source was our lab notebook with body weights records of sacrificed mice. We have restricted ourselves to 28 chromosome substitution strains and time period from January 2005 to December 2007. Information about 523 mice (both males and females, sacrificed between 75 and 81 days) was collected.

Our second data source was Mouse Phenome Database (MPD), http://www.jax.org/phenome, an open web-based repository of phenotypic data on commonly used and genetically diverse inbred strains of mice and their derivatives. There were three large datasets where we were able to recover litter IDs from mouse IDs: Lake1, Svenson2 and Tordoff3.

Lake1 (MPD accession number: 199) was a multi-system analysis of mouse physiology of C57BL/6J-Chr#^{A}/NaJ chromosome substitution strain panel collected by Jeffrey Lake, Leah Rae Donahue and Muriel T Davisson. The purpose was to examine the phenotypic outcome of chromosome substitution for multiple parameters such as hematology, blood chemistry, lung function, blood pressure and pulse, and electrocardiogram. This survey contains 374 mice from 23 strains.

Svenson2 (Gregorová et al., 2008, MPD accession number: 219) was an analogical multi-system physiological survey of mouse physiology in chromosome substitution strain panel. However, it was devoted to C57BL/6J-Chr#^{PWD} consomics. The survey contains 399 mice from 18 strains.

Tordoff3 (Tordoff et al., 2007; MPD accession number: 103) was a survey of calcium and sodium intake, blood pH and calcium level, and bone and body composition data in 40 inbred mouse strains to gain insight into how food and beverage consumption contribute to diseases such as obesity, hypertension and diabetes. This survey contains 790 mice.

4.2 Statistical Analysis

The response variable (quantitative trait) Y_i of an animal i in an experimental group $g(i)$ and a litter $l(i)$ was modeled by ANOVA model with a random litter effect as follows

$$Y_i = \mu_{g(i)} + \varphi_{l(i)} + \varepsilon_i, \quad (1)$$

where $\mu_\bullet$ is a group fixed effect, $\varphi_\bullet \sim N(0, \sigma_l^2)$ is a random litter effect and $\varepsilon_\bullet \sim N(0, \sigma_e^2)$ is a random noise, e.g. Gaussian independent, identically distributed random variables with zero means and constant variance.

Residual variability explained by LE (or attributed to LE) is defined as follows

$$\sigma_l^2/(\sigma_l^2 + \sigma_e^2) \quad (2)$$

Standard error (SE) of residual variability explained by LE can be approximated from σ_l^2 and σ_e^2 by delta method. The distribution of this fraction is far from bell shape and the calculated SE should be used as (only) approximation of true value.

Testing for a difference between group means is a standard test for presence of fixed effect in mixed-effect model as discussed e.g. in (Verbeke and Molenberghs, 2000, p. 55). Testing for random litter

effect is a bit more challenging. Two approaches were implemented:

- Likelihood ratio test as discussed in (Verbeke and Molenberghs, 2000, p. 65): the test statistic is a difference in log-likelihood between models with and without random effect multiplied by two. Under the null hypothesis ($\sigma_l^2 = 0$) it is asymptotically distributed as a mixture (weights ½ and ½) of chi-squared distribution with 1 degree of freedom and constant 0.
- Permutation test: 1000 permutations of observations within each experimental group are performed to see how much exceptionally high is the test statistic (the observed residual variability explained by LE). P-value is a fraction of randomly generated test statistics greater than actually observed test statistic.

All calculations were done in R 2.9.2, *nlme* package was used for LE inference in mixed models.

4.3 Simulation Study

In the first scenario a random sample of 100 000 cases was generated for each considered value of $\sigma_l^2/(\sigma_l^2 + \sigma_e^2)$ (from 0.00 to 0.75 by 0.05). For each case two random vectors were generated such that observation *i* of litter *l(i)* was computed as follows

$$Y_i = \varphi_{l(i)} + \varepsilon_i, \quad (3)$$

where $\varphi_{l(i)}$ and ε_i were sampled from distributions N(0, σ_l^2) and N(0, σ_e^2), respectively.

For each case T-test was performed and resulting p-value recorded. The percentage of cases with p-value below 5% was reported as Type-I-error.

In the second scenario for the same set of considered values of $\sigma_l^2/(\sigma_l^2 + \sigma_e^2)$ and a temporary suggestion for possible minimal sample size *n* we generated 10 000 samples of two vectors of length *n* such that observation *i* of litter *l(i)* was computed as follows

$$Y_i = \varphi_{l(i)} + \varepsilon_i + 2\omega\sigma_e, \quad (4)$$

where ω equals zero for the first vector and one for the second vector; $\varphi_{l(i)}$ and ε_i were sampled from distributions N(0, σ_l^2) and N(0, σ_e^2), respectively.

For each case we compared means of vectors by ANOVA with a random litter effect and record the p-value. If the percentage of cases with p-value below 5% was lower than 80% then *n* was increased by 1 and the whole procedure was repeated.

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Table 1: Litter effect and its statistical significance in three MPD surveys (first part): dataset, variable name and description (in MPD notation), residual variability (%) explained by LE as defined in (2), its standard error (SE) and p-value of a test for a submodel without LE (likelihood-ratio and permutation tests).

Dataset	Variable	Description	% explained	SE (approx.)	p-value (LR test)	p-value(perm. test)
Lake1	WBC	## 19901 .... WBC .... total white blood cell (WBC, leukocyte) count (units	17%	9%	0.018	0.003
Lake1	RBC	## 19902 .... RBC .... total red blood cell (RBC, erythrocyte) count (units pe	27%	9%	0.000	< 0.001
Lake1	mHGB	## 19910 .... mHGB .... measured hemoglobin (HGB) .... g/dL	1%	6%	0.404	0.454
Lake1	HCT	## 19912 .... HCT .... hematocrit (HCT) .... %	26%	8%	0.000	0.002
Lake1	MCV	## 19913 .... MCV .... mean RBC corpuscular volume (MCV) .... fL	33%	8%	0.000	< 0.001
Lake1	MCH	## 19914 .... MCH .... mean RBC corpuscular hemoglobin content (MCH) ..	22%	8%	0.001	< 0.001
Lake1	MCHC	## 19915 .... MCHC .... mean RBC corpuscular hemoglobin concentration (	27%	8%	0.000	< 0.001
Lake1	CHCM	## 19916 .... CHCM .... RBC corpuscular hemoglobin concentration mean (	67%	6%	0.000	< 0.001
Lake1	RDW	## 19917 .... RDW .... RBC corpuscular distribution width .... %	60%	7%	0.000	< 0.001
Lake1	HDW	## 19918 .... HDW .... hemoglobin concentration distribution width (HDW	74%	5%	0.000	< 0.001
Lake1	NEUT	## 19980 .... NEUT .... neutrophils (NEUT) count (units per volume x 103) .	16%	7%	0.003	0.019
Lake1	LYM	## 19981 .... LYM .... lymphocytes (LYMP) count (units per volume x 103) .	17%	9%	0.017	0.002
Lake1	MONO	## 19982 .... MONO .... monocytes (MONO) count (units per volume x 103	39%	8%	0.000	< 0.001
Lake1	EOS	## 19983 .... EOS .... eosinophils (EOS) count (units per volume x 103) .... r	22%	9%	0.001	0.004
Lake1	LUC	## 19984 .... LUC .... large unstained cells (LUC) count (units per volume x	56%	7%	0.000	< 0.001
Lake1	BASO	## 19985 .... BASO .... basophils (BASO) count (units per volume x 103)	44%	8%	0.000	< 0.001
Lake1	reticulocytes	## 21917 .... Retic .... reticulocytes (Retic) count (units per volume x 109)	51%	8%	0.000	0.001
Lake1	pct_NEUT	## 19903 .... pct_NEUT .... percent neutrophils (% of total WBC) .... %	15%	7%	0.003	0.025
Lake1	pct_LYM	## 19904 .... pct_LYM .... percent lymphocytes (% of total WBC) .... %	16%	7%	0.001	0.008
Lake1	pct_MONO	## 19905 .... pct_MONO .... percent monocytes (% of total WBC) .... %	40%	8%	0.000	< 0.001
Lake1	pct_EOS	## 19906 .... pct_EOS .... percent eosinophils (% of total WBC) .... %	31%	9%	0.000	0.001
Lake1	pct_LUC	## 19907 .... pct_LUC .... percent large unstained cells (% of total WBC)	56%	7%	0.000	< 0.001
Lake1	pct_BASO	## 19908 .... pct_BASO .... percent basophils (% of total WBC) .... %	45%	8%	0.000	< 0.001
Lake1	pct_Retic	## 19986 .... pct_Retic .... percent reticulocytes (% of total RBC) .... %	49%	8%	0.000	< 0.001
Lake1	cHGB	## 19911 .... cHGB .... calculated hemoglobin (HGB) .... g/dL	39%	8%	0.000	< 0.001
Lake1	PLT	## 19919 .... PLT .... platelet (PLT) count (units per volume x 103) .... n/?L	30%	8%	0.000	< 0.001
Lake1	MPV	## 19920 .... MPV .... mean platelet volume .... fL	56%	8%	0.000	0.003
Lake1	AST	## 19929 .... AST .... aspartate aminotransferase (plasma AST) .... mg/dL	0%	0%	1.000	1.000
Lake1	CHOL	## 19925 .... CHOL .... total cholesterol (plasma CHOL) .... mg/dL	21%	8%	0.001	< 0.001
Lake1	GLU	## 19927 .... GLU .... glucose (plasma GLU, 4h fast) .... mg/dL	26%	9%	0.000	< 0.001
Lake1	HDL	## 19926 .... HDL .... high density lipoprotein cholesterol (plasma HDL)	29%	9%	0.000	< 0.001
Lake1	TFA	## 19930 .... TFA .... total fatty acids (plasma TFA) .... mg/dL	47%	8%	0.000	< 0.001
Lake1	TBIL	## 19931 .... TBIL .... total bilirubin (plasma TBIL) .... mg/dL	0%	0%	1.000	1.000
Lake1	TG	## 19928 .... TG .... triglycerides (plasma TG) .... mg/dL	16%	7%	0.004	0.002
Lake1	QRS	## 19941 .... QRS .... interval between beginning and end of QRS complex	0%	0%	1.000	1.000
Lake1	PR	## 19942 .... PR .... interval between peak of P-wave to R-wave (PR) .... m	7%	7%	0.145	0.286
Lake1	PQ	## 19943 .... PQ .... interval between peak of P-wave to Q-wave (PQ) .... r	9%	7%	0.088	0.189
Lake1	QT	## 19944 .... QT .... interval between peak of Q-wave to end of T-wave (Q	0%	0%	1.000	1.000
Lake1	QTc	## 19945 .... QTc .... rate-corrected QT .... ms	2%	7%	0.374	0.093
Lake1	QT_Dis	## 19946 .... QT_Dis .... QT interval in a string of signals .... ms	15%	7%	0.004	0.036
Lake1	QTc_Dis	## 19947 .... QTc_Dis .... rate corrected QT dispersion .... ms	14%	7%	0.007	0.101
Lake1	HRV	## 19949 .... HRV .... heart rate variability, beats per minute .... n/min	0%	0%	1.000	1.000
Lake1	HR_cv	## 19950 .... HR_cv .... heart rate coefficient of variance .... percent	0%	0%	1.000	1.000
Lake1	bp	## 19953 .... bp .... systolic blood pressure .... mmHg	35%	9%	0.000	< 0.001
Lake1	bp_sd	## 19954 .... bp_sd .... systolic blood pressure variability across tests .... m	15%	8%	0.015	0.006
Lake1	pulse	## 19951 .... pulse .... pulse rate (beats per minute) .... n/min	57%	7%	0.000	< 0.001
Lake1	pulse_sd	## 19952 .... pulse_sd .... pulse rate variability across tests (beats per min	23%	8%	0.000	< 0.001
Lake1	BF_roomair	breath frequency response, room air	40%	9%	0.000	< 0.001
Lake1	BF_saline	breath frequency response, saline	2%	6%	0.399	0.436
Lake1	BF5	breath frequency response to MCh	13%	8%	0.026	0.031
Lake1	BF10	breath frequency response to MCh	19%	8%	0.002	0.002
Lake1	BF20	breath frequency response to MCh	24%	8%	0.000	< 0.001
Lake1	TV_saline	tidal volume response, saline	0%	6%	0.480	0.516
Lake1	TV5	tidal volume response to MCh	19%	8%	0.002	0.018
Lake1	TV10	tidal volume response to MCh	21%	8%	0.000	0.002
Lake1	TV20	tidal volume response to MCh	31%	8%	0.000	< 0.001
Lake1	Penh_roomai	Penh response to MCh	25%	9%	0.000	0.003
Lake1	Penh_saline	Penh response to MCh	4%	7%	0.294	0.140
Lake1	Penh5	Penh response to MCh	20%	8%	0.001	0.005
Lake1	Penh10	Penh response to MCh	32%	10%	0.000	< 0.001
Lake1	Penh20	Penh response to MCh	29%	9%	0.000	< 0.001
Svenson2	WBC	## 21901 .... WBC .... total white blood cell (WBC, leukocyte) count (units	36%	10%	0.000	< 0.001
Svenson2	RBC	## 21902 .... RBC .... total red blood cell (RBC, erythrocyte) count (units pe	18%	9%	0.007	0.005
Svenson2	mHGB	## 21909 .... mHGB .... hemoglobin (HGB) .... g/dL	4%	6%	0.211	0.267
Svenson2	HCT	## 21910 .... HCT .... hematocrit (HCT) .... %	31%	9%	0.000	0.005

Table 2: Litter effect and its statistical significance in three MPD surveys (second part): dataset, variable name and description (in MPD notation), residual variability explained (%) by LE as defined in (2), its standard error (SE) and p-value of a test for a submodel without LE (likelihood-ratio and permutation tests).

Dataset	Variable	Description	% explained	SE (approx.)	p-value (LR test)	p-value(perm. test)
Svenson2	MCV	## 21911 .... MCV .... mean RBC corpuscular volume (MCV) .... fL	55%	8%	0.000	< 0.001
Svenson2	MCH	## 21912 .... MCH .... mean RBC corpuscular hemoglobin content (MCH) ..	0%	0%	1.000	1.000
Svenson2	MCHC	## 21913 .... MCHC .... mean RBC corpuscular hemoglobin concentration (	5%	6%	0.177	0.334
Svenson2	CHCM	## 21914 .... CHCM .... RBC corpuscular hemoglobin concentration mean (	51%	8%	0.000	< 0.001
Svenson2	RDW	## 21915 .... RDW .... RBC corpuscular distribution width (RDW) .... %	44%	9%	0.000	< 0.001
Svenson2	HDW	## 21916 .... HDW .... hemoglobin concentration distribution width (HDW	57%	8%	0.000	< 0.001
Svenson2	PLT	## 21919 .... PLT .... platelet (PLT) count (units per volume x 103) .... n/?L	5%	7%	0.175	0.116
Svenson2	MPV	## 21920 .... MPV .... mean platelet volume (MPV) .... fL	34%	11%	0.001	0.012
Svenson2	NEUT	## 21921 .... NEUT .... neutrophil (NEUT) count (units per volume x 103) ...	19%	11%	0.013	0.215
Svenson2	LYM	## 21922 .... LYM .... lymphocyte (LYMP) count (units per volume x 103) ...	35%	10%	0.000	< 0.001
Svenson2	MONO	## 21923 .... MONO .... monocyte (MONO) count (units per volume x 103)	41%	10%	0.000	< 0.001
Svenson2	LUC	## 21926 .... LUC .... large unstained cells (LUC) count (units per volume x	22%	8%	0.000	0.003
Svenson2	BASO	## 21925 .... BASO .... basophils (BASO) count (units per volume x 103)	44%	9%	0.000	0.004
Svenson2	pctNEUT	## 21903 .... pctNEUT .... percent neutrophils (% of total WBC) .... %	26%	9%	0.000	0.112
Svenson2	pctLYM	## 21904 .... pctLYM .... percent lymphocytes (% of total WBC) .... %	23%	9%	0.000	0.088
Svenson2	pctMONO	## 21905 .... pctMONO .... percent monocytes (% of total WBC) .... %	29%	10%	0.000	< 0.001
Svenson2	pctLUC	## 21907 .... pctLUC .... percent large unstained cells (% of total WBC)	21%	9%	0.001	0.031
Svenson2	pctBASO	## 21908 .... pctBASO .... percent basophils (% of total WBC) .... %	29%	9%	0.000	0.100
Svenson2	pctRetic	## 21992 .... pctRetic .... percent reticulocytes (% of total RBC) .... %	37%	9%	0.000	< 0.001
Svenson2	Retic	## 21917 .... Retic .... reticulocytes (Retic) count (units per volume x 109)	37%	9%	0.000	< 0.001
Svenson2	cHGB	## 21918 .... cHGB .... calculated hemoglobin (HGB) .... g/dL	15%	8%	0.015	0.018
Svenson2	AT3	## 21941 .... AT3 .... antithrombin III (AT III) anticlotting factor .... % of nor	30%	9%	0.000	< 0.001
Svenson2	Fib	## 21942 .... Fib .... blood fibrinogen .... mg/dL	20%	9%	0.004	0.024
Svenson2	F8	## 21943 .... F8 .... clotting factor VIII .... % of normal human value	34%	9%	0.000	< 0.001
Svenson2	TG	## 21962 .... CHOL .... total cholesterol (plasma CHOL) .... mg/dL	16%	8%	0.011	0.028
Svenson2	HDLD	## 21965 .... HDL .... high density lipoprotein cholesterol (plasma HDL)	24%	9%	0.001	< 0.001
Svenson2	AST	## 21967 .... AST .... aspartate aminotransferase (plasma AST, SGOT) .... m	32%	9%	0.000	0.006
Svenson2	FFA	## 21969 .... FFA .... free fatty acids (plasma FFA) .... mEq/L	47%	9%	0.000	< 0.001
Svenson2	TBIL	## 21971 .... TBIL .... total bilirubin (plasma TBIL) .... mg/dL	13%	8%	0.032	0.041
Svenson2	BMD	## 21983 .... BMD .... bone mineral density (BMD) g/cm2	20%	10%	0.008	0.003
Svenson2	BMC	## 21984 .... BMC .... bone mineral content (BMC) .... g	37%	9%	0.000	< 0.001
Svenson2	bone_area	???	33%	9%	0.000	< 0.001
Svenson2	tissue_area	???	20%	9%	0.002	0.002
Svenson2	RST	???	3%	8%	0.347	0.110
Svenson2	total_wt	## 21989 .... total_wt .... total body tissue mass .... g	16%	8%	0.017	0.007
Svenson2	fat_wt	## 21991 .... fat_wt .... body fat tissue weight (calculated) .... g	8%	8%	0.120	0.038
Svenson2	lean_wt	## 21990 .... lean_wt .... lean body tissue mass .... g	20%	10%	0.005	0.008
Svenson2	pct_fat	## 21988 .... pct_fat .... percent of tissue mass that is fat .... %	14%	9%	0.024	0.008
Tordoff3	bw_start	## 10305 .... bw_start .... body weight at start of testing .... g	32%	7%	0.000	< 0.001
Tordoff3	bw_end	## 10306 .... bw_end .... body weight at end of testing .... g	22%	6%	0.000	< 0.001
Tordoff3	bw_chg	## 10307 .... bw_chg .... fold change in body weight .... fold	17%	7%	0.000	0.144
Tordoff3	CaCl2_pref7	## 10308 .... CaCl2_pref7 .... preference for 7.5mM CaCl2 solution .... %	10%	5%	0.001	0.004
Tordoff3	CaCl2_pref25	## 10309 .... CaCl2_pref25 .... preference for 25mM CaCl2 solution .... %	5%	4%	0.077	0.082
Tordoff3	CaCl2_pref75	## 10310 .... CaCl2_pref75 .... preference for 75mM CaCl2 solution .... %	9%	4%	0.001	0.004
Tordoff3	CaLa_pref7	## 10311 .... CaLa_pref7 .... preference for 7.5mM CaLa solution .... %	4%	3%	0.069	0.092
Tordoff3	CaLa_pref25	## 10312 .... CaLa_pref25 .... preference for 25mM CaLa solution .... %	6%	4%	0.027	0.049
Tordoff3	CaLa_pref75	## 10313 .... CaLa_pref75 .... preference for 75mM CaLa solution .... %	2%	3%	0.228	0.229
Tordoff3	NaCl_pref75	## 10315 .... NaCl_pref75 .... preference for 75mM NaCl solution .... %	16%	5%	0.000	< 0.001
Tordoff3	NaCl_pref225	## 10316 .... NaCl_pref225 .... preference for 225mM NaCl solution .... %	13%	5%	0.000	< 0.001
Tordoff3	NaLa_pref25	## 10317 .... NaLa_pref25 .... preference for 25mM NaLa solution .... %	4%	3%	0.097	0.114
Tordoff3	NaLa_pref75	## 10318 .... NaLa_pref75 .... preference for 75mM NaLa solution .... %	14%	6%	0.000	0.002
Tordoff3	NaLa_pref225	## 10319 .... NaLa_pref225 .... preference for 225mM NaLa solution .... %	15%	6%	0.000	0.001
Tordoff3	bleeding_tim	## 10320 .... bleeding_time .... time from tail cut to 1/2 tube of blood coll	15%	5%	0.000	0.111
Tordoff3	ionized_Ca	## 10321 .... ionized_Ca .... blood ionized calcium .... mg/dL	36%	6%	0.000	< 0.001
Tordoff3	pH	## 10322 .... pH .... blood pH .... pH	28%	6%	0.000	< 0.001
Tordoff3	adj_ionized_C	## 10323 .... adj_ionized_Ca .... blood ionized calcium adjusted to pH 7.4 .	39%	6%	0.000	< 0.001
Tordoff3	total_calcium	## 10324 .... total_calcium .... plasma total calcium .... mg/dL	21%	5%	0.000	< 0.001
Tordoff3	BMD	## 10326 .... BMD .... bone mineral density g/cm2	29%	6%	0.000	< 0.001
Tordoff3	BMC	## 10327 .... BMC .... bone mineral content .... g	21%	6%	0.000	< 0.001
Tordoff3	lean_wt	## 10328 .... lean_wt .... calculated weight of lean tissue .... g	44%	6%	0.000	< 0.001
Tordoff3	fat_wt	## 10329 .... fat_wt .... calculated weight of fat tissue .... g	30%	6%	0.000	< 0.001
Tordoff3	total_wt	## 10330 .... total_wt .... total weight (lean + fat) .... g	44%	6%	0.000	< 0.001
Tordoff3	pct_fat	## 10331 .... pct_fat .... percent fat .... %	13%	5%	0.000	< 0.001
Tordoff3	pct_lean	## 10332 .... pct_lean .... percent lean .... %	13%	5%	0.000	< 0.001