Original Research

Weekly Biological Variability of Urinary Organic Acids

RS Lord, PhD;* C Nelson-Dooley, MS; C Morris, BS; JA Bralley, PhD

Metametrix Clinical Laboratory, 3425 Corporate Way, Duluth, GA

Use of LC-MS/MS methods has improved sample preparation and increased throughput for the measurement of 40 or more organic acids in urine. In order to assess the significance of abnormalities that might be attributed to nutritional inadequacies or other metabolic disturbances, the week-to-week variation of results due to normal physiological responses needs to be established. This study determined the biological variability for 37 organic acids plus hippuric acid, D-arabinitol and 8-hydroxy-2'-deoxyguanosine in overnight urine specimens from eight weekly samples submitted by 22 healthy adults. For the 40 analytes, CV_b values varied from 12.3 to 74.3. Fourteen of the analytes had CV_b values less than 30 and another 19 of them were less than 50. Multiple analytes displayed the property of increasing variability with concentration that may be characteristic of most intermediary metabolites. Linear regression line slopes for CV_b vs. concentration were tabulated to assist the use of this information. The 40 analytes display biological variability in the range of disease risk markers such as serum lipoprotein cholesterol concentrations, cancer markers and thyroid hormones. The likelihood of a single measurement being representative of the true mean concentration varies with the analyte and the level found. Data reported here demonstrate reliability of results of urinary organic acid profiling performed under the reported analytical conditions.

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Key Words: *Organic acids, biological variability, LC-MS-MS methods, kynurenic acid, citric acid, formiminoglutamic acid, tricarballylic acid, D-lactic acid, D-arabinitol*

INTRODUCTION

Concentrations of organic acids in urine have been measured for diagnosis of inborn errors of metabolism and as markers of essential nutrient status, neurotransmitter disturbances, toxicant effects and small intestinal microbial overgrowth.[1-8] Elevations of specific organic acids have been reported as markers of nutritional deficiencies, including xanthurenic acid (vitamin B_6),[9] formiminoglutamic acid (folic acid),[10] and 3-hydroxyisovaleric acid (biotin).[11] Neurotransmitter metabolites in urine have been found useful in studies of cancer[12] and behavioral disorders,[13] and exposure to xenobiotic compounds has been monitored by measuring excreted metabolic products.[14] Rationales for using organic acids as markers of various disorders have been reviewed.[1-15]

Variability of urinary analytes has been the subject of multiple reports.[16-19] When 10 overnight urine samples were collected by 10 patients with non-insulin-dependent diabetes mellitus, urinary albumin excretion showed total variability of 12%.[20] A subsequent study found creatinine-normalized urine albumin variability that rose from 19% for normal controls to 61% for children with type I diabetes based on results from 3 consecutive morning urine samples.[21]

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***Corresponding Author:** Chief Science Officer, Metametrix Clinical Laboratory, Duluth, GA 30096. (Email: rslord@metametrix.com)

Knowledge of biological variation (CV_b) can assist decisions about significance of results because, as CV_b increases, the likelihood that any single result approximates the true mean value decreases, and repeated, independent measurements may be required for accurate assessment of risk. Knowledge of biological variation provides a means for assessing the usefulness of population-based reference ranges. For example, it would be useful to know that a result found to be in the 3rd quintile of a reference population would be expected to fall less than one quintile away for a specimen obtained a week later. Such knowledge would assist observations of analyte patterns when multiple, physiologically related analytes are reported. This study was conducted to determine the CV_b of analytes in a profile of urinary organic acids.

METHODS

Study Subjects

Twenty two healthy individuals (8 males and 14 females) of ages ranging from 23 to 65 who had stable diets and lifestyles, and who were not using any prescription medications were enrolled. Subject heights, weights, BMIs and fasting insulin levels are shown in **Table 1**. Regular use of supplementary vitamins or oral contraceptives was allowed throughout the study period. All subjects were free of diagnosed endocrine, metabolic and immune disorders. None of the women became pregnant during the study.

Table 1. Subject demographics and metabolic parameters.

	Age	Gender	Height inches	Weight lbs.	BMI	Fasting Insulin (mIU/mL)	Average Creatinine (mg/dL)
1	32	M	75	210	26.3	2.0	193
2	32	F	63	220	39.1	22.5	66
3	29	F	67	125	19.6	6.8	94
4	44	F	63	115	20.4	2.9	100
5	65	M	71	180	25.2	9.6	65
6	34	F	60	105	20.5	5.0	83
7	30	F	66	173	28.0	7.3	104
8	32	F	64	145	24.9	6.2	270
9	51	M	69	133	19.7	2.0	71
10	25	F	68	160	24.4	8.0	60
11	23	F	65	144	24.0	6.5	132
12	36	F	68	178	27.1	15.4	241
13	32	M	74	220	28.3	10.5	185
14	24	F	64	145	24.9	7.3	199
15	31	M	68	138	21.0	2.0	223
16	30	F	63	170	30.2	8.7	124
17	28	F	64	160	27.5	6.0	184
18	48	M	68	145	22.1	5.6	135
19	33	F	67	143	22.4	12.4	98
20	23	F	61	135	25.6	3.1	158
21	24	M	68	130	19.8	2.8	225
22	31	M	72	210	28.5	22.2	185

Study Protocol

The study protocol was approved by an IRB prior to initiation of any steps. Subjects were instructed concerning the necessity of maintaining normal diet and lifestyle during the eight weeks of the study. At these sessions, the design and intention of the study were explained in printed and verbal format, and informed consent was obtained. All subjects completed a food frequency questionnaire as required for nutrient intake analysis by Viocare software. In addition, on each day of specimen collection, participants completed a survey including dietary and nutritional supplement intake, and they were asked to record any current symptoms or unusual events. No special dietary restrictions other than maintaining normal habits were imposed.

Participants were asked to follow a standard protocol for collection of overnight urine specimens every Tuesday morning for 8 consecutive weeks. All urine collected in a 2 liter plastic bottle from bedtime to morning arising was mixed by swirling, and two 10 ml specimens were transferred to separate tubes containing 1 mg thymol. The specimens were delivered to the laboratory the morning of collection and stored on ice prior to preparation for analysis.

Each week of collection, specimens were analyzed in a single batch within 32 hrs. of collection. At the end of the eight weeks, one subject submitted an entire overnight urine collection on which replicate analyses were performed to determine within-run and between-run analytical variability. This specimen was analyzed in 5 replicates performed three times in every-other day intervals. As an alternative source of analytical variability data, replicate values were obtained during the span of the study for control solutions. Specimens submitted too late for processing the same day were stored at 2 – 8C for processing on the next day. According to stability studies performed at the laboratory as part of routine method validation procedures, the urine samples were stable for 1 week when stored at 2 - 8 ℃.

Table 2. Panel Compositions for LC/MS-MS Methods. These panels account for 35 of the reported analytes. Separate methods were used as described for 8-hydroxy-2'-deoxyguanosine, D-arabinitol, sulfate and L- and D-lactic acids.

Panel 1 (electrospray negative mode)	**Panel 2 (electrospray negative mode)**	**Panel 3 (electrospray positive mode)**
cis-Aconitic acid	Adipic acid	Formiminoglutamic acid
Citric acid	Hippuric acid	5-Hydroxyindoleacetic acid
Ethylmalonic acid	Homovanillic acid	2-Methylhippuric acid
Fumaric acid	4-Hydroxyphenylacetic acid	Pyroglutamic acid
Glucaric acid	4-Hydroxyphenyllactic acid	
3-Hydroxybutyric acid	Indoxyl sulfuric acid	
3-Hydroxyisovaleric acid	Kynurenic acid	
Hydroxymethyglutaric acid	Suberic acid	
Isocitric acid	Vanilmandelic acid	
2-Ketoglutaric acid	Xanthurenic acid	
2-Ketoisocaproic acid		
2-Ketoisovaleric acid		
2-Keto-3-methylvaleric acid		
Malic acid		
Methylmalonic acid		
Orotic acid		
Picolinic acid		
Pyruvic acid		
Quinolinic acid		
Succinic acid		
Tricarballylic acid		

Specimens were divided into aliquots used for seven procedures for measuring individual analytes or panels of related analytes. Three LC/MS-MS methods were used to analyze the panels shown in Table 2 with analytical parameters and deuterated internal standards optimized for each set of compounds. Details of methods have been published for Panel 2[22] and Panel 3.[23] Panel 1 was performed by a method similar to that used for Panel 2, including isotopic dilution of specimens and quantitation from matrix-matched calibration curves. Resolution of analytes was achieved by reverse-phase liquid chromatography with detection by tandem mass spectrometry in electrospray positive or negative ionization mode. Two other previously published LC/MS-MS methods were used to determine 8-hydroxy-2'-deoxyguanosine[24] and L- and D-lactate.[25] D-Arabinitol was measured on an Olympus AU400 using a single channel, self-blanking enzyme assay method (Arabinitech-Auto, manufactured by Marukin-Bio) without pretreatment. Sulfate was measured using a barium chloride turbidometric procedure, and creatinine was measured spectrophotometrically by the alkaline picrate method. According to standard laboratory procedures, two levels of controls were run at the beginning and middle of each batch for all methods. Analytical performance was confirmed by demonstration of control levels within 2 standard deviations of the means.

Statistical Methods

Total test variability (CV_T) was calculated as the average of coefficients of variation for each analyte over all subjects. Biological variability (CV_b) values for the measured compounds were calculated as the difference between (CV_T) and analytical variability (CV_a), using $CV_b = ((CV_T)^2-(CV_a)^2)^{1/2}$.[26] Since subject specimens were not analyzed in replicate it was not possible to derive CV_a from those measurements. Instead, CV_a values for most analytes were derived from the mean concentrations and standard deviations of data from 5 replicate analyses on three specimens from a single subject. Most analyte concentrations in that subject were close to the averages for the other subjects. However, for a few analytes CV_a could not be calculated from the single subject specimen because the levels were below quantitative limits of detection. In those instances, variability of data from the between-run replicates of the normal control specimen during the eight weeks of the study was used to as CV_a, as noted in the footnote to **Table 3**. Analyte concentrations in the normal control are adjusted to produce upper normal range concentrations.

Table 3. Biological variabilities of analytes are shown in order of increasing CV_b (concentrations in mcg/mg creatinine).

Analyte	CV_T	CV_a	**CV_b**	Average Concentration (Biol. Var. Range)	Laboratory 95% Reference Range	SD v. Conc. Slope
Picolinic acid	16	9.7	**12.3**	6.1 (5.7-6.5)	1.8 – 11.2	0.14
Isocitric acid	18	3.9	**17.5**	74.7 (68.2-81.2)	1.0 – 110	0.41
Quinolinic acid	21	10.4	**17.8**	2.8 (2.6-3)	< 5.8	0.16
Ethylmalonic acid	20	9.2	**18.3**	2.1 (1.9-2.3)	< 4.4	0.43
cis-Aconitic acid	19	3.9	**19.1**	37.2 (33.6-40.8)	1.0 – 74	0.29
Methylmalonic acid	22	9.7	**19.4**	1.1 (1.0-1.2)	< 2.0	0.24
Vanilmandelic acid	21	3.4	**20.9**	2.7 (2.4-3)	1.0 – 5.7	0.23
Sulfuric acid	22	2.5	**21.8**	1380 (1230-1530)	762 – 2778	0.25
8-Hydroxy-2-deoxyguanosine	26	4.4	**25.5**	3.6 (3.1-4.1)	< 7.6	0.25
Glucaric acid	31	16.4	**25.9**	4.3 (3.7-4.9)	< 14.9	0.21
Hydroxymethylglutaric acid	28	5	**27.6**	3.4 (2.9-3.9)	< 5.2	0.15
3-Hydroxyisovaleric acid	29	7.9	**28.1**	6.4 (5.5-7.3)	< 7.9	0.35
2-Methylhippuric acid	35	19.2	**29.3**	0.04 (0.03-0.05)	< 0.073	--
Citric acid	34	5.7	**29.5**	386 (329-443)	9.0 - 670	0.16
Homovanillic acid	30	3.7	**30.1**	3.6 (3.1-4.1)	0.8 – 13	0.01
Pyruvic acid*	34	14	**30.6**	3.2 (2.7-3.7)	< 4.9	0.21
L-Lactic acid	37	15.2	**33.5**	9.4 (7.8-11.0)	3.0 – 47	0.74
Pyroglutamic acid	34	3.5	**33.5**	25.5 (21.2-29.8)	29 - 85	1.04
2-Ketoglutaric acid	49	34.3	**35.2**	14.5 (11.9-17.1)	< 35	0.17
3-Hydroxybutyric acid	40	17	**36.2**	1.7 (1.4-2.0)	< 9.9	0.04
4-Hydroxyphenyllactic acid	37	6	**36.7**	0.6 (0.49-0.71)	< 1.5	0.22
Xanthurenic acid	37	5.4	**36.9**	0.7 (0.57-0.83)	< 0.74	0.51
Malic acid	50	32.7	**38.2**	0.53 (0.43-0.63)	< 3.1	0.41
Tricarballylic acid	40	11.2	**38.3**	0.51 (0.41-0.61)	< 1.4	0.52
2-Ketoisocaproic acid*	40	8	**39.6**	0.31 (0.25-0.37)	< 0.52	0.78
D-Arabinitol	40	0.7	**40.4**	23.4 (18.7-28.1)	< 73	1.33
4-Hydroxyphenylacetic acid	41	5	**40.6**	14.4 (11.5-17.3)	< 34	0.54
5-Hydroxyindoleacetic acid	42	1.9	**42.3**	2.9 (2.3-3.5)	1.6 – 9.8	0.65
Adipic acid	44	7.2	**43.1**	5.5 (4.3-6.7)	< 8.3	0.44
Kynurenic acid	43	2.1	**43.4**	1.9 (1.5-2.3)	< 2.7	0.41
Indoxyl sulfuric acid*	44	7	**43.5**	47.2 (36.9-57.5)	< 74	0.07
2-Ketoisovaleric acid*	47	8	**46.1**	0.27 (0.21-0.33)	< 0.49	0.89
2-Keto-3-methylvaleric acid	56	30	**48.2**	0.31 (0.24-0.38)	< 1.1	1.38
Succinic acid	61	37.2	**48.4**	1.7 (1.3-2.1)	< 20.9	0.64
Formiminoglutamic acid	66	5.6	**48.4**	0.86 (0.7-1.1)	< 2.2	0.01
Suberic acid	52	4.4	**52.0**	1.5 (1.1-1.9)	< 3.2	0.62
D-Lactic acid	63	15.8	**60.9**	1.5 (1.04-1.96)	< 7.0	1.21
Hippuric acid	64	1.6	**64.2**	283 (192-374)	< 1150	0.57
Orotic acid	72	24.9	**67.2**	0.44 (0.29-0.59)	< 1.01	0
Fumaric acid	82	36.4	**74.3**	0.34 (0.21-0.47)	< 1.35	0.01

* Analytes in the replicate specimen at concentrations too low to allow variance calculation, for which normal control variances were used as CV_a. Concentrations as mcg/mg creatinine.

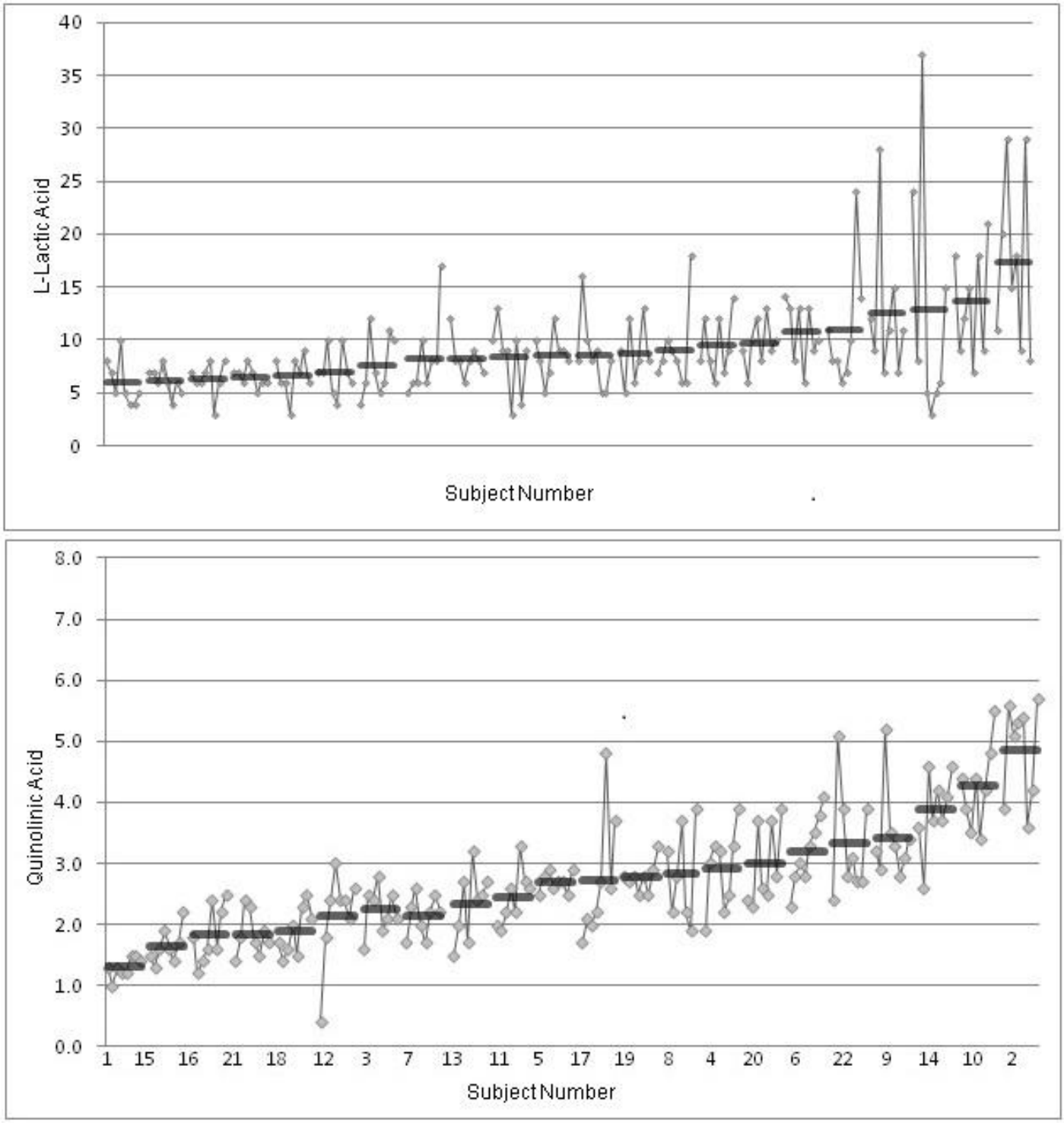

Figure 1. Eight weekly levels are plotted for each of the 22 subjects and displayed in order of mean concentration (mcg/mg creatinine). Short, horizontal grey bars show mean levels for each subject.

RESULTS

Body mass index (BMI) values for the subjects ranged from 19.6 to 39.1, and their fasting insulin values ranged from 2.0 to 22.5 (see Table 1). Although some subjects had less than ideal values for both of these parameters, the overall subject population was judged to represent the general outpatient US population in this regard. Average creatinine concentrations in the 8 weekly urine specimens ranged from 66 to 241 mg/dL. Fasting insulin levels above 15 microIU/mL were found in subjects 2, 12 and 22, but no apparent effects on analyte concentrations or variability were associated with them.

Values for essential nutrient intake based on food frequency questionnaire responses and calculated by the Viocare Pronutra software were at or above recommended intakes for those nutrients with potential to directly influence specific urinary organic acids. Nutrients considered most important in this regard were vitamins B_1, B_2, B_3, B_5, B_{12}, biotin, folic acid and protein. Values for the all other analyzed nutrients fell in ranges that are at or above reported averages for Americans.[27]

Data from the laboratory's commercial patient database were used to assess the effects of creatinine normalization. When organic acid profile concentrations from a large set of patient specimens were normalized to creatinine concentrations, the average CV_T for all analytes fell by 17%. These changes are presumed primarily to reflect the reduction of subject variance due to hydration offsetting positive contributions from creatinine CV of 2.3%. All results from the study specimens are expressed as mcg/mg creatinine.

For all analytes, average subject concentration, CVT, CVa, and CVb values are shown in **Table 3**. During week 5 for subject 2 multiple, uniquely large excursions of results were found for 5 analytes (3-hydroxybutyric, 3-hydroxyisovaleric, citric, isocitric and cis-aconitic acids). The results for these analytes differed from the means of the other 7 weeks for subject 2 by an average of 10.5 standard deviations. The week 5 data for subject 2 were rejected when the data excursions were found to coincide with an unusually stressful period of personal and work-related events during that week, reported by the subject. The resulting CVb values for all analytes ranged from 12.3 for picolinic acid to 74.3 for fumaric acid. In order to evaluate the significance of variations within the healthy subjects, reference ranges from the laboratory database composed of an adult outpatient population with widely diverse conditions are included in **Table 3**. Also listed in **Table 3** are the average analyte concentrations with ranges expressed as average ± CVb and linear curve fit slopes of SD v. concentration. For the large majority of analytes, the healthy subject ranges were within the laboratory 95% reference range limits. Generally, the exceptions are analytes with average healthy subject concentrations less than 1 mcg/mg creatinine.

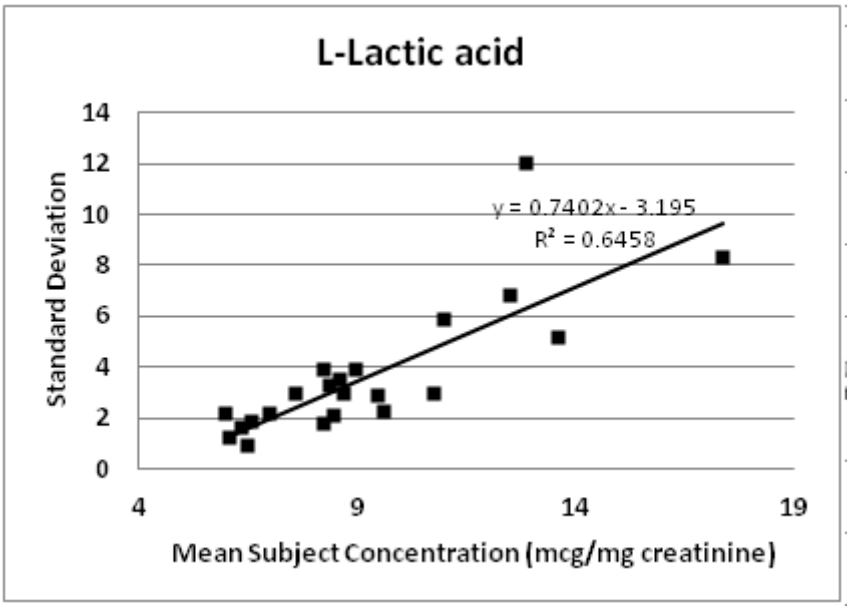

Figure 2. Plot of standard deviations vs. mean concentrations for the 22 subjects for L-lactic acid. Similar linear relationships of various regression line slopes were found for all other analytes except for 2-methylhippuric acid that uniquely required an exponential curve fit (see text).

Figure 1 shows all weekly values for (a) L-lactic and (b) quinolinic acid for each subject, presented in order of increasing subject mean concentrations. A greater tendency for L-lactic acid than for quinolinic acid for CV_b to vary with mean concentration is apparent in these data. In order to present the degree of this trend for all analytes, linear regression curve trend line slopes for plots of standard deviation vs mean concentrations were determined as shown in **Table 3**. **Figure 2A-2B** show the curves and linear regression lines for lactic and quinolinic acids, and figure 2c shows the exponential curve fit required to fit the data for 2-methylhyppuric acid.

DISCUSSION

The nature of biological variation for many disease risk factors has been examined.[17,18,28] Serum lipids are one of the most thoroughly analyzed types of clinical laboratory data. Composite estimates of biological variability for total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC) and

triglyceride (TG) by meta-analysis of 30 published studies was done in order to determine the number of patient specimens required to obtain reliable values.[29] Variability was found to depend on the number of subjects. The results led to suggesting a minimum of two specimens per patient for TC and four for HDLC, LDLC, and TG for reliable estimate of average levels. Inter-individual biological variation for cancer markers CA 19-9, carcinoembryonic antigen and alpha-fetoprotein were determined to be 27.2, 30.8 and 26.6%, respectively.[30] Similarly, the variability for serum thyroid stimulating hormone, free T3, and free T4 in Turkish people were 37.2, 22.3, and 13.2% respectively.[31]

Reports of values for CV_b have led to conclusions regarding the number of samples required for reliable estimates of serum lipids[29] and the medical usefulness of urine for diagnosis and screening of pathologies.[19] When CV_b was determined from twice weekly measurements in 20 healthy adults, values for cholesterol, HDL cholesterol, apo A-I, and apo B were less than 8%, cholesterol was less than 10%, and triglyceride was 28%. Intra-subject CV_b for Lp(a) ranged from 1% to 50% among the 20 subjects in a direct concentration-dependent manner.[26] Similar direct concentration-dependent variation in CV_b was found for most analytes in this study. However, the overall mean CV_b values can provide a measure of the usefulness of population-based reference ranges.

Sample preparation can have large effects on analyte variability. The GC-MS methods used in most early studies of urinary organic acids required steps of extraction, transfer, evaporation and derivitization that are error-prone. In addition, for profiles containing numerous analytes, methods requiring extractions and derivitizations are likely to have low recoveries for some analytes due to issues of solubility, volatility, or lack of reactivity. These are principal reasons for the superiority of LC-MS/MS methods where minimal sample preparation is needed. Further method accuracy and reliability is achieved by performing sub-profiles as necessary to allow LC and tandem MS conditions suitable for specific analyte types and by utilizing deuterated internal standards to correct for deviations of instrumental performance.[22,25,32] The methods used in this study incorporated these approaches to achieve the analytical performances reported.

For most analytes in this study, analytical variations (CV_a) were small compared to CV_T values. Four analytes had CV_a values above 20% due to their concentrations in the replicate specimen being near their limits of detection where analytical variability generally rises (see **Table 3**). The kynurenin pathway products, picolinic and quinolinic acids that reflect states of inflammation had some of the lowest CV_b values (12.3 and 17.8, respectively) in the healthy individuals of this study. On the other hand, indican and D-Lactic and hippuric acids, compounds that can be influenced by normal fluctuations of intestinal bacteria growth had some of the highest CV_b values (43.5, 60.9 and 64.2, respectively). Other compounds of moderate to higher variability such as succinic, fumaric and suberic acids may be sensitive to macronutrient intake, illustrating the potential for using challenge compounds that can raise metabolic throughput to achieve improved clinical significance of abnormal results.

When biological variations are compared to the laboratory reference ranges, the subject data is found generally to fall consistently below 95 percentile limits. For fumaric acid, the analyte with the highest CV_b, overall variation 0f 74.3% about the mean of 0.4 mcg/mg creatinine indicates that, on average, values consistently fall under the upper reference limit of 1.35 mcg/mg creatinine, showing consistency of normal results in healthy individuals.

The L-lactic acid data for all subjects in **Figure 1A** shows general characteristics that are representative of most of the analytes studied. Concentrations tended to fall in the range of 6 to 20 mcg/mg creatinine, which is normal relative to the laboratory 95% reference interval of 3-47 mcg/mg creatinine. The well-known physiological regulatory action that resists sustained elevated L-lactic acid may explain some variations.[33] For example, in 2 of the first 3 weeks for subject 11, levels of 24 and 37 mcg/mg creatinine were found, followed by 4 weeks of values below 7.8 mcg/mg creatinine. Similarly, subjects 6 and 20 had CV_b's well above the overall average because their results never stayed in the higher ranges for more than a single week. Such effects may produce CV_b values for normal biochemical intermediates that increase with mean values because normalizing physiological forces tend to act more aggressively when concentrations rise. Such data may lead to further refinement of reference ranges by identifying individuals with sub-clinical metabolic weaknesses who should be excluded from reference populations. Blood L-lactate is a metabolic marker that can signal poor homeostatic regulation at levels less than frank elevations characteristic of high disease risk.[34]

The linear trend line slopes of concentration vs. mean plots listed in **Table 3** range from near zero for formiminoglutamate to 1.33 for the intestinal yeast marker D-arabinitol. The data plotted in **Figure 2A** for L-lactic acid with a standard deviation increase of 0.74/mcg/mg creatinine is representative of those analytes that have higher tendencies to display this effect. The slope values provide a means of weighing each analytes relative tendency to display variability that increases with concentration. One potential use of this information is to generate weighting factors for interpretation of multiple-analyte profiles. For example, a result slightly above the 95%ile limits for fumaric acid would be weighted less than a similar result for quinolinic acid. The phenomenon of increasing variability with concentration for biochemical pathway intermediates arises due to homeostatic influences. As the concentrations of such analytes like suberate and ethylmalonate increase in liver, muscle and other tissues, compensatory events tend to come into play to normalize their levels. In the case of D-arabinitol, the high variability may be due to short bursts of intestinal yeast growth that are suppressed by normal immune responses in healthy individuals. In highly abnormal states such as inherited metabolic disorders, normal biochemical intermediates can be consistently elevated, producing

hallmark features such as phenylpyruvic aciduria found in PKU.

No value is shown for 2-methylhippurate in Table 3 because an exponential trend line was required to fit the CV_b vs. concentration data. This compound, a sensitive marker of xylene exposure, was consistently found in low concentrations with low variance for most subjects. The two subjects with the highest 2-methylhippuric acid CV_b reported, on interrogation, exposure events of house painting or cleaning fluid use that occurred shortly prior to the single week excursions of 2-methylhippurate. Rapid xylene clearance following sporadic episodes of exposure among individuals with relatively low average exposure may explain this behavior. Thus, on any week where elevated results were found, levels routinely fell to lower levels by the following Monday evening, producing the exponential curve fit requirement.

Biological variability data is useful for defining the number of specimens needed to approximate true average analyte concentration.[26] On the other hand, it can be used to estimate the probability of any single measurement being within a specified range of the true average. In routine clinical practice, decisions are frequently made based on single measurements as general awareness of variability is taken into account. When multiple, related compounds are measured, pattern analysis can strengthen interpretations, because random fluctuations are less likely to cause similar perturbations of multiple analytes. An example in this profile is the set of lactic, pyruvic, and alpha-ketoglutaric acids that become elevated due to thiamin deficiencies.[35]

CONCLUSION

Routine determination of normal biochemical intermediates in urine over the entire physiological range is challenging due to the necessity of maintaining accurate calibrations at low as well as high levels. In order to produce data of sufficient accuracy for clinical inspection of mild elevations and, in some cases, suppressed excretion below low normal limits, laboratories must develop rugged methods with sufficient sensitivity and analytical dynamic range. Ruggedness here implies that the assay performance is independent of the person performing the test and the results are consistent over extended time intervals. These data show for the first time the feasibility of achieving such analytical performance in reporting a profile of 40 organic acids in human urine. The phenomenon of variability increasing with concentration needs further investigation and it suggests the need for innovative ways of expressing results so that they may be properly interpreted no matter where they fall relative to normal ranges.

CONFLICT OF INTEREST
None.

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