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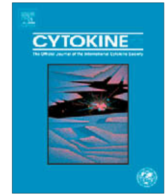

Day-to-day variation in plasma interleukin-6 concentrations in older adults

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ABSTRACT

Interleukin-6 (IL-6) is a pro-inflammatory cytokine commonly used in studies as a means of assessing chronic inflammatory status. Despite the use of plasma IL-6 as a marker of chronic inflammation few studies exist that examine the variability of plasma IL-6 within and between individuals. The purpose of this study was to assess inter- and intra-variability of plasma IL-6 concentration in men and postmenopausal women. Sixteen healthy postmenopausal women and 5 men completed the 2-week study. Fasted venous blood samples were obtained on three consecutive mornings for two consecutive weeks (six blood draws per participant). Mean plasma IL-6 values were 2.00 ± 1.74 pg/mL. Intra-variability was not significantly different ($p > 0.05$) however inter-variability was significantly different ($p < 0.05$). The index of individuality (II) was 0.20 and the standard error of the mean (SEM) was determined to equal 0.16 pg/mL (0.32 pg/mL; 1.96 SEM). An II of 0.20 demonstrates the need to carefully evaluate changes in plasma IL-6 concentration instead of utilizing population-based reference norms. In an older adult population until plasma IL-6 differences exceed 0.32 pg/mL such values could be considered normal fluctuation between trials and most likely not attributable to a nutrition intervention.

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1. Introduction

Interleukin-6 (IL-6) is a pro-inflammatory cytokine commonly used in epidemiological studies as a means of measuring chronic inflammatory status. Chronic inflammation has been linked with conditions including cardiovascular disease [1,2], cancer [3–5], arthritis [6], obesity [7,8], and Type 2 diabetes mellitus [9–11]. Sources of variation for biochemical analytes include biological (e.g. age and body weight), preanalytical (e.g. specimen collection), analytical (e.g. bias and imprecision), and postanalytical (e.g. reporting of results) causes. Specifically, plasma IL-6 concentrations may be influenced by a person's age [12], weight [8], disease conditions [1–3,6,11], medications/supplements [13,14], alcohol consumption [15,16], composition of the last meal eaten [17–19], and activity level [20,21]. Despite these sources of variation, biochemical analytes are used to evaluate serial changes in individual analytes and assess the clinical utility of population-based reference intervals [22].

In 1974, Harris introduced the concept of the index of individuality (II) as the ratio of the total within subject variation ($CV_{w/i}$) to between-subject ($CV_{b/t}$) variation [23]. A low II (<0.6) means the analyte has marked individuality ($CV_{w/i} < CV_{b/t}$) whereas a high II (>1.4) indicates the analyte has little individuality ($CV_{w/i} > CV_{b/t}$). Considering the sources of biological variation, the ideal analyte would have a small $CV_{w/i}$ and $CV_{b/t}$ resulting in a high II which would make population-based reference values useful in the clinical setting [24]. Most tests have marked individuality (low II) thus comparing these analytes with previous values is advantageous.

Few studies exist that examine the variability of IL-6 within and between individuals. In order to use blood concentrations of IL-6 as an inflammatory biomarker, it is important to know the day-to-day variability of this cytokine to determine whether a single fasted sample is sufficient or if consecutive day blood samples are needed to represent an individual's IL-6 status. Furthermore, it is unknown how much variability occurs day-to-day that must be overcome to detect a change in inflammatory status as a result of an intervention (e.g. dietary or pharmaceutical). The purpose of this study was to assess both inter- and intra-variability of plasma IL-6 concentrations in men and postmenopausal women. We hypothesized that there would be little day-to-day variability within subjects but significant variability between subjects resulting in a low II over the six days of blood sampling.

2. Materials and methods

2.1. Sample

Sixteen postmenopausal women (aged 50–81) and five men (aged 44–72) were recruited from the community via flyers and

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a newspaper advertisement. Subjects phoned the Nutrition Research Laboratory and answered an oratory screening questionnaire to determine eligibility. All subjects signed a consent form approved by the Montana State University Institutional Review Board prior to participating in the study. Subjects were eligible for inclusion if they were either male and between 40 and 75 years of age, or female and postmenopausal. Postmenopausal status was defined as absence of a menstrual cycle for at least 12 consecutive months (self-reported). Exclusion criteria included consumption of anti-inflammatory medications (e.g. ibuprofen and aspirin) or supplements (e.g. fish oils) within the previous 2 weeks, diagnosis of a chronic inflammatory condition (e.g. diabetes mellitus and cardiovascular disease), current use of hormone therapy, or menstrual cycle within the past 12 months. Subjects who reported exercising on a regular basis were included if they agreed to refrain from exercising for the 24 h prior to each blood draw. Additionally, prior to each blood draw subjects were asked whether they had experienced any cold or flu symptoms in the previous 24 h; reporting symptoms would have excluded them from the study.

2.2. Design

The study design is a prospective cohort study. Subjects reported to Bozeman Deaconess Outpatient Clinic in a fasted state (water only for the previous eight hours) between the hours of 7 and 8 a.m. for blood draws for three consecutive days for two consecutive weeks, for a total of six blood draws. Blood was drawn from the antecubital vein into EDTA vacutainers (Becton Dickinson; San Jose, California) by an experienced phlebotomist. Blood was immediately transported to the Montana State University Nutrition Research Lab and centrifuged at 700 g for 15 min at 12 °C; plasma was subsequently stored at −80 °C. Plasma IL-6 concentrations were analyzed using R&D Systems Inc. Quantikine® Human IL-6α Immunoassay diagnostic kit (Minneapolis, MN). Manufacturer protocols were followed. As stated in the manufacturer's instructions, the minimum detectable dose (MDD) of IL-6 ranged from 0.016 to 0.110 pg/mL. The mean MDD was 0.039 pg/mL. The intra-assay coefficient of variance was 5%; no interassay CV was determined as all samples from an individual were run at the same time to minimize variance attributable to between assay analysis.

2.3. Statistical analysis

To evaluate the stability of measures across all trials, a two-way analysis of variance was performed to separate the soruces of variability due to intra-individual ($CV_{w/i}$) and inter-individual ($CV_{b/t}$) effects. Following this, components of the analysis were extracted and utilized in standard calculations of the index of individuality (II) based on the ratio of the coefficients of variation (CV) for the within and between subjects effects as proposed by Harris [23]; as well as the coefficient of reliability and the standard error measurement for the IL-6 data. With this analysis, both the interaction (subjects × trials) and main effect for trials were evaluated as indicators of stability. For statistical analysis, SPSS software was utilized (SPSS for Windows, version 14.0.0. SPSS, Inc., Chicago, IL 2005). Statistical significance for all tests was set at the level of $p < 0.05$.

3. Results

For descriptive characteristics of the subjects, see Table 1. Twenty-one subjects participated in the study; sixteen women and five men. All 21 subjects completed the study (none reported cold or flu symptoms in the 24 h prior to the blood draw). All subjects reported their ethnicity as Caucasian. Subjects included four previous smokers and one current smoker. The current smoker agreed not to smoke during the 10 h prior to each of the blood draws. Average weekly alcohol intake was 0.5 drinks (0–3 drinks).

Table 1
Subject Characteristics.[a]

	All subjects	Women	Men
Number of subjects	21	16	5
Age (years)	59 ± 9.4	59.1 ± 10.4	58.4 ± 6.8
Height (cm, self-reported)	167.6 ± 9.4	164.6 ± 9.4	177.3 ± 7.9
Weight (kg, self-reported)	73.8 ± 20.0	67.5 ± 20.5	94.1 ± 21.1
BMI (kg/m^2)	26.0 ± 5.5	24.8 ± 5.8	29.7 ± 5.1
Medical history			
Diabetes mellitus	0	0	0
CVD	0	0	0
Hypertension	3	1	2
Cirrhosis/hepatitis	0	0	0
High cholesterol	6	4	2
Angina	0	0	0
Anemia	0	0	0
Cancer	0	0	0
Gout	0	0	0
Thyroid	2	2	0

[a] Data is reported as mean ± standard deviation.

Average plasma IL-6 values were 2.00 ± 1.74 pg/mL. There were no plasma IL-6 concentration differences between the men and women so data were analyzed collectively. Two-way analysis of variance of the IL-6 data revealed significant differences between subjects, but an absence of significant effects across trials or any significant subject × trials interaction. Day-to-day variation within (represented by the average value for each of the six days) and between (represented by the error bars on each single day) subjects is represented in Fig. 1. Corresponding to this stability of measures across trials, an II of 0.20 ($CV_{w/i}/CV_{b/t} = II$; therefore 37.14/185.85 = 0.20) and a coefficient of reliability of $r = 0.96$ was revealed. In addition, the standard error of measurement (SEM) was found to equal 0.16 pg/mL or 8% of the grand mean for the sample. The power for these analyses was determined to be 0.92.

4. Discussion

In the present study, intra-individual (within) subject IL-6 concentrations were not significantly different from one day to the next, while inter-individual (between) subject IL-6 concentrations varied significantly. As expected, the variability between subjects is greater than the variability within subjects.

An II below 0.60 is considered to be "low" in magnitude and suggests that the day-to-day variation of IL-6 measures tends to stay within a relatively narrow range for most individuals, in comparison to the overall variation in concentration that is seen across the general population. This means that the evaluation of serial changes of IL-6 concentrations within an individual may be more meaningful in detecting a change in health status than to use a single comparison to a population-based reference standard.

Using the standard error of measurement, calculation of a 95% confidence interval can provide insight in terms of the normal range of trial-to-trial differences in IL-6 measures that can occur within a typical subject. These results suggest that until differences exceed 0.32 pg/ml (1.96 SEM) such values could be considered to be normal between trial variations, and most likely not attributable to other effects (e.g. diet, exercise or anti-inflammatory medications). This information may be particularly useful to those designing research studies with outcome measures utilizing plasma IL-6 concentrations as a biomarker of inflammation.

Very few studies have examined the intra-individual and inter-individual variability of IL-6, and to our knowledge this is the first

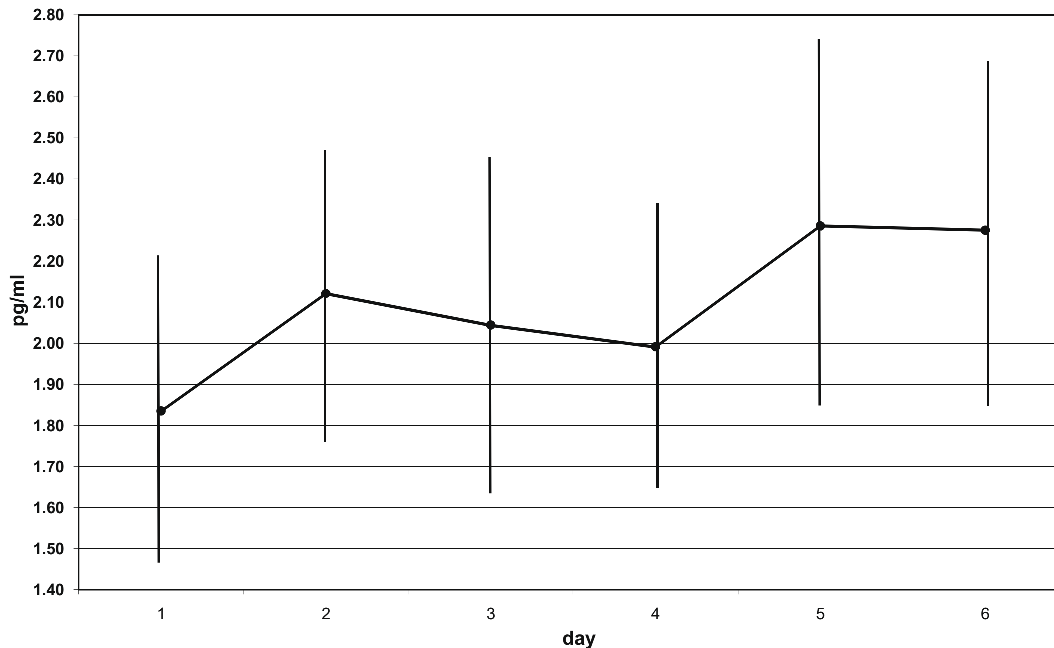

Fig. 1. Inter- and intra-variability of plasma interleukin-6 concentrations.

study to assess day-to-day variation from multiple samples collected in a short time span. A previous study conducted by Dugue et al. measured serum IL-6 concentrations in 22 healthy persons over a 3.5 h period [17]. Blood was drawn after an overnight fast (8 a.m.), after volunteers ate breakfast (9:30 a.m.), and two hours after consumption of breakfast (11:30 a.m.). Results showed a significant ($p < 0.05$) difference in serum IL-6 concentrations between the 8:00 and 9:30 a.m. blood draws (1.75 ng/l and 1.36 ng/l, respectively) and a significant ($p < 0.01$) difference in IL-6 concentrations between the 9:30 and 11:30 a.m. blood draws (1.36 ng/l and 1.91 ng/l, respectively) but no significant difference in serum IL-6 concentration between the 8:00 and 11:30 a.m. draws. Notably, this demonstrates that serum IL-6 concentration is influenced by food intake, as the IL-6 concentration immediately following breakfast was different from both the serum concentration in a fasted state and the serum concentration two hours postprandial. These results indicate that, ideally, serum IL-6 should be measured after an overnight fast or at the very least a 2-h fast. Utilizing the fasted baseline IL-6 values, an II of 0.70 was found. An II between 0.60 and 1.4 indicates that the biomarker may be compared against a population-based reference [23]. Despite an acceptable II from baseline samples this study did not actually assess changes in IL-6 from day-to-day.

Ho et al. measured plasma IL-6 concentrations from one sample at baseline, as well as at 12 and 24 months in 36 women (19.5 ± 3.2 years) [25]. An intraclass correlation coefficient (ICC), (between subject variance/total variance) of 0.48 was calculated. An ICC of 0 indicates no correlation in IL-6 values from the same individual over time whereas an ICC of 1 demonstrates a perfect correlation. However, the authors did not indicate whether the blood draws were done in a fasted or fed state [17], or if they controlled for the phase of the menstrual cycle [26,27]. Both factors could influence plasma IL-6 concentrations.

Finally, a study conducted by Cava et al. measured serum IL-6 concentrations once per month for 6 consecutive months in 15 subjects in a fasted state [28]. Subjects included 4 males (aged 30.7 ± 5.7 years) and 11 females (aged 39.6 ± 11.1 years). Subjects continued their regular lifestyle during the study and were asked to avoid strenuous exercise after 10 p.m. the evening prior to the blood draw. An II of 1.4 was determined; this is at the upper limit of the II range that suggests it is appropriate to compare an isolated result with population-based reference values for diagnostic purposes; however the reliability coefficient ($R = 0.37$) is low. The authors suggested that due to a low R value, more than one sample should be collected from each patient to determine the patient's "true" IL-6 concentration. Also, assuming some of the subjects were perimenopausal based on the age of the subjects, no mention was made regarding the phase of each woman's menstrual cycle. Serum IL-6 concentrations have been shown to change with menstrual cycle phases, and sampling of subjects needs to occur during the same phase for all subjects [26,27,29]. By using postmenopausal women for the current study, we avoided the menstrual cycle fluctuations and negated the issue of oral contraceptive use, as plasma IL-6 concentrations have been found to directly correlate with the progestin dose of oral contraceptives [30].

The II provides insight into the ratio of within subject to between subject variability. If the II is between 0.6 and 1.4 comparing a subject's plasma value to a reference standard is deemed appropriate however an II <0.6 or >1.4 suggests the need for serial measurements and the change from pre- to post-treatment is relevant. The present study demonstrated the need for serial measurements due to a low II; furthermore there are not currently reference values for IL-6. A "normal" IL-6 value (or range) has yet to be determined. Future studies are needed to identify a normal IL-6 value particularly as more inflammatory research is conducted. The II identified in the present study (II = 0.2) is different than other studies (0.6 and 1.4). This demonstrates that the within subject and/or between subject variability is influenced by many other factors (e.g. meal timing and menstrual phase). In attempts to clarify these discrepant findings, any future studies that investigate the nature of variation in IL-6 measures will need to carefully control for such factors. This study is limited to the population assessed (older adults). It is suggested that the II and SEM for plasma IL-6 concentrations be determined a priori in the population to be studied.

Inflammation has been linked to numerous chronic diseases and plasma IL-6 is commonly used to assess chronic inflammation. To the best of our knowledge, this is the first study that investigates the individual day-to-day variation in multiple samples of

plasma IL-6 from consecutive days and proposes an average daily fluctuation in concentration that must be overcome in order to attribute an individual's change in concentration to an intervention. Although the within-subject change in IL-6 was not statistically significant, this fluctuation may be clinically relevant. Therefore based on the results of this study and in combination with others, it is suggested that multiple samples be collected (specifically two or three fasted samples from consecutive days) and evaluated for change. Because day-to-day variation of IL-6 is influenced by common daily behaviors including dietary intake, alcohol intake, menstrual cycle, medications, and illness it is essential to control for all of these. Utilizing a similar design, future studies are needed to assess biological variation within and between subjects for other inflammatory biomarkers (e.g. C-reactive protein and tumor necrosis factor alpha).

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