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Intra- and Inter-individual Variabilities in Endogenous Cortisol 6β-Hydroxylation Clearance as an Index for in Vivo CYP3A Phenotyping in Humans

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Running title: Intra- and Inter-individual Variabilities in CYP3A Activity

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Abbreviations: CYP3A, cytochrome P450 3A; CLm(6β), 6β-hydroxylation clearance of endogenous cortisol; AUC, area under the plasma concentration-time curve; X(6β), urinary excreted amount of 6β-hydroxycortisol; AUC(F), area under the plasma concentration-time curve of cortisol; 6β-OHF, 6β-hydroxycortisol; F, cortisol; 6β-OHF/F, urinary ratio of 6β-hydroxycortisol to cortisol; GC-MS-SIM, gas chromatography-mass spectrometry-selected ion monitoring; HPLC, high-performance liquid chromatography; PXR, nuclear pregnane X receptor; CV, coefficient of variation
ABSTRACT:

The present study was designed to evaluate the in vivo activity of cytochrome P450 3A (CYP3A) in 49 healthy Japanese subjects aged 22-58 years using endogenous cortisol 6β-hydroxylation clearance, a novel biomarker for CYP3A-phenotyping. The 6β-hydroxylation clearances (CLm(6β)) in 49 healthy subjects were 2.40 ± 0.79 ml/min with an approximately 4-fold inter-individual variability of CYP3A activity. The mean clearance in 24 female subjects was 2.50 ± 0.89 ml/min, and the value was higher than that in 25 male subjects (2.30 ± 0.69 ml/min) by approximately 9%. Furthermore, changes of the 6β-hydroxylation clearances (CLm(6β)) in 14 healthy subjects were followed once at 10:00-12:00 in the morning at least every 2 or 3 days over a period of 36 - 53 days, indicating 1.5 ~ 3.4-fold day-to-day intra-individual variability of the CYP3A activity. The mean value for CLm(6β) in each subject for 36 - 53 days was 2.54 ± 0.76 ml/min (n = 14). We also evaluated the 6β-hydroxylation clearances (CLm(6β)) every 2 hours from 8:00 to 20:00 in 26 healthy subjects. Within-day intra-individual variability of the clearance was 1.1 ~ 2.5-fold (2.45 ± 0.91 ml/min, n = 26) and no characteristic diurnal rhythms were observed in the in vivo activity of CYP3A.
Introduction

Cytochrome P450 3A (CYP3A) metabolizes a number of xenobiotics including therapeutic drugs, environmental chemicals, and dietary components, as well as endogenous substrates such as steroids and bile acids. CYP3A is the most abundant cytochrome P450 enzyme, and is involved in the metabolism of approximately 50% of all drugs currently used in humans (Watkins, 1994; Wilkinson, 1996; Streetman et al., 2000). Marked inter-individual variabilities of the catalytic function to metabolize CYP3A substrates have been demonstrated. However, the genetic polymorphism of CYP3A cannot explain the variability in CYP3A-mediated metabolism. Therefore, the prospective use of CYP3A phenotyping or evaluating in vivo CYP3A activity has been suggested as an attractive approach to prediction of optimal dosage range to improve the therapeutic outcome and to minimize adverse effects (Zaigler et al., 2000; Dahl, 2002).

We have previously reported evidence for the validity of endogenous cortisol 6β-hydroxylation clearance as a new index for in vivo CYP3A phenotyping in humans (Furuta et al., 2001; 2003). It is calculated using the amount of urinary excreted 6β-hydroxycortisol (6β-OHF) divided by the area under the plasma concentration-time...
curve (AUC) of cortisol (F). The inhibitory effects of clarithromycin on *in vivo* CYP3A activity have clearly been demonstrated by the 6β-hydroxylation clearance of endogenous cortisol, but not by a conventional index, urinary ratio 6β-OHF/F (Furuta et al., 2003). The 6β-hydroxylation clearance can precisely assess *in vivo* CYP3A activity even when there are intra- and inter-individual variations in the metabolic clearance of cortisol, mediated by enzymes other than CYP3A, and in the renal clearance of cortisol. However, it is still unclear when and how the activity of CYP3A changes throughout the day, week and month in humans.

In the present study, we investigated the intra- and inter-individual variabilities of *in vivo* CYP3A activity in Japanese subjects, using endogenous cortisol 6β-hydroxylation clearance (CLm(6β)). The 6β-hydroxylation clearances (CLm(6β)) were examined in 49 healthy subjects aged 22-58 years. The present study was also designed to evaluate the *in vivo* activity of CYP3A every 2 hours from 8:00 to 22:00 in 26 healthy adult subjects. Furthermore, changes of the 6β-hydroxylation clearances (CLm(6β)) in 14 healthy subjects were followed once at 10:00-12:00 in the morning at least every 2 or 3 days over a period of 36 - 53 days. The method is the safest and simplest phenotyping procedure to sequentially follow *in vivo* enzyme activity throughout the day, week and month, since the
administration of a probe drug is not required. Our technique also can avoid potential physiological changes that may be caused by administration of a probe drug.
Materials and Methods

**Chemicals and reagents.** 6β-Hydroxycortisol (6β-OHF, 6β,11β,17α,21-tetrahydroxypregen-4-ene-3,20-dione), and 6α-hydroxycorticosterone (6α,11β,21-trihydroxypregen-4-ene-3,20-dione) were purchased from Steraloids (Wilton, NH, USA). Cortisol was purchased from Sigma (St. Louis, MO, USA). Stable isotopically labeled cortisol *i.e.*, [1,2,4,19-13C4]cortisol (cortisol-13C4) for use as analytical internal standard was synthesized in this laboratory (Furuta et al., 2000a). The isotopic compositions of the labeled cortisol was >97.3 atom%. All other chemicals and solvents were of analytical-reagent grade and were used without further purification.

**Study subjects.** The study was approved by the Tokyo University of Pharmacy and Life Sciences Human Subjects Review Board and written informed consent was obtained from all subjects. All subjects were Japanese and nonsmokers. No individual was receiving any medication or herbal supplements. All subjects abstained from alcohol and grapefruit juice for at least 4 weeks before and during the study period. In Study I, the cortisol 6β-hydroxylation clearances (CLm(6β)) were measured in 49 healthy adult subjects aged 22-58 years (28.3 ± 8.8 years) (25 men and 24 women). In Study II, 26 healthy adult
subjects (Subject I ~ XXVI) aged 22 to 53 years (28.8 ± 8.7 years) (16 men and 10 women) participated in the study of partial diurnal variation of \textit{in vivo} CYP3A activity. Blood samples (5 ~ 10 ml) were obtained every 30 min or 1 hour between 10:00 ~ 16:00, 8:00 ~ 22:00, 9:00 ~ 20:00 or 9:00 ~ 22:00. In Study III, 14 healthy adult subjects (Subject 1 ~ 14) aged 23 ~ 58 years (30.5 ± 10.7 years) (2 men and 12 women) participated in the study of day-to-day variation of \textit{in vivo} CYP3A activity. Blood samples (5 ~ 10 ml) were obtained at 10:00, 11:00, and 12:00 everyday, every second or third day for 36 ~ 53 days. Heparinized blood was collected in glass tubes and centrifuged. Urine samples were obtained at a timed period of 2 hours, and the volume and pH of the urine samples were recorded. The plasma and urine samples were stored at –20⁰C until analysis. Plasma concentrations of cortisol were analyzed by GC/MS using cortisol-13C4 as internal standard (Furuta et al., 2000b) and urinary excreted amounts of 6β-OHF were analyzed by HPLC using 6α-hydroxycorticosterone as internal standard (Furuta et al., 2004; Shibasaki et al., 2012). The inter- and intra-assay coefficients of variation (C.V.) were < 1.80% for GC-MS-SIM and < 2.5% for HPLC, respectively.

\textbf{6β-Hydroxylation clearance of endogenous cortisol for phenotyping \textit{in vivo} CYP3A activity.} Fractional metabolic clearance specific for the 6β-hydroxylation of endogenous cortisol...
cortisol (CLm(6β)) was used for phenotyping in vivo CYP3A activity. Since plasma concentrations of endogenously secreted cortisol (F) are considered steady state, the amount of the metabolite 6β-OHF produced from cortisol (F) in body can be expressed as CLm(6β) x AUC(F), where AUC(F) is the area under the concentration-time curve of cortisol. The metabolite is then excreted as an unconjugated form in urine. Therefore, the amount of urinary excreted 6β-hydroxycortisol (X(6β)) is expressed as CLm(6β) x AUC(F). In the present study, the 6β-hydroxylation clearance of cortisol (CLm(6β), Equation 1) was calculated as the amount of urinary excreted 6β-hydroxycortisol (X(6β)) during the 2-hour urine collection period divided by the corresponding 2-hour area under the concentration-time curve of cortisol (AUC(F)) (Furuta et al., 1996, 2001; 2003):

\[
\text{CLm}(6\beta) = \frac{X(6\beta)}{\text{AUC}(F)} \quad (1)
\]
RESULTS

Inter-individual variability of in vivo CYP3A activity (Study I). Fig. 1 shows the distributions of cortisol 6β-hydroxylation clearances (CLm(6β)) in 49 healthy subjects (25 men and 24 women) aged 22-58 years (28.3 ± 8.8 years). The values were in the range of 1.23 and 4.75 ml/min with 3.9-fold inter-individual variability of CYP3A activity. The mean clearance was 2.40 ± 0.79 ml/min. The clearances in most subjects were in the range of 1.5 - 3.5 ml/min (Fig. 1). The mean clearance for 24 female subjects was 2.50 ± 0.89 ml/min and 2.30 ± 0.69 ml/min for 25 male subjects.

Within-day variability of in vivo CYP3A activity (Study II). Fig. 2 shows the time courses of cortisol 6β-hydroxylation clearance (CLm(6β)) every 2 hours from 8:00 to 22:00 in 26 healthy subjects (Subject I ~ XXVI) (16 men and 10 women) aged 22 ~ 53 years (28.8 ± 8.7 years). Of 26 subjects (Table 1), the highest value was 5.14 ml/min for Subject XXV and the lowest was 0.81 ml/min for Subject XXI (Table 1). The mean values of CLm(6β) in each subject were 1.23 ~ 4.79 ml/min (n = 26, 2.45 ± 0.91 ml/min) with 3.9-fold (=4.79/1.23) inter-individual variability of CYP3A activity (Table 1). Within-day intra-individual variabilities were 1.1 ~ 2.5-fold (CV = 4.0 ~ 34.2%) (Table 1). There was
no characteristic diurnal rhythm in 6β-hydroxylation clearance of cortisol (Fig. 2).

**Day-to-day variability of in vivo CYP3A activity (Study III).** Fig. 3 shows the time courses of 6β-hydroxylation clearance of cortisol (CLm(6β)) once at 10:00 - 12:00 in the morning at least every 2 or 3 days over a period of 36 ~ 53 days in 14 healthy subjects (Subject 1 ~ 14) aged 23-58 years (30.5 ± 10.7 years). Of 14 subjects, the highest value was 5.85 ml/min for Subject 13 and the lowest was 0.91 ml/min for Subject 5 (Table 2). The mean values of CLm(6β) in each subject for 36 ~ 53 days were 1.76 ~ 4.17 ml/min (n = 14; 2.54 ± 0.76 ml/min) with 2.4-fold (=4.17/1.76) inter-individual variability of CYP3A activity (Table 2). Day-to-day intra-individual variabilities of the 6β-hydroxylation clearance (CLm(6β)) were 1.5 ~ 3.4-fold (CV = 11.2 ~ 30.2%) (Table 2).
DISCUSSION

It is well-known that marked inter-individual variabilities of the catalytic function to
metabolize CYP3A substrates have been demonstrated. However, the genetic polymorphism
of CYP3A cannot explain the variability in CYP3A-mediated metabolism. The
physiological changes through the day or over a longer period may affect the variability of
*in vivo* enzyme activity within an individual and could potentially influence drug
metabolism and pharmacokinetics. Recently, human chronokinetic studies have been
reported to partly explain chronopharmacodynamic phenomena (Bruguerolle, 1998). The
time of drug administration is a possible factor in the metabolic and pharmacokinetic
variations. However, systematic investigation of the variability of *in vivo* CYP3A activity
are difficult to perform in the same individuals throughout the day by administration of a
probe drug, such as midazolam, erythromycin, omeprazole, etc. The urinary excretion ratio
of 6β-hydroxycortisol to cortisol (6β-OHF/F) has been extensively used as a useful
non-invasive index for evaluating not only *in vivo* CYP3A activity in drug-drug interactions
but the statistical correlation of CYP3A activity with genetic and/or environmental factors,
disease and ethnic differences, etc. (Wilkinson, 1996; Ged et al., 1989; Monsarrat et al.,
1998; Pichard-Garcia et al., 2000). However, the correlations between morning spot and
24-hour urinary ratios have been mixed. Some results have shown the absence of any
changes in the ratio of $6\beta$-OHF/F in spot urine throughout the day (Galteau et al., 2003;
Micuda et al., 2007). Conversely one report has indicated a diurnal rhythm with increased
levels in the evening (Ohno et al., 2000). It should be noted that the urinary ratio $6\beta$-OHF/F
is valid as the index for \textit{in vivo} CYP3A activity only when the renal clearance of cortisol is
consistent (Furuta et al., 2001; 2003; Peng et al., 2011), because the urinary ratio $6\beta$-OHF/F
is a function of two independent parameters, metabolic clearance specific for
$6\beta$-hydroxylation and renal clearance of cortisol.

By using a probe drug in phenotyping, the clearance of the drug should provide the best
estimate of \textit{in vivo} catalytic activity of the enzyme of interest. If the probe drug has multiple
metabolic pathways, the fractional metabolic clearance corresponding to the pathway of
interest should be an appropriate measure (Watkins, 1994). In our previous paper, we
provided evidence for the validity of the $6\beta$-hydroxylation clearance of endogenous cortisol
($\text{CL}_{m}(6\beta)$) as an appropriate index for phenotyping the \textit{in vivo} CYP3A activity by using
stable isotope methodology (Furuta et al., 2001; 2003). The method can precisely assess \textit{in
vivo} CYP3A activity even when there are intra- and inter-individual variations in cortisol
metabolic clearance mediated by enzymes other than CYP3A and in the renal clearance of cortisol. In this case, the clearance $\text{CL}_{m}(6\beta)$ can be estimated as the index for phenotyping the \textit{in vivo} CYP3A activity under the assumption that the metabolite 6\beta-hydroxycortisol does not undergo further metabolism (Furuta et al., 1996). On the other hand, cortisol reversibly converts to cortisone by 11\beta-hydroxysteroid dehydrogenase (Draper, et al., 2005). Cortisone is then partly metabolized to 6\beta-hydroxycortisone, by CYP3A, that may interconvert to 6\beta-hydroxycortisol by 11\beta-hydroxysteroid dehydrogenase \textit{in vivo}. A recent study (Peng et al, 2011), however, demonstrated that there was no significant difference in the magnitude of inhibition of CYP3A activity by itraconazole, estimated by either the formation clearance of 6\beta-hydroxycortisol from cortisol or the combined clearance of 6\beta-hydroxycortisol from cortisol and 6\beta-hydroxycortisone from cortisone, indicating that the formation clearance of 6\beta-hydroxycortisol should reflect the combined formation clearance of 6\beta-hydroxycortisol and 6\beta-hydroxycortisone. It seems reasonable to assume that there is a negligible contribution of 6\beta-hydroxycortisone to the formation clearance of 6\beta-hydroxycortisol in phenotyping the \textit{in vivo} CYP3A activity.

In the present study, we investigated the inter-individual variabilities of \textit{in vivo} CYP3A activity in 49 healthy subjects aged 22-58 years, using 6\beta-hydroxylation clearance of
endogenous cortisol (CL_{m(6\beta)}). Furthermore, we followed the time courses of the 6\beta-hydroxylation clearance (CL_{m(6\beta)}) every 2 hours from 8:00 to 22:00 in 26 healthy subjects and once at 10:00-12:00 in the morning at least every 2 or 3 days over a period of 36 - 53 days in 14 healthy subjects, in order to evaluate within-day and day-to-day intra-individual variabilities in the \textit{in vivo} CYP3A activity.

The present study demonstrated less than 3.9-fold inter-individual variabilities of the clearance (CL_{m(6\beta)}) in the Study I - III. There were no significant intra-individual variabilities in the values of CL_{m(6\beta)} in the within-day (1.1 \sim 2.5\text{-}fold, CV = 4.0 \sim 34.2\%) and day-to-day studies (1.5 \sim 3.4\text{-}fold, CV = 11.2 \sim 30.2\%), respectively (Table 1 and 2). The mean value of cortisol 6\beta-hydroxylation clearances (CL_{m(6\beta)}) in 49 healthy subjects (Study I) was 2.40 \pm 0.79 ml/min (CV = 32.9\%), which was consistent with the mean clearance values in 26 subjects obtained from the Study II (within-day: 2.45 \pm 0.91 ml/min) and in 14 subjects from the Study III (day-to-day: 2.54 \pm 0.76 ml/min). The intra-individual variability of \textit{in vivo} CYP3A activity could be due to dietary and environmental or hormonal factors. No characteristic within-day and day-to-day variations or rhythms were observed in the 6\beta-hydroxylation clearance (CL_{m(6\beta)}), and the clearances in most subjects were in the range of 1.5 - 3.5 ml/min (Fig. 1 - 3). Yin et al. (2004) reported that there was a
large inter-individual variability with a 30-fold difference and a small intra-individual variability (CV = 31.1%) in the urinary ratio $6\beta$-OHF/F. This indicates that the urinary ratio $6\beta$-OHF/F should be affected by a wide inter-individual variability in the renal clearance of cortisol (Furuta et al., 2001; 2003).

The present study also demonstrated that the mean clearance in 24 female subjects (2.50 ± 0.89 ml/min) was higher than that in 25 male subjects (2.30 ± 0.69 ml/min) by approximately 9% (Fig. 1), which is consistent with previous studies using CYP3A substrates or markers (Horsmans et al., 1992; Harris et al., 1995; Inagaki et al., 2002; Zhu et al., 2003; Cotreau et al., 2005; Lutz et al., 2010). However, there was no significant sex-related difference in our study (p = 0.37). The presence of the female sex steroids estrogen and progesterone in vivo may play a role in modulation of this sex-related difference. Higher clearance rates of drugs in women could be a result of CYP3A transcriptional regulation by endogenous steroid hormones through the nuclear pregnane X receptor (PXR). Successive measurements of estrogen and progesterone in relation to CYP3A activity during the menstrual cycle are of great interest, and will provide further insights into sex-based differences in drug metabolism and pharmacokinetics.
day-to-day) variabilities in the *in vivo* CYP3A activity using 6β-hydroxylation clearance of endogenous cortisol (CLm(6β)). The results presented here should provide important and useful information for predicting individual drug metabolism and dispositions in various patients or identifying individuals with important variants affecting CYP3A activity in humans.
Authorship Contributions

Participated in research design: Shibasaki, Hosoda, Goto, Suzuki, Yokokawa, Ishii, and Furuta

Conducted experiments: Hosoda, Goto, and Suzuki

Contributed new reagents or analytic tools: Yokokawa and Ishii

Performed data analysis: Shibasaki, Hosoda, Goto, Suzuki, Yokokawa, Ishii, and Furuta

Wrote or contributed to the writing of the manuscript: Shibasaki and Furuta
References


Figure legends

Fig. 1 Distributions of endogenous cortisol 6β-hydroxylation clearances (CL$m(6β)$) in 49 healthy subjects (25 men and 24 women).

Fig. 2 Time courses of endogenous cortisol 6β-hydroxylation clearances (CL$m(6β)$) for phenotyping in vivo cytochrome P450 3A (CYP3A) activity in humans. Within-day variabilities (8:00 ~ 22:00) of in vivo CYP3A activity in Subject I ~ XXVI; Each value of CL$m(6β)$ is represented at a mid-point time in the 2-hour urine collection period (8:00 ~ 10:00, 10:00 ~ 12:00, 12:00 ~ 14:00, 14:00 ~ 16:00, 16:00 ~ 18:00, 18:00 ~ 20:00, and 20:00 ~ 22:00).

Fig. 3 Time courses of endogenous cortisol 6β-hydroxylation clearances (CL$m(6β)$) for phenotyping in vivo cytochrome P450 3A (CYP3A) activity in humans. Day-to-day variabilities of in vivo CYP3A activity in Subject 1 ~ 14; Each value of CL$m(6β)$ is represented at a mid-point time (11:00) in the 2-hour urine collection period (10:00 ~ 12:00).
TABLE 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Cortisol 6β-Hydroxylation Clearance (CLm(6β))</th>
<th>CV (%)</th>
<th>Range (ml/min)</th>
<th>Variability (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>25</td>
<td>Man</td>
<td>1.83 ± 0.35</td>
<td>19.0</td>
<td>1.49 – 2.32</td>
<td>1.6</td>
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<tr>
<td>II</td>
<td>29</td>
<td>Man</td>
<td>1.96 ± 0.28</td>
<td>14.1</td>
<td>1.58 – 2.27</td>
<td>1.4</td>
</tr>
<tr>
<td>III</td>
<td>27</td>
<td>Man</td>
<td>2.99 ± 0.80</td>
<td>26.7</td>
<td>2.23 – 4.56</td>
<td>2.0</td>
</tr>
<tr>
<td>IV</td>
<td>53</td>
<td>Man</td>
<td>2.17 ± 0.36</td>
<td>16.5</td>
<td>1.51 – 2.62</td>
<td>1.7</td>
</tr>
<tr>
<td>V</td>
<td>24</td>
<td>Man</td>
<td>2.25 ± 0.22</td>
<td>9.9</td>
<td>1.89 – 2.48</td>
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<tr>
<td>VI</td>
<td>23</td>
<td>Man</td>
<td>1.55 ± 0.16</td>
<td>10.5</td>
<td>1.32 – 1.69</td>
<td>1.3</td>
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<tr>
<td>VII</td>
<td>26</td>
<td>Man</td>
<td>2.11 ± 0.29</td>
<td>13.7</td>
<td>1.61 – 2.32</td>
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<td>VIII</td>
<td>25</td>
<td>Man</td>
<td>1.93 ± 0.33</td>
<td>17.2</td>
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<tr>
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<td>24</td>
<td>Man</td>
<td>2.17 ± 0.40</td>
<td>18.4</td>
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<td>1.73 ± 0.26</td>
<td>15.3</td>
<td>1.25 – 1.97</td>
<td>1.6</td>
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<tr>
<td>XI</td>
<td>25</td>
<td>Man</td>
<td>3.47 ± 0.33</td>
<td>9.4</td>
<td>2.97 – 3.85</td>
<td>1.3</td>
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<tr>
<td>XII</td>
<td>25</td>
<td>Man</td>
<td>3.48 ± 0.26</td>
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<tr>
<td>XIII</td>
<td>53</td>
<td>Man</td>
<td>1.77 ± 0.24</td>
<td>13.5</td>
<td>1.44 – 2.00</td>
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<tr>
<td>XIV</td>
<td>35</td>
<td>Man</td>
<td>1.78 ± 0.11</td>
<td>5.9</td>
<td>1.68 – 1.90</td>
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<tr>
<td>XV</td>
<td>24</td>
<td>Man</td>
<td>2.49 ± 0.37</td>
<td>15.0</td>
<td>2.15 – 2.91</td>
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<tr>
<td>XVI</td>
<td>24</td>
<td>Man</td>
<td>2.77 ± 0.16</td>
<td>5.7</td>
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<td>XVII</td>
<td>22</td>
<td>Woman</td>
<td>4.79 ± 0.19</td>
<td>4.0</td>
<td>4.66 – 5.01</td>
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<td>XVIII</td>
<td>23</td>
<td>Woman</td>
<td>1.80 ± 0.28</td>
<td>15.4</td>
<td>1.41 – 2.19</td>
<td>1.6</td>
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<tr>
<td>XIX</td>
<td>22</td>
<td>Woman</td>
<td>2.75 ± 0.65</td>
<td>23.8</td>
<td>2.28 – 3.50</td>
<td>1.5</td>
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<tr>
<td>XX</td>
<td>23</td>
<td>Woman</td>
<td>1.52 ± 0.52</td>
<td>34.2</td>
<td>0.85 – 2.10</td>
<td>2.5</td>
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<td>XXI</td>
<td>45</td>
<td>Woman</td>
<td>1.23 ± 0.31</td>
<td>25.3</td>
<td>0.81 – 1.57</td>
<td>1.9</td>
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<td>31</td>
<td>Woman</td>
<td>1.58 ± 0.48</td>
<td>30.2</td>
<td>1.19 – 2.24</td>
<td>1.9</td>
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<td>XXIII</td>
<td>28</td>
<td>Woman</td>
<td>2.38 ± 0.50</td>
<td>20.9</td>
<td>1.86 – 2.97</td>
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<td>Woman</td>
<td>3.38 ± 0.31</td>
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<td>3.13 – 3.81</td>
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<td>XXV</td>
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<td>Woman</td>
<td>4.25 ± 0.63</td>
<td>14.9</td>
<td>3.65 – 5.14</td>
<td>1.4</td>
</tr>
<tr>
<td>XXVI</td>
<td>24</td>
<td>Woman</td>
<td>3.69 ± 0.52</td>
<td>14.0</td>
<td>3.36 – 4.28</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Mean ± SD 28.8 ± 8.7 2.45 ± 0.91 15.8 ± 7.6 1.5 ± 0.3
<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Mean ± SD (ml/min)</th>
<th>CV (%)</th>
<th>Range (ml/min)</th>
<th>Variability (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>Woman</td>
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<td>19.6</td>
<td>1.01 - 2.45</td>
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Mean ± SD 30.5 ± 10.7 2.54 ± 0.76 20.0 ± 4.5 2.30 ± 0.53