Estradiol reference intervals in women during the menstrual cycle, postmenopausal women and men using an LC-MS/MS method

Verdonk, Sara J E; Vesper, Hubert W; Martens, Frans; Sluss, Patrick M; Hillebrand, Jacquelien J; Heijboer, Annemieke C

Published in:
Clinica chimica acta; international journal of clinical chemistry

DOI:
10.1016/j.cca.2019.04.062

Published: 01/08/2019

Document Version
Publisher's PDF, also known as Version of record

Citation for pulished version (APA):
Verdonk, S. J. E., Vesper, H. W., Martens, F., Sluss, P. M., Hillebrand, J. J., & Heijboer, A. C. (2019). Estradiol reference intervals in women during the menstrual cycle, postmenopausal women and men using an LC-MS/MS method. *Clinica chimica acta; international journal of clinical chemistry*, *495*, 198-204. https://doi.org/10.1016/j.cca.2019.04.062

Clinica Chimica Acta 495 (2019) 198–204

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Estradiol reference intervals in women during the menstrual cycle, postmenopausal women and men using an LC-MS/MS method

Sara J.E. Verdonk[a], Hubert W. Vesper[b], Frans Martens[a,d], Patrick M. Sluss[c], Jacquelien J. Hillebrand[d], Annemieke C. Heijboer[a,d,*]

[a] Amsterdam UMC, Vrije Universiteit Amsterdam, Endocrine Laboratory, Department of Clinical Chemistry, Amsterdam Gastroenterology & Metabolism, Amsterdam, Netherlands
[b] Centers of Disease Control and Prevention, 4770 Buford Highway, NE F25, Atlanta 30341-3724, Georgia
[c] Clinical Pathology Core Laboratory, Massachusetts General Hospital, Boston, MA, USA
[d] Amsterdam UMC, University of Amsterdam, Endocrine Laboratory, Department of Clinical Chemistry, Amsterdam, Netherlands

ARTICLE INFO

Keywords:
Estrogens
Liquid chromatography
Mass spectrometry
Premenopausal women
Menstrual cycle

ABSTRACT

Background: For optimal medical decision-making, harmonized reference intervals for estradiol for different ages and both sexes are needed. Our aim was to establish reference intervals using a highly accurate and traceable LC-MS/MS method and to compare these with reference intervals in literature.
Methods: Estradiol was measured in serum obtained daily during the menstrual cycle of 30 healthy premenopausal women and in serum of 64 men and 33 postmenopausal women. The accuracy of our LC-MS/MS method was demonstrated by a method comparison with the CDC reference method.
Results: Our LC-MS/MS method was traceable to the reference method. Estradiol reference interval during the early follicular phase (days −15 to −6) was 31–771 pmol/L; during the late follicular phase (days −5 to −1) 104–1742 pmol/L; during the LH peak (day 0) 275–2864 pmol/L; during the early luteal phase (days +1 to +4) 95–1188 pmol/L; during mid luteal phase (days +5 to +9) 151–1941 pmol/L; during late luteal phase (days +10 to +14) 39–1769 pmol/L. The reference interval for men was 12–136 pmol/L and for postmenopausal women < 26 pmol/L.
Conclusions: The established estradiol reference intervals can be used for all traceable LC-MS/MS methods for medical-decision making.

1. Introduction

Estradiol (17-β estradiol) is the primary ovarian hormone in the development of the female reproductive system. Estradiol also has additional functions, such as the inhibition of bone resorption [1]. The concentration of the hormone is relatively low in men as well as in women before puberty and after the menopause, yet increases in women during puberty. During the menstruation cycle estradiol concentration varies due to the feedback mechanism regulated by the hypothalamic-pituitary-gonadal axis [2]. Estradiol concentrations can be measured to determine production shortages in women, as well as excess in both women and men. The hormone level is frequently measured for diagnostic purposes; in women having complaints of dysregulated menstrual cycles or premature menopause, in women before starting anti-hormonal treatment used to treat breast cancer and before starting and during an IVF treatment, in girls with early or late onset of puberty and in men with clinical signs of gynecomastia [3,4]. For proper diagnosis an accurate measurement of estradiol is highly important [5]. Currently, the concentration of estradiol is often measured using direct immunoassays, despite their inaccuracy in the low concentration range due to cross reactivity and/or matrix interference [3,6,7]. Moreover, inconsistent calibration presents another source of inaccuracy in measuring estradiol concentrations [8].

Liquid chromatography tandem mass spectrometry (LC-MS/MS) was recently introduced in clinical laboratories as a method to determine steroid hormone concentrations, including estradiol [8]. The major advantage of a well-validated and correctly operated LC-MS/MS method is the high specificity, compared to direct immunoassays that

Abbreviations: LC-MS/MS, Liquid chromatography–mass spectrometry; CDC, Centers of Disease Control and Prevention; LH, Luteinizing Hormone; IVF, In Vitro Fertilization; VUmc, VU University Medical Center; UPLC, Ultra performance liquid chromatography

* Corresponding author at: Endocrine Laboratory, Amsterdam UMC, location AMC, Meibergdreef 9, 1105 AZ Amsterdam, Netherlands.
E-mail address: a.heijboer@amsterdamumc.nl (A.C. Heijboer).

https://doi.org/10.1016/j.cca.2019.04.062
Received 25 September 2018; Received in revised form 9 April 2019; Accepted 10 April 2019
Available online 11 April 2019
0009-8981/

often suffer from cross reactivity [5]. In recent years, the sensitivity of the LC-MS/MS methodologies has improved so that lower concentrations of estradiol in men, children and postmenopausal women can be accurately measured [9]. For optimal medical decision-making, harmonized reference intervals for estradiol for all ages and both sexes are needed [5]. Reference intervals have been established in the past but mostly using direct immunoassays. Due to the inaccuracy of these immunoassays, these reference intervals are of limited value in interpreting estradiol concentrations obtained using an LC-MS/MS method [10].

Estradiol reference intervals using an LC-MS/MS method have been established earlier for men and postmenopausal women [9], but complete data on estradiol concentrations in women during their menstrual cycle are lacking. The aim of this study was to establish reference intervals using a highly accurate and well standardized LC-MS/MS method in premenopausal women during the whole menstrual cycle as well as in men and postmenopausal women and to compare these with reference intervals in literature.

2. Materials and methods

Our study consisted of three parts. First, a method comparison was performed to verify the accuracy and standardization of the LC-MS/MS method used at the Amsterdam UMC, location VU University Medical Center (VUmc). The method comparison was performed with the reference LC-MS/MS method of the Centers of Disease Control and Prevention (CDC). The measurements performed at the Centers for Disease Control and Prevention (CDC) laboratory for method comparison was determined not to constitute engagement in human subject research. Secondly, reference intervals were established for premenopausal women, postmenopausal women and men. Finally, a comparison with estradiol reference intervals published earlier was performed.

2.1. Part 1: comparison of LC-MS/MS method to reference method

A comparison was performed between the LC-MS/MS method of the VUmc and the reference LC-MS/MS method of the CDC. During the method comparison, for target concentrations > 73 pmol/L, a bias of ± 12.5% was allowed and in target concentrations ≤73 pmol/L a maximum bias of ± 9 pmol/L was allowed [8].

2.1.1. Specimens

For the method comparison, 20 specimens from the Hormone Standardization (HoST) Program of the CDC were used (Centers for Disease Control and Prevention (CDC), 2014). These 20 specimens had been obtained from 20 healthy donors and processed according to the CLSI protocol C37 [11]. The specimens were obtained from both men and women to ensure a sufficient range in estradiol concentrations. The specimens covered a range in estradiol concentration between 9.9 and 983 pmol/L (=2.7–268 pg/mL). The specimens were assigned a reference value by the LC-MS/MS reference method of the laboratory at the CDC.

2.1.2. LC-MS/MS method

Estradiol concentrations were measured in serum using the LC-MS/MS method of the VUmc. In short, a stable, isotopically labelled internal standard (13C3-labelled estradiol, obtained from IsoSciences, King of Prussia, PA, USA) was added to every specimen (sample, control, calibrator).

Estradiol was extracted from 150 µL of sample using a 4:1 (volume/volume) mixture of hexane and ether. The supernatant was subsequently dried under a stream of nitrogen (45 °C) and reconstituted in 75 µL of a 1:1 (volume/volume) mixture of methanol and water. Analysis of the samples was performed using tandem mass spectrometry with a 2D Xevo TQ-S mass spectrometer (Waters Corp., Milford, MA) with electrospray in negative mode using an injection volume of 50 µL. Separation was achieved on two (C4 and C18) Acquity UPLC analytical columns (Waters Corp), 2.1 × 50 mm, 1.7-µm particle size, with a methanol:water gradient elution at a flow rate of 0.6 and 0.4 mL/min (water containing 100 µM NH4F). Total analysis time was 8.2 min. The monitored transitions were *m/z* 271 to 145 and 183 and m/z 274 to 148 and 186 for estradiol and the internal standard, respectively.

The LC-MS/MS method has a lower limit of quantification of 4 pmol/L (CV% of 16%). The intra assay variation was < 5% between 20 and 1700 pmol/L and the inter assay variation was 9% at 21 pmol/L and < 7% at 179 and 760 pmol/L. All samples were run in duplicate.

2.1.3. Statistical analysis

The method comparison was analyzed using a Passing and Bablok regression analysis, a Bland-Altman analysis and a Pearson-correlation coefficient. The analysis was performed using MedCalc (version 11.6.0.0, Oostende, Belgium).

2.2. Part 2: establish reference intervals

2.2.1. Specimens

To calculate reference intervals, blood was collected from healthy premenopausal women, postmenopausal women and men. The venous blood was drawn into 10 mL glass vacutainers (no preservatives added). The blood was allowed to clot at 2–8 °C for 1–12 h. Hereafter, the serum was separated from clot by centrifugation (3000-3200 *g* for 10 min), aliquoted into polypropylene tubes and stored capped at −25 to −35 °C. The serum specimens were transported on dry ice to the VUmc for measurement of estradiol.

2.2.2. Reference intervals for premenopausal women during the menstrual cycle

Serum specimens were taken from 30 healthy women every day during one menstrual cycle. The women were recruited at the Massachusetts General Hospital in Boston. All women provided written informed consent and the protocol was approved by the Partners Healthcare internal review board (IRB). Blood specimens were drawn daily every morning starting the first day of the menses until the first day of the next menses. Depending on the length of the menstrual cycle, approximately 28 specimens were collected per woman.

Women were included if they had at least two previous menstrual cycles. In addition to gender their age and smoking habits were registered. Women were excluded if they used hormonal contraceptives and if there was an indication of an abnormal endocrine system found at physical examination. Physical examination consisted of an examination of the ovaries using ultrasound, inspection of the thyroid size and consistency, examination of the breasts for masses and checking for hirsutism. Furthermore, women were screened to have no abnormalities at auscultation of the chest, normal heart sounds, soft and non-tender abdomen, no masses or visceromegaly, normal extremities and a normal central and peripheral nervous system. After the physical examination, nine women were excluded. Of these women four were excluded because of hirsutism, three were excluded because of acanthosis nigricans, one was excluded because of nodules present at head/eyes/ears/nose/throat exam, and one was excluded because of heart murmurs. Subjects were also questioned on use of medication. The included subjects did not use medication other than multi-vitamins, allergy medication or over the counter pain reducing medicine. Therefore after physical examination 32 women were included. Of these 32, two were excluded for having a non-ovulatory menstrual cycle. Non-ovulatory was judged by the absence of elevated progesterone concentration during the luteal phase. LH and progesterone were determined immunochemically using AxSYM (Abbott Diagnostics) at the Massachusetts General Hospital. An LH peak of > 9.1 IU/L was set as day 0: the reference day. The LH peak which induces ovulation was confirmed by progesterone levels of > 5.1 nmol/L (1.6 ng/mL) during

the subsequent luteal phase and observed by ultrasonography in 30 out of 32 women. In all specimens estradiol was measured using the VUmc LC-MS/MS method described under Part 1.

2.2.3. Reference interval in postmenopausal women and in men

To establish reference intervals in postmenopausal women and men, serum specimens were collected of 97 healthy individuals of whom 64 were men and 33 were postmenopausal women. The specimens were drawn by a group of volunteers consisting mainly of employees of the Academic Medical Centre of the University of Amsterdam, who were recruited with flyers. The inclusion criteria for men were age between 20 and 70 years and self-reported good health. Inclusion criteria for women were age between 55 and 70 years old and self-reported good health as well. Specimens were drawn between September 2014 and October 2016. The Medical Ethics Committee of the Academic Medical Center approved the study and informed consent was obtained from all participants. In all specimens estradiol was measured using the VUmc LC-MS/MS method described under Part 1.

2.2.4. Statistical analysis

The reference intervals for premenopausal women were calculated using Linear Regression (SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp)). The ovulation day was used as the reference day. Because of deviation to the right, the natural logarithm of estradiol was used to calculate the 95% reference intervals. The values were calculated per day as well as per phase of the menstrual cycle: follicular phase, ovulation phase and luteal phase. The 95% reference intervals in postmenopausal women and men were calculated using the Robust method (CLSI C28-A3) using MedCalc (version 18.5, Oostende, Belgium).

2.3. Part 3: comparison with literature

To compare our calculated reference intervals, PubMed was searched for reference intervals from earlier studies. Only studies that measured estradiol in either serum or plasma were included. Furthermore, studies had to have at least 20 healthy donors to be able to calculate reference intervals correctly [11]. Reference intervals had to be calculated for one or more of the following subgroups: premenopausal women, postmenopausal women or men. Studies that calculated reference intervals for premenopausal women were only included if they used the ovulation day as reference day. The data extracted from each article were: authors, year of publication, number of subjects, demographics of subjects, analytical method used, calculated reference interval, the statistical method used for the calculation. Neither the analytical nor the statistical method used to establish the reference interval was used as exclusion criteria.

3. Results

3.1. Part 1: comparison of LC-MS/MS method to reference method

Comparison between the LC-MS/MS method of the VUmc and the reference LC-MS/MS method of the CDC 20 serum specimens with estradiol concentrations between 9.9 and 983 pmol/L by Passing and Bablok regression analysis showed a slope of 1.12 (1.10–1.16), intercept of −1.13 (−4.28–1.28), and Pearson r of 0.9994 (0.9984–0.9998) (p-value < 0.0001). The Bland-Altman analysis showed that on average the LC-MS/MS method of the VUmc measured 10.7% too high. Based on this standardization difference, the VUmc LC-MS/MS method was adjusted by mathematical conversion, which led to a comparison of $Estradiol_{VUmc}$ = 1.01 $Estradiol_{CDC}$ – 0.97 pmol/L, also shown in Fig. 1.

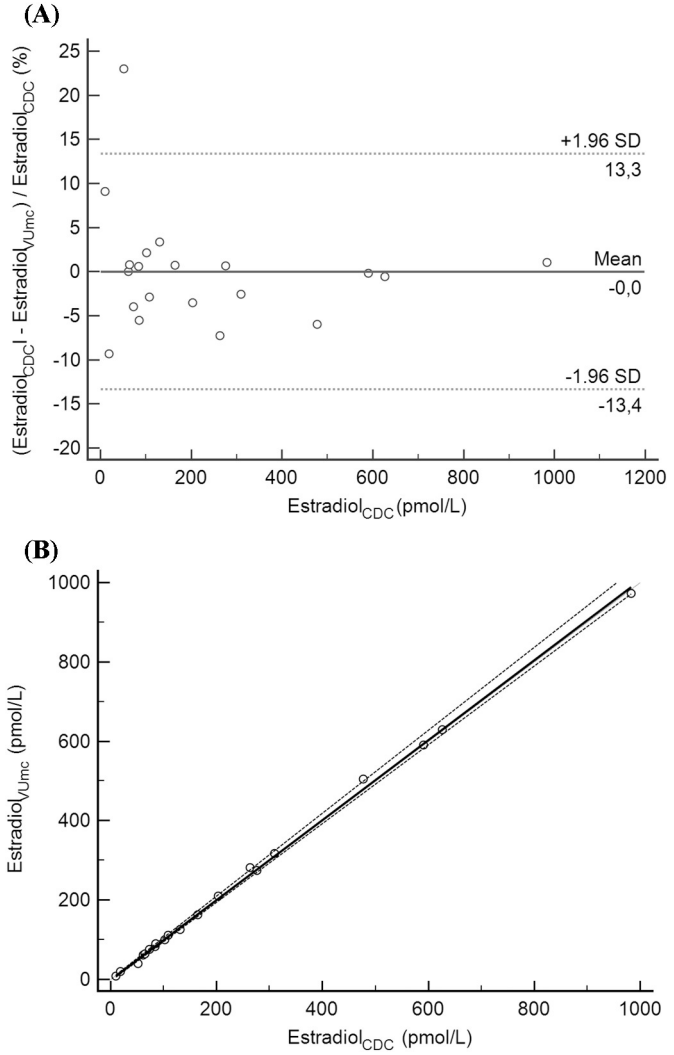

Fig. 1. Method comparison using the Bland-Altman analysis (A). Method comparison using a Passing and Bablok regression analysis (B). To convert to pg/mL, the estradiol concentration should be divided by 3.67.

Table 1
Demographics of the 30 premenopausal women.

Demographics (n = 30)	Mean (Range), or percentage
Length menstruation cycles in days	28.13 (22–35)
Age in years	26.67 (18–39)
BMI[a] in kg/m^2	23.47(18–28.8)
Race	
Caucasian	63%
Hispanic	17%
Black	7%
Other	13%
Self-reported smoking status	
Non-smokers	77%
Ex-smokers	13%
Smokers	10%

[a] BMI was calculated in 29 of the 30 women included in the study.

3.2. Part 2: establish reference intervals

3.2.1. Reference intervals for premenopausal women during the menstrual cycle

In total 30 women were included in the analysis. Of these women, 25 had specimens collected during every day of the menstrual cycle. The other five women had an incomplete number of specimens. In total

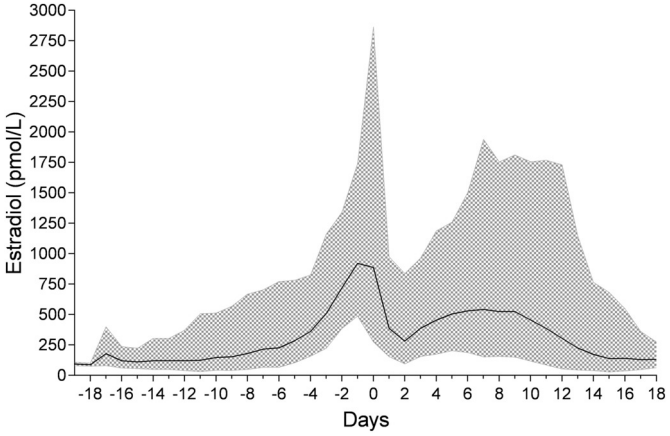

Fig. 2. Daily mean estradiol concentration in 30 premenopausal women. Solid line: presents the mean concentration of Estradiol. The grey areas around the solid line: present the reference interval (the 95% range). To convert to pg/mL, divide the estradiol concentration by 3.67.

Table 2
Reference interval for premenopausal women per phase of the menstrual cycle.

Phase of cycle	Days from LH peak	Estradiol (pmol/L)	
		Reference interval[a]	
		Lower limit	Upper limit
Early follicular	−15 to −6	31	771
Late follicular	−5 to −1	104	1742
LH peak	0	275	2864
Early luteal	+1 to +4	95	1188
Mid-luteal	+5 to +9	151	1941
Late luteal	+10 to +14	39	1769

[a] This is a summary table of the reference intervals found per day of the menstrual cycle (for reference intervals per day see Table 3). The lower limit represents the lowest 2,5th percentile value found at any of the days belonging to this phase of the menstrual cycle. Whereas the upper limit represents the highest 97,5th percentile value found at any of the days belonging to this phase.

ten specimens were missing. Demographics of the included 30 women are shown in Table 1.

Data for normal cycling women by day of the cycle are presented in Table 3 and Fig. 2. Shown are the mean, 2.5th and 97.5th percentiles of estradiol concentration in pmol/L [12]. Additionally reference intervals during different phases of the menstrual cycle are shown in Table 2. Estradiol concentration reference intervals for every phase of the menstrual cycle are presented as 95% of the total range. The reference interval during the early follicular phase (days −15 to −6) was 31–771 pmol/L; during the late follicular phase (days −5 to −1) 104–1742 pmol/L; during the LH peak (day 0) 275–2864 pmol/L; during the early luteal phase (days +1 to +4) 95–1188 pmol/L; during the mid-luteal phase (days +5 to +9) 151–1941 pmol/L; during the late luteal phase (days +10 to +14) 39–1769 pmol/L.

3.2.2. Reference interval in postmenopausal women and in men

In total, specimens of 64 men and 33 postmenopausal women were used. The men had a mean age of 46 (22–63) years. The women had a mean age of 60 (55–68) years. Reference intervals are presented as median and 2.5th and 95th percentile values in Table 4. The percentiles can be interpreted as the reference interval for the group presented. The reference interval, not stratified for age, for men is 12–136 pmol/L (90%CI lower limit −5-31; 90% CI upper limit: 117–153). Data per age group are shown in Table 4. The reference interval for postmenopausal women is < 26 (90% CI 20–32) pmol/L.

3.3. Part 3: comparison with literature

In total nine studies were included. Reference intervals found in previous studies for premenopausal women, postmenopausal women and men are presented respectively in Tables 3 and 4. The reference intervals calculated in our study are presented in the tables as well.

4. Discussion

The aim of the current study was to establish reference intervals for premenopausal women across the menstrual cycle and verify reference intervals for postmenopausal women and men, using a highly accurate LC-MS/MS method that is adjusted to a reference method.

We showed that the LC-MS/MS method for estradiol of the VUmc was concordant with the Reference Measurement Procedure (RMP) for estradiol developed by the Centers of Disease Control and Prevention [13]. The RMP produces measurement results that are traceable to SI according to ISO 17511 and meets ISO requirements for reference methods as indicated in its listing in the Joint Committee for Traceability in Laboratory Medicine (JCTLM) database. This RMP serves as a higher-order standard for establishing measurement traceability and provides an accuracy base against which routine methods can be compared [13]. Although our LC-MS/MS method correlated extremely well with the RMP our LC-MS/MS method showed a constant bias of 10.7% which can be seen as calibration bias. For this reason, we corrected for the calibration bias by aligning our results to the RMP and thus made our results traceable to the RMP. It is highly important that all methods are standardized to make sure that the results can be compared to one other. This is necessary in research when comparing estradiol concentrations from literature, but also in diagnostics to be able to use reference intervals obtained with a different method [14]. Standardization is one of the issues that needs attention, not only in case of immunoassays but also in case of LC-MS/MS methods [15,16]. As our LC-MS/MS method was adjusted based on the comparison with the reference method, our reference values in premenopausal women can be used for all traceable LC-MS/MS methods for medical decision-making.

Our study was the first to establish reference intervals for premenopausal women daily during the whole menstrual cycle using an LC-MS/MS method. The reference values found by our study suggest that there is a large range in estradiol values between premenopausal women. Nelson et al. used an LC-MS/MS method to establish reference values as well [17]. However, they calculated one range for the whole cycle, which has an upper limit that is lower than found in our study. As this study does not describe the statistical method for the calculation of the reference interval, we cannot draw any conclusions on this difference. Other studies used automated immunoassays of Abbott Diagnostics [2,6,18]. The reference intervals obtained by these three studies differ from one another while they used a similar measurement method, which makes it difficult to draw a conclusion about the difference between intervals established using immunoassays and LC-MS/MS. One of the differences between these studies is the way the reference interval was calculated. This results in the fact that comparability of reference intervals in literature is hampered by the variability in the statistics used to calculate the reference intervals.

The reference interval we established in the current study for postmenopausal women (< 26 pmol/L; total range 4.1–38 pmol/L) is highly comparable to the other studies that used LC-MS/MS methods to obtain their reference intervals (< 37 and < 35 pmol/L) [17,19]. The studies using immunoassays obtained a higher upper limit for the reference interval, with the Architect immunoassay even leading to a 3 times higher upper limit [6,20]. These higher upper limit of the reference interval is probably related to the inaccuracy and imprecision of estradiol immunoassays at this concentration range [8–10,14,21].

The reference intervals for men established in our study are comparable to the reference intervals found in another study [22]. This

Table 3
Reference intervals for premenopausal women found in previous research.

Author + year of publication	Estradiol reference intervals in pmol/L (in phases)						Statistical analysis of reference interval	N	Method
	Early follicular phase (days -15 to -6)	Late follicular phase (days -5 to -1)	Ovulation day (day 0)	Early luteal phase (days +1 to +4)	Mid luteal phase (days +5 to +9)	Late luteal phase (days +10 to +14)			
Nelson, 2004 *(17)*	55-1285 pmol/L						Not stated	92	LC-MS/MS
Dighe, Moy, Hayes, & Sluss, 2005 *(18)*	Cycle day / Reference interval: -15 128 – 275; -14 143 – 253; -13 154 – 319; -12 165 – 261; -11 176 – 246; -10 180 – 275; -9 191 – 301; -8 224 – 330; -7 242 – 352; -6 279 – 400; -5 341 – 503; -4 411 – 591; -3 554 – 786; -2 731 – 1013; -1 907 – 1325; 0 778 – 1068			Cycle day / Reference interval: +1 352 – 613; +2 334 – 532; +3 385 – 595; +4 463 – 697; +5 459 – 701; +6 474 – 716; +7 496 – 720; +8 444 – 650; +9 477 – 727; +10 474 – 683; +11 393 – 661; +12 338 – 554; +13 264 – 437; +14 184 – 349; +15 132 – 422			98% Confidence Interval around the mean	51	Abbott AxSYM (immunoassay)
Stricker et al., 2006 *(2)*	78-266	195-1147	482-1425	178-566	276-762	101-787	90% range	20	Abbott Architect i2000 (chemoluminiscence immunoassay)
Sluss et al., 2008 *(6)*	88-1042		209-2591*	95-1266			90% range	42	Abbott Architect i2000 (chemoluminiscence immunoassay)
Verdonk et al (this study)	Cycle day / Reference interval: -15 56 – 224; -14 48 – 304; -13 49 – 305; -12 40 – 372; -11 31 – 508; -10 42 – 512; -9 41 – 568; -8 49 – 669; -7 67 – 705; -6 67 – 771; -5 104 – 782; -4 158 – 826; -3 222 – 1161; -2 386 – 1341; -1 488 – 1742; 0 275 – 2864			Cycle day / Reference interval: +1 154 – 975; +2 95 – 837; +3 155 – 964; +4 174 – 1188; +5 203 – 1260; +6 188 – 1502; +7 152 – 1941; +8 156 – 1755; +9 151 – 1812; +10 118 – 1757; +11 84 – 1769; +12 53 – 1732; +13 44 – 1148; +14 39 – 766			95% range	30	LC-MS/MS

*In the study of Sluss et al. [6], the reference interval presented at the ovulation day included specimens gathered at the days −1 and +1 as well. These days were not included in the follicular phase or luteal phase.

latter study, also using an LC-MS/MS method, used a high sample size and calculated reference intervals for different age categories. Categorizing in age groups does not seem necessary when looking at the differences in reference intervals. The reference intervals in the different age groups do not differ much from one another. The reference intervals calculated by the other studies, some using LC-MS/MS and some using immunoassays [6,17,19,23], were also quite comparable. Due to differences in presentation of the reference intervals and an unclear description or sometimes no description at all of the statistical analysis used to calculate these reference intervals makes it difficult to draw hard conclusions. This shows that not only accuracy of the used analytical method is important, but that also information about the distribution of for instance age and a clear description of statistics are crucial.

A limitation of our study is the relatively small sample size per group. Although we used specimens from a total of 127 subjects to establish reference intervals, the sample size in the different subgroups is a much smaller. Specimens from 30 premenopausal women, 33 postmenopausal women and 64 men were included. The men and postmenopausal women were considered healthy based on self-report.

Table 4
Reference intervals for postmenopausal women and men found in previous studies.

Author + year of publication	Age range	Estradiol median in pmol/L	Estradiol reference intervals in pmol/L	Statistical analysis of reference interval	N	Method
Reference intervals for postmenopausal women:						
Nelson, [17]	not stated	–	< 37	Not stated	30	LC-MS/MS
Sluss et al., [6]	not stated	< 55	< 55–103	Median + 90% range	50	Abbott Architect i2000 (chemoluminiscence immunoassay)
Clendenen et al., [20]	53–64 yr.	26	15–59	Median + 90% range	293	radioimmunoassay (RIA)
Pauwels et al., [19]	53–88 yr.	12	< 35	Median + total range	30	LC-MS/MS
Verdonk et al. (this study)	55–68 yr.	11	< 26	Median + Robust method (CLSI C28-A3)	33	LC-MS/MS
Reference intervals for men:						
Nelson, [17]	–	–	37–147	Not stated	32	LC-MS/MS
Sluss et al., [6]	–	84	< 55–161	Median + 90% range	101	ARCHITECT i2000 (chemoluminiscence immunoassay)
Pauwels et al., [19]	33–87 yr.	61	32–118	Median + 'complete' range	25	LC-MS/MS
		69	21–124	Median + 'complete' range		Roche Modular E170 (electrochemiluminescence immunoassay)
Jasuja et al., [22]	30–39 yr.	92	38–146[a]	Mean (SD)	10	LC-MS/MS
	40–49 yr.	94	2–186[a]	Mean (SD)	147	LC-MS/MS
	50–59 yr.	94	30–158[a]	Mean (SD)	515	LC-MS/MS
	60–69 yr.	99	33–165[a]	Mean (SD)	475	LC-MS/MS
Szyska-Skrobot et al., [23]	18–39 yr.	114	32–196[a]	Mean (SD)[b]	80	Immulite 1000 (chemiluminescence immunoassay)
Verdonk et al. (this study)	All ages	74	12–136	Median + Robust method (CLSI C28-A3)	64	LC-MS/MS
	20–29 yr.	78	2–149	Median + Robust method (CLSI C28-A3)	13	LC-MS/MS
	30–39 yr.	87	34–136	Median + Robust method (CLSI C28-A3)	11	LC-MS/MS
	40–49 yr.	80	23–129	Median + Robust method (CLSI C28-A3)	9	LC-MS/MS
	50–59 yr.	72	< 161	Median + Robust method (CLSI C28-A3)	21	LC-MS/MS
	60–63 yr.	63	36–90	Median + Robust method (CLSI C28-A3)	10	LC-MS/MS

[a] These reference intervals are calculated based on the mean ± 2SD presented in the mentioned studies, thus presenting the 95% range.
[b] The study of Szyska-Skrobot et al. [23] presented in their article a reference range calculated using the mean ± 1SD. To make the reference interval more comparable to the other reference intervals presented above the reference interval was calculated using the mean ± 2SD.

Postmenopausal women were included based on the fact that there age was above 55 years.

Our study has some important strengths, including the daily sampling of premenopausal women and the LC-MS/MS method used to measure estradiol. A total of 834 specimens were obtained from the 30 premenopausal women, resulting in a relatively large number of specimens. These specimens gathered daily during one menstrual cycle allowed us in this study to calculate reference intervals per day as well as per phase. Daily reference intervals are essential when assessing dynamic changes in the hypothalamic-pituitary-ovarian axis or endeavoring to determine functional ovarian reserve during the early follicular phase. The premenopausal women underwent physical and biochemical examination to ensure that they had a normal menstrual cycle and did not have any hormonal abnormalities. The reference intervals from our study were established with an accurate, standardized LC-MS/MS method. The calculated reference intervals can be used in other standardized LC-MS/MS methods for medical decision-making in the clinic.

In conclusion, we showed that we used an accurate LC-MS/MS method to measure estradiol. In addition, using this method we established reference intervals for estradiol for premenopausal women during the whole menstrual cycle. Reference intervals were verified and compared to literature for men and postmenopausal women. Comparisons with reference intervals from literature are hampered by the variety in statistics used in the calculation of reference intervals.

Our reference intervals can be used for all standardized LC-MS/MS methods for medical decision-making.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2019.04.062.

Disclosure summary

The authors have nothing to disclose.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

References

[1] B.L. Clarke, S. Khosla, Female reproductive system and bone, Arch Biochem Biophisics 503 (2010) 118–128.
[2] R. Stricker, R. Eberhart, M.C. Chevailler, F.A. Quinn, P. Bischof, R. Stricker, Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT® analyzer, Clin. Chem. Lab. Med. 44 (2006) 883–887.
[3] M.M. Kushnir, A.L. Rockwood, J. Bergquist, et al., High-sensitivity tandem mass spectrometry assay for serum estrone and estradiol, Am. J. Clin. Pathol. 129 (2008) 530–539.
[4] H. Ketha, A. Girtman, R.J. Singh, Estradiol assays - the path ahead, Steroids 99 (2015) 39–44.
[5] W. Rosner, S.E. Hankinson, P.M. Sluss, H.W. Vesper, M.E. Wierman, Challenges to the measurement of estradiol: an endocrine society position statement, J. Clin. Endocrinol. Metab. 98 (2013) 1376–1387.
[6] P.M. Sluss, F.J. Hayes, J.M. Adams, et al., Mass spectrometric and physiological validation of a sensitive, automated, direct immunoassay for serum estradiol using the Architect®, Clin. Chim. Acta 388 (2008) 99–105.
[7] D.J. Handelsman, J.D. Newman, M. Jimenez, R. McLachlan, G. Sartorius, G.R.D. Jones, Performance of direct estradiol immunoassays with human male serum samples, Clin. Chem. 60 (2014) 510–517.
[8] H.W. Vesper, J.C. Botelho, M.L. Vidal, Y. Rahmani, L.M. Thienpont, S.P. Caudill, High variability in serum estradiol measurements in men and women, Steroids 82 (2014) 7–13.
[9] A.M.M. Faqehi, D.F. Cobice, G. Naredo, et al., Derivatization of estrogens enhances specificity and sensitivity of analysis of human plasma and serum by liquid chromatography tandem mass spectrometry, Talanta 151 (2016) 148–156.
[10] L.M. Demers, Testosterone and estradiol assays: current and future trends, Steroids 73 (2008) 1333–1338.
[11] G.L. Horowitz, S. Altaie, J.C. Boyd, EP28-A3c: defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline—third edition, Clin Lab Stand Inst 28 (October) (2010) 12.
[12] S.J.E. Verdonk, H.W. Vesper, F. Martens, P.M. Sluss, J.J. Hillebrand, A.C. Heijboer, Data from: Estradiol Reference Intervals in Women during the Menstrual Cycle, Postmenopausal Women and Men Using an LC-MS/MS Method: Supplemental Table 1. Reference Interval for Premenopausal Women per Day of the Menstrual Cycle, Will be deposited in a repository. Deposited (2019).
[13] J.C. Botelho, A. Ribera, H.C. Cooper, H.W. Vesper, Evaluation of an isotope dilution HPLC tandem mass spectrometry candidate reference measurement procedure for Total 17-β Estradiol in human serum, Anal. Chem. 88 (2016) 11123–11129.
[14] F.Z. Stanczyk, J.S. Lee, R.J. Santen, Standardization of steroid hormone assays: why, how, and when? Cancer Epidemiol. Biomark. Prev. 16 (2007) 1713–1719.
[15] R.M. Büttler, F. Martens, F. Fanelli, et al., Comparison of 7 published LC-MS/MS methods for the simultaneous measurement of testosterone, androstenedione, and dehydroepiandrosterone in serum, Clin. Chem. 61 (2015) 1475–1483.
[16] R.M. Büttler, F. Martens, M.T. Ackermans, et al., Comparison of eight routine unpublished LC-MS/MS methods for the simultaneous measurement of testosterone and androstenedione in serum, Clin. Chim. Acta 454 (2016) 112–118.
[17] R.E. Nelson, S.K. Grebe, D.J. O'Kane, R.J. Singh, Liquid chromatography-tandem mass spectrometry assay for simultaneous measurement of estradiol and estrone in human plasma, Clin. Chem. 50 (2004) 373–384.
[18] A.S. Dighe, J.M. Moy, F.J. Hayes, P.M. Sluss, High-resolution reference ranges for estradiol, luteinizing hormone, and follicle-stimulating hormone in men and women using the AxSYM assay system, Clin. Biochem. 38 (2005) 175–179.
[19] S. Pauwels, L. Antonio, I. Jans, et al., Sensitive routine liquid chromatography-tandem mass spectrometry method for serum estradiol and estrone without derivatization, Anal. Bioanal. Chem. 405 (2013) 8569–8577.
[20] T.V. Clendenen, K.L. Koenig, R.E. Shore, M. Levitz, A.A. Arslan, A. Zeleniuch-Jacquotte, NIH public access, Cancer Epidemiol. Biomark. Prev. 18 (2009) 275–281.
[21] J.S. Lee, B. Ettinger, F.Z. Stanczyk, et al., Comparison of methods to measure low serum estradiol levels in postmenopausal women, J. Clin. Endocrinol. Metab. 91 (2006) 3791–3797.
[22] G.K. Jasuja, T.G. Travison, M. Davda, et al., Age trends in estradiol and estrone levels measured using liquid chromatography tandem mass spectrometry in community-dwelling men of the Framingham heart study, Journals Gerontol - Ser A Biol Sci Med Sci 68 (2013) 733–740.
[23] D. Szyska-Skrobot, K. Marchlewska*, R. Walczak-Jędrzejowska, et al., Free and bioavailable fractions of sex steroids may influence bones in young men, depending on age and oestradiol level, Endokrynol Pol 65 (2014) 357–364.