Female sex hormones do not influence arterial wall properties during the normal menstrual cycle

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1. In previous studies, the elastic properties of the common carotid artery were found to differ between men and women. In these studies, however, the phase of the menstrual cycle was not taken into consideration. It was the aim of the present study to investigate the effect of changing ovarian hormone levels during the normal menstrual cycle on the arterial wall properties of female large arteries.

2. We investigated the elastic right common carotid artery and the muscular right common femoral artery of normotensive young (18-35 years) female subjects (n = 12). The arterial distensibility and cross-sectional compliance coefficients were determined by the use of a specially designed ultrasonic wall-tracking device and measurements of automatic brachial artery cuff blood pressure. The phase of the menstrual cycle was assessed by ultrasonographic evaluation and measurement of 17β-oestradiol and progesterone blood plasma levels.

3. The distensibility coefficient and the cross-sectional compliance coefficient of both the common carotid and the common femoral artery did not change significantly during the normal menstrual cycle despite evidently changing ovarian hormone levels.

4. We conclude that the menstrual cycle does not influence the arterial wall properties of either the elastic common carotid artery or the muscular common femoral artery.

INTRODUCTION

It has been known for quite some time that arteries, especially the larger elastic ones, become stiffer with increasing age [1-5]. For example, from the third decade of age on, distensibility and compliance of the elastic common carotid artery decrease linearly with age, the reduction in compliance being less steep than the reduction in distensibility [5]. The less pronounced decrease in compliance can be explained by the increase in arterial diameter observed with increasing age [4, 6, 7]. In two of these studies, it was shown that the distensibility of the aorta [2] and of the carotid artery [4] was significantly lower in male subjects than in age-matched females. François [8] found stiffer carotid, brachial and leg arteries in men than in women. In contrast, the findings of Riley et al. [9] indicated that the carotid artery is less distensible in females than in males. Similar results were obtained in a study by Van Merode et al. [10], who found distensibility and compliance to be significantly lower in females than in males. An explanation for these discrepancies may be that the phase of the menstrual cycle and the use of oral contraceptives were not considered in these studies. It is possible that female sex hormones influence arterial wall properties, because sex hormone receptors have been shown to be present in the artery wall in both endothelium and vascular smooth-muscle cells [11].

The aim of the present study was to investigate whether arterial wall properties change during the normal menstrual cycle in relation to changes in the levels of female sex hormones during this cycle.

MATERIALS AND METHODS

Subjects

The study was performed on 12 presumed healthy female volunteers, ranging in age from 18 to 35 years. According to the criteria of Vollman [12] the duration of the menstrual cycle had to be between 28 and 32 days. Oral contraceptives were not taken at least 3 months before the study. Before subjects entered the study a physical examination was performed, measuring height, weight and blood pressure. The carotid and femoral arteries were screened for atherosclerotic disease using B-mode imaging (Mark IV; ATL, Bethell, WA, U.S.A.). No
intima-media thickening or atherosclerotic disease could be detected. Blood was drawn from an ante-
cubital vein for the analysis of 17β-oestradiol, pro-
gesterone, haemoglobin, thyroid-stimulating hor-
mone, testosterone and prolactin. Six to eight
examinations were performed during each menstrual
cycle with an average interval of 4 days, involving
the most important phases of the menstrual cycle
(see Fig. 2).

The study design was approved by the Medical
Ethics Committee of the Academic Hospital Maa-
stricht and Maastricht University, and informed con-
sent was obtained from all subjects before they
entered the study.

Study design

All measurements were performed after 10–15
min of rest in the supine position until blood pres-
sure stabilized. Arterial blood pressure was recorded
non-invasively in the right brachial artery, using a
semi-automated oscillometric device (Dinamap; Criti-
ticon, Tampa, FL, U.S.A.). With this method the
pulse pressure in the brachial artery can be assessed
reliably [13]. In previous studies, the pulse pressure
in the brachial artery has been proven to correlate
well with the pulse pressure in the carotid artery
[14]. The correlation between brachial artery and
femoral artery pulse pressure, however, is relatively
poor (C. Willekes, S. Samyo, A. P. G. Hoeks and R.
S. Reneman, unpublished work). Because we com-
pared brachial artery blood pressure at the same
place at different moments in time in the same sub-
ject, and the brachial and femoral artery are both
muscular vessels, the error made was systematic in
nature and did not influence the outcome of our
study. The ultrasound investigations were performed
with the subjects in the recumbent position and the
head tilted at an angle of 45° to the contralateral
side when examining the right common carotid
artery. The elastic common carotid artery, 2–3 cm
proximal to the flow divider, and the muscular femo-
ar artery, 2 cm proximal to the flow divider, were
examined in a random order. The investigations
were limited to the right common carotid and femo-
ar arteries, because previous studies in our labora-
tories have shown that there is no significant
difference between the right and left common caro-
tid arteries and the right and left common femoral
arteries as far as vessel wall properties are concen-
trated (C. Willekes, M. Kool, A. P. G. Hoeks and R.
S. Reneman, unpublished work).

The room temperature was kept constant
(23 ± 2°C) and the measurements were performed at
the same time of the day to avoid diurnal variations
in arterial wall properties. No alcohol, cigarettes or
caffeine-containing beverages were consumed by the
subjects at least 3 hours before the ultrasound
examination. This was considered to be appropriate,
because in a previous study we showed that the
effects of cigarette-smoking on arterial wall prop-
erties are only short-term; no long-term differences
could be demonstrated between smokers and non-
smokers [15].

The phase of the menstrual cycle was assessed by
ultrasonographic follicle size measurement, using a
5 MHz curved array probe attached to an ultrasound
system (Ultramark IX; ATL, Bethell, WA, U.S.A.).
Before each vascular ultrasound examination the
subjects were asked to empty their bladder, as a full
bladder was found to cause stimulation of the symp-
pathetic nervous system reducing arterial wall dis-
tensibility (C. Willekes, A. P. G. Hoeks and R. S.
Reneman, unpublished work).

At the end of each examination, blood was
sampled from an antecubital vein. After centrifuga-
tion the plasma was stored at −20°C. Analysis of
plasma 17β-oestradiol and progesterone was per-
formed in duplicate, using commercially available
radioimmunoassay kits (Diagnostic Products Cor-
poration, Los Angeles, CA, U.S.A.). The mean
intra-assay coefficient of variation, as calculated
from replicate determinations, was 4–5% and 3–5% for
17β-oestradiol and progesterone, respectively.
Interassay coefficients of variation were 12% and
10% for 17β-oestradiol and progesterone, respec-
tively. Evaluation of vascular data was only per-
formed in menstrual cycles showing adequate follicle
growth and eventual ovulation.

Methods

Arterial wall properties were determined by
means of a vessel-wall-tracking system, as described
in detail by Hoeks et al. [16, 17]. The system used in
the present study consists of a conventional ultra-
sound imaging system (Ultramark IV) and a data-
acquisition system connected to a personal
computer (Pie Medical, Maastricht, The Nether-
lands). A 7.5 MHz transducer was used to produce a
two-dimensional B-mode image of the vessel of
interest. An M-line perpendicular to the vessel was
selected. After the echo system was switched to
M-mode, storage of data started. During three to
five cardiac cycles, radiofrequency (RF) signals were
digitized and temporarily stored in a 1 Mbyte
memory. The positions of the anterior and posterior
arterial walls were marked by the observer by plat-
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arterial walls were marked by the observer by plac-
ing two data windows on the first RF signal stored
in the scope of the personal computer. Line after line the data were transferred to a personal computer. The cumulative change in phase
between the successive RF lines was calculated for
the anterior and the posterior wall windows, and the
position of the sample gates was continuously adjus-
ted according to the detected displacement (track-
ing). After processing all lines, the displacement of
the anterior and posterior walls was displayed (Fig.
1). The difference in displacement between the ante-
rior and posterior walls represents arterial dis-
Arterial wall properties and the menstrual cycle

Fig. 1. Displacement curves of the anterior (ant) and posterior (pos) walls of the common carotid artery in a healthy young female (age 20 years). The bottom trace is the difference between the displacements of both arterial walls and represents the change in arterial diameter during the cardiac cycle. The first sign on the distension tracing refers to the trigger of the R-wave of the ECG (x), after which detection of end diastole and peak systole (I) starts. Abbreviations: b, heart beat; dist, distension.

The mean values (+SDs) of DC and CC and arterial blood pressure for the three most important phases of the menstrual cycle (phase 1, 3 and 5) are shown in Table 1. These phases were chosen

RESULTS

Screening of the subjects for thyroid-stimulating hormone, testosterone, prolactin and haemoglobin in venous blood plasma revealed no abnormalities.

The mean values (+SDs) of DC and CC and arterial blood pressure for the three most important phases of the menstrual cycle (phase 1, 3 and 5) are shown in Table 1. These phases were chosen

tension, which is the change in arterial diameter during the cardiac cycle. This procedure is an off-line analysis and provides data on arterial end-diastolic diameter (D) and distension (ΔD) for each captured heart beat. Arterial blood pressure (see above) was recorded every 3 min, and the mean of the three measurements nearest to the distension measurement was taken as the subject's reading. Pulse pressure (ΔP) was defined as systolic minus diastolic blood pressure. From D, ΔD and ΔP, the cross-sectional arterial wall distensibility (DC) and compliance (CC) coefficients were calculated according to the following equations [5]:

\[
DC = \frac{2\Delta D/D}{\Delta P} \tag{1}
\]

\[
CC = \pi D(\Delta D/2\Delta P) \tag{2}
\]

With the wall-tracking system, displacements of a few micrometres can be resolved [18] and D, ΔD, ΔD/D, DC and CC can be assessed reliably [19]. The arterial wall properties, as determined in this way, reflect a combination of passive elastic characteristics of the wall and active components induced by smooth-muscle cells. The intra-observer intra-session coefficient of variation (SD/mean × 100%) for the determination of D, ΔD/D, DC and CC of the common carotid artery was 4.5%, 7.9%, 8.3% and 9.1%, respectively. In the common femoral artery the intra-observer intra-session coefficients of variation were 2.7% (D), 12.4% (ΔD/D), 13.4% (DC) and 12.5% (CC). Intra-observer inter-session variability was comparable with intra-observer intra-session variability for all vessels.

Figure 2 shows the division into phases after correction for time of ovulation, being the central event for every menstrual cycle. A total of five phases were selected according to the height of the plasma levels of 17β-oestradiol and progesterone. In phase 1, 17β-oestradiol and progesterone are low, while in phase 2 17β-oestradiol rises and progesterone is still low. In phase 3, 17β-oestradiol reaches its maximum value and a slow rise in progesterone is observed. In phase 4, 17β-oestradiol decreases while progesterone continues to increase until the maximum value is reached in phase 5. In this phase, 17β-oestradiol shows a second peak which, however, is not as high as the first one observed just before ovulation.

Wilcoxon non-parametric statistical analysis was performed, using SPSS software packages, to compare paired measurements in the menstrual cycle. Bonferroni correction for the level of statistically significant values was used for repeated analysis. A level of P<0.016 was considered to be statistically significant \(P<0.05/[g(g-1)/2]\), where g is the number of pairwise comparisons} [20].
because in phase 1 both 17β-oestradiol and progesterone are low, and in phase 3 17β-oestradiol is at its maximum value while progesterone, although increasing, is still low. Finally, in phase 5, progesterone reaches its highest value, while 17β-oestradiol, although still elevated, is lower than in phase 3.

No significant statistical differences in arterial wall properties were found between these three phases, despite evidently different plasma levels of 17β-oestradiol and progesterone. Also, systolic and diastolic blood pressure were not significantly different between the three phases. There was a tendency to a lower diastolic blood pressure towards the end of the menstrual cycle, but this decrease did not reach the level of significance after Bonferroni correction. Pulse pressure and mean arterial pressure, calculated from systolic and diastolic pressure, did not change significantly under the influence of changes in the levels of either 17β-oestradiol and progesterone. Heart rate showed a slight, but non-significant, increase towards the end of the menstrual cycle.

We also tested the above-mentioned variables in the remaining two phases of the menstrual cycle (phases 2 and 4), but again no statistically significant differences were found.

**DISCUSSION**

The findings in the present study show that compliance and distensibility of the common carotid and common femoral arteries do not change during the

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**Table 1.** End-diastolic diameter (D), relative distensibility (ΔD/D × 100%), DC and CC of the right common carotid and right common femoral arteries, as well as systolic (Ps) and diastolic (Pd) arterial blood pressure, pulse pressure (AP), mean arterial pressure (MAP) and heart rate (HR) during the three most important phases of the menstrual cycle. Mean values (± SD) are shown (n = 12)

<table>
<thead>
<tr>
<th>Phase</th>
<th>Carotid artery</th>
<th>Femoral artery</th>
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<td>D (mm)</td>
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<td>65 (±4)</td>
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<td>64 (±5)</td>
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<td>62 (±4)</td>
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<td>12.7 (±2.2)</td>
<td>48 (±5)</td>
<td>12.4 (±2.6)</td>
<td>47 (±4)</td>
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<td>50 (±4)</td>
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<td>40.5 (±9.1)</td>
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<td>39.7 (±9.7)</td>
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<td>40.5 (±8.4)</td>
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normal menstrual cycle, despite pronounced changes in plasma levels of $17\beta$-oestradiol and progesterone. Therefore, we may conclude that the different phases of the menstrual cycle cannot be held responsible for the discrepancies between the various studies as far as the differences in arterial wall properties between men and women are concerned. In some studies the arteries were found to be less distensible in men than in women [2, 4, 8], whereas in others women were found to have less distensible arteries [9, 10].

It may be argued that the discrepancies between the various studies result from differences in the methods used to assess arterial wall compliance and distensibility. Indeed, Laogun and Gosling [2] and François [8], who found the distensibility to be lower in men than in women, determined the pulse wave velocity over a considerable distance along the arterial tree to calculate distensibility by means of the Moens–Korteweg equation. Riley et al. [9] and Van Merode et al. [10], who found less distensible arteries in women than in men, determined arterial wall distensibility and compliance locally with the use of a vessel-wall-tracking system. Recently, however, Hansen et al. [4], using a similar device, found the distensibility to be lower in men than in women, albeit only in young and old subjects.

The discrepancies between the findings in the different studies are also not explained by considering different parts of the arterial tree. The common carotid artery was found to be less distensible in women than in men by Riley et al. [9] and Van Merode et al. [10], and more distensible in women by François [8] and Hansen et al. [4]. It is of interest to note that, in the study of Riley et al. [9], the elastic modulus was found to be higher in men than in women, suggesting reduced elasticity of the common carotid artery wall in men, which is discordant with the increased distensibility. It should be noted, however, that the elastic modulus characterizes the material properties of the arterial wall, while distensibility is a functional parameter. Changes in these two parameters are not necessarily in line with each other [21].

A role for the use of oral contraceptives cannot be explained, as this was not considered in any of the studies discussed.

It is important to note that in all of the studies discussed the parameters measured to characterize arterial wall properties show a large variability, with differences often within 1 SD of the mean. Moreover, most of the studies were performed on a relatively small group of subjects using statistical analyses applicable only to larger populations displaying a normal distribution. This raises the question as to whether there are differences in arterial wall properties between males and females and whether the discrepancies between the various studies are real.

Whether the absence of changes in arterial wall properties during the menstrual cycle is a consequence of the short-term hormonal variations in this situation remains to be elucidated. The possibility that the effect of female sex hormones on arterial wall properties is a slow process with a time-constant cannot be excluded.

In conclusion, no significant differences in arterial wall properties of the elastic common carotid artery and the muscular common femoral artery could be demonstrated during the menstrual cycle, despite pronounced changes in plasma levels of $17\beta$-oestradiol and progesterone.

REFERENCES