



Menstrual cycle and respiratory symptoms in a general Nordic-Baltic population

Journal:	<i>American Journal of Respiratory and Critical Care Medicine</i>
Manuscript ID:	Blue-201206-11120C.R1
Manuscript Type:	OC - Original Contribution
Date Submitted by the Author:	28-Sep-2012
Complete List of Authors:	Macsali, Ferenc; Haukeland University Hospital, Department of Obstetrics and Gynecology Svanes, Cecilie; Institute of Medicine, University of Bergen, Bergen Respiratory Research Group Sothorn, Robert; University of Minnesota, College of Biological Sciences Benediktsdottir, Bryndis; University of Iceland, Medical Faculty, Dept of Respiratory Medicine and Sleep Line, Bjorge; University of Bergen, Institute of Clinical Medicine Dratva, Julia; University of Basel, Inst of Social and Preventive Medicine Franklin, Karl; Umeå University, Dept of Surgery and Dept of Medicine Holm, Mathias; University of Gothenburg, Dept of Occupational and Environmental Medicine Janson, Christer; Uppsala University, j Dept of Medical Sciences: Respiratory Medicine and Allergology Johannessen, Ane; Haukeland University Hospital, Centre for Clinical Research Lindgren, Eva; Uppsala University, j Dept of Medical Sciences: Respiratory Medicine and Allergology Omenaas, Ernst; Institute of Medicine, University of Bergen, Bergen Respiratory Research Group Schlünssen, Vivi; Aarhus University, Dept of Environmental and Occupational Medicine Zemp, Elizabeth; University of Basel, Inst of Social and Preventive Medicine Real, Francisco; Institute of Medicine, University of Bergen, Bergen Respiratory Research Group
Keywords:	Menstrual cycle, RHINE, Respiratory symptoms, Asthma, Sex hormones

Menstrual cycle and respiratory symptoms in a general Nordic-Baltic population

Ferenc Macsalı MD^{ab}, Cecilie Svanes MD, PhD^{bc}, Robert B Sothorn PhD^d, Bryndis Benediktsdottir MD, PhD^e, Line Bjørge MD, PhD^{af}, Julia Dratva MD, PhD^g, Karl A Franklin MD, PhD^h, Mathias Holm MD, PhDⁱ, Christer Janson MD, PhD^j, Ane Johannessen PhD^k, Eva Lindberg MD, PhD^j, Ernst R Omenaas MD, PhD^{bk}, Vivi Schlünssen MD, PhD^l, Elizabeth Zemp MD, PhD^g, Francisco Gómez Real MD, PhD^{ab}

^a Dept of Gynecology and Obstetrics, Haukeland University Hospital, Bergen, Norway

^b Bergen Respiratory Research Group, Inst of Medicine, University of Bergen, Norway ^c Dept of Occupational Medicine Haukeland University Hospital, Bergen, Norway

^d College of Biological Sciences, University of Minnesota, St. Paul, MN, USA

^e University of Iceland, Medical Faculty. Dept of Respiratory Medicine and Sleep, Landspítali University Hospital, Iceland

^f Inst of Clinical Medicine, University of Bergen, Norway

^g Inst of Social and Preventive Medicine, University of Basel, Basel, Switzerland

^h Dept of Surgery and Dept of Medicine, Umeå University, Sweden

ⁱ Dept of Occupational and Environmental Medicine, University of Gothenburg, Sweden

^j Dept of Medical Sciences: Respiratory Medicine and Allergology, Uppsala, Sweden

^k Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway

^l Dept of Environmental and Occupational Medicine, Aarhus University, Denmark

Corresponding author: Ferenc Macsalı, Department of Gynecology and Obstetrics, Haukeland University Hospital, 5021 Bergen, Norway

E-mail: ferenc.macsali@med.uib.no

Phone: + 47 55 974200

Fax: + 47 55 974968

Running title: Menstrual cycle and respiratory symptoms

Word count: 3493; methods 488

Keywords: Menstrual cycle; RHINE; respiratory symptoms; asthma; sex hormones.

Abbreviations

BHR: Bronchial hyper reactivity

BMI: Body mass index

CRP: C-reactive protein

ECRHS: European Community Respiratory Health Survey

DLCO: Lung diffusion capacity

FENO: Fractional exhaled Nitric Oxide

FSH: Follicle stimulating hormone

IgE: Immunoglobulin E

IL: interleukin

IR: Insulin resistance

LH: Luteinizing hormone

RHINE: Respiratory Health in Northern Europe

WHO: World Health Organization

Authorship

FM: conception and design, analysis and interpretation, drafting the manuscript for important intellectual content

CS: conception and design, analysis and interpretation, drafting the manuscript for important intellectual content

RBS: conception and design, analysis and interpretation, drafting the manuscript for important intellectual content

BB: drafting the manuscript for important intellectual content

LB: drafting the manuscript for important intellectual content

JD: drafting the manuscript for important intellectual content

KAF: drafting the manuscript for important intellectual content

MH: drafting the manuscript for important intellectual content

CJ: drafting the manuscript for important intellectual content

AJ: drafting the manuscript for important intellectual content

EL: drafting the manuscript for important intellectual content

ERO: drafting the manuscript for important intellectual content

VS: drafting the manuscript for important intellectual content

EZ: drafting the manuscript for important intellectual content

FGR: conception and design, analysis and interpretation, drafting the manuscript for important intellectual content

Abstract

Rationale: There is little knowledge of variations in respiratory symptoms during the menstrual cycle in a general population, and potential modifying factors are not investigated.

Objective: To investigate menstrual cycle variation in respiratory symptoms in a large general population, using chronobiology methodology, and stratifying by BMI, smoking and asthma status.

Methods: 3926 women with regular cycles ≤ 28 days and not taking exogenous sex hormones, answered a postal questionnaire regarding the first day of last menstruation and respiratory symptoms last three days. Moving 4-day means were computed to smooth uneven records of daily sampling; best-fitting 28-day composite cosine curves were applied to each time series to describe rhythmicity.

Measurements and main results: Significant rhythmic variations over the menstrual cycle were found in each symptom for all subjects and subgroups. Wheezing was higher on cycle days 10-22, with a mid-cycle dip near the time of putative ovulation (~days 14-16) in most subgroups. Shortness of breath was higher on days 7-21, with a dip just prior to mid-cycle in many subgroups. Cough was higher just following putative ovulation for asthmatics, $\text{BMI} \geq 23 \text{ kg/m}^2$ and smokers, or just prior to ovulation and menses onset for low symptomatic subgroups.

Conclusions: Respiratory symptoms varied significantly during the menstrual cycle and were most frequent from the mid-luteal to mid-follicular stages, often with a dip near the time of ovulation. The patterns varied by BMI, smoking and asthma status. These relations link respiratory symptoms with hormonal changes through the menstrual cycle and imply a potential for individualized chronotherapy for respiratory diseases.

Word count: 250

Introduction

Emerging understanding of the role of sex hormones in respiratory health represent a major advance in respiratory epidemiology the last decade(1). Sex hormones influence respiratory health throughout a woman's life-span (2-8). A considerable scientific effort addresses menstrual cycle variations in respiratory health, and a recent publication by Farha et al(9) showed that airflow and gas transfer vary over the menstrual cycle. Menstrual cycle variations have been described for respiratory symptoms(10-12), asthma exacerbations(11, 12), hospital admissions for asthma(13, 14), peak expiratory flow rate (PEFR)(15-20), bronchial hyperreactivity (BHR)(21), and gas diffusion capacity(9, 22); mainly studied in asthmatic women. Parameters of inflammatory and immunological responses like total IgE(23), CRP(24), IL4(25) and fractional exhaled nitric oxide (FENO)(26) vary during the menstrual cycle, as does insulin levels and insulin sensitivity (27).

The understanding of menstrual cycle variations is important, given the potential to throw light on airways physiology and the potential for chronotherapy for a subgroup of women with respiratory diseases like asthma. However, there is little knowledge on menstrual cycle variation in respiratory symptoms in a general population, and respiratory symptoms are far more common than asthma. No study of menstrual cyclicity in respiratory outcomes addresses potential modifying factors like body mass index (BMI) and smoking, both related with respiratory health and sex hormones(28).

Chronobiological methodology is generally not used in the above cited literature on respiratory health, but provides the most sensitive approach to investigate menstrual cycle variations. It is not biologically plausible to categorize data for complex rhythmical processes; categorization implies a risk of losing important information and biasing the identification of peaks and troughs.

The course and timing of menstruation is steered through a complex endocrine interplay between hormones originating from the hypothalamus, the pituitary gland and the ovaries. In the 10 to 14 days following menses (follicular phase), there is a complex process of endocrine and paracrine sequential actions that usually leads to one mature follicle. Estrogen increases until ovulation, follicle stimulating hormone (FSH) rises but has a short dip before a new rise around ovulation and luteinizing hormone (LH) initiates ovulation and has a large surge around ovulation. At ovulation the basal body temperature rises 0.5 C°. The luteal or secretory phase occurs in the second half of the cycle. There is a surge in circulating progesterone 4 to 8 days after ovulation, FSH and LH decrease, and estrogen first decreases and then has a secondary smaller peak. All hormones are approaching their nadir at menstruation except FSH, which is slowly rising. Figure 1 shows an idealized scheme of the menstrual cycle(29).

Hormonal factors are closely linked with metabolic factors and obesity. Thus, accounting for metabolic factors, like body mass index (BMI) (30), is highly relevant when investigating hormonal effects on respiratory health (3, 5, 7, 31). Smoking is important for respiratory health and has anti-estrogenic effects(28), and should be considered when studying hormonal factors in respiratory health. Whether asthmatics and non-asthmatics exhibit different menstrual cycle patterns of symptoms is not known.

We hypothesize that respiratory symptoms in a general population vary according to the menstrual cycle, and that the cyclical pattern varies according to characteristic like BMI, smoking and asthma status. Thus, the aims of the present study were to i) investigate whether respiratory symptoms in a general population vary rhythmically during the menstrual cycle and assess the magnitude of such variation, and ii) investigate whether the cyclical pattern of symptoms differs according to BMI, smoking and asthma status. This was addressed in a large population-based multi-centric study, where respiratory symptoms within the last three days were reported independently of last menses onset, and analysis for periodicity were based on chronobiological methodology using data from each day over a 28 day cycle rather than pre-defined cut-points or sub-spans.

Some of the results of these studies have been previously reported in the form of an abstract. (32)

Methods

Respiratory Health in Northern Europe (RHINE; www.rhine.nu) is a population-based multi-center postal questionnaire study, including 8592 women (response rate 77%). Written consent was obtained from participants; local ethics committees approved the study.

Respiratory symptoms the last three days were defined by “yes”/“no” answers to the questions “Have you had wheezing or whistling in your chest at any time in the last three days?”, “Have you been woken by an attack of shortness of breath at any time in the last three days?”, and “Have you been woken by an attack of coughing, at any time, in the last three days?”. Menstrual cycle day was calculated from reported date of the first day of the last menses onset to the questionnaire date. BMI was computed from reported height and weight (in kg/m²). Smoking was categorized in current versus non-smokers (never-/ ex-smokers). The question “Have you ever had asthma diagnosed by a doctor?” defined asthma status.

To analyze a hormonally well-defined group of women with regular natural menstrual cycles, the following were excluded: pregnant (n=260), using oral contraception (n=1,023) or HRT (n=634), irregular menstruation (n=1360), oligomenorrhea (cycle length ≥ 35 days; n=762), postmenopausal (n=158), ages >55 years (n=138), error in reported menses onset (n = 101).

The percentage of women reporting a symptom on each day of the menstrual cycle was calculated as N with symptom divided by N reporting on each day. We calculated 4-day moving averages to smooth the data, due to daily fluctuations in number of women on some days. The numbers of women reporting on each cycle day were evenly distributed.

Analysis of each time series for menstrual periodicity was accomplished by the single cosinor procedure (33) using percent incidence of each variable on days 1-28 of the menstrual cycle. Single cosinor modeling involves the least-squares fitting of cosine curves with periods that are expected to characterize the dataset. This involved the approximation of each time series data by the least-squares linear regression fitting of a single component (28 days) or multiple component (28 days plus 1, 2 or 3 harmonic periods) (34). A p-value for rejecting the zero-

amplitude assumption was determined by an F-test of variance accounted for by the fit of the single or compound period waveform versus the variance accounted for by a straight line approximation of the arithmetic mean. Rhythm detection was considered statistically significant if $p \leq 0.05$ for each fitted period in the cosine model separately and overall.

Rhythm characteristics determined from the best-fitting cosine include: the 'mesor' (the middle of the cosine, representing an adjusted 28-day average); 'amplitude' (half the distance from the peak and trough of the best-fitting curve); and 'phase' of the cosine ($\emptyset$, in days after 00:00h on day 1 of mesor). The peak of the fitted cosine, representing the calculated average time of high values in the data, is termed the 'orthophase ($o\emptyset$)' for a multiple-component cosine, while the 'bathypase ($b\emptyset$)' indicates the lowest values of the cosine waveform.

RESULTS

Demographic characteristics are presented in Table 1. Number of women with menstrual cycles longer than 28 days dropped abruptly and were excluded ($n=230$); demographic characteristics were unaltered when excluding these (Table 1). Thus, data from 3926 women with cycle lengths ≤ 28 days were analyzed. The mean age was 39 years, the median BMI was 23 kg/m^2 , and 28.5% of the women were current smokers. 7.7% reported doctor's diagnosed asthma. In the last 3 days, 5.6% had experienced wheezing, 1.8% shortness of breath, and 6.1% cough.

The cosinor analyses are presented as figures, with numerical parameters of rhythm estimates in tables. The methodology did not allow for analyses of interaction, thus stratified analyses are presented for subgroups. Upon inspection of cosine analyses and graphs, we noted that a 28-day plus 3 harmonic period's cosine most closely approximated the menstrual cycle waveform in the daily incidence of respiratory symptoms. Accordingly, these numerical results are listed in Table 2, and the four-component curve is shown superimposed over the 4-day smoothed daily averages in Figures 2-4.

The overall prevalence of wheeze in the last three days was 5.5% (rhythm adjusted mean as opposed to arithmetic mean); 6.6% when $\text{BMI} \geq 23 \text{ kg/m}^2$ and 4.4% when $\text{BMI} < 23 \text{ kg/m}^2$, and 22.2% for women with diagnosed asthma and 5.3% for those without diagnosed asthma. Smokers had a prevalence of 10.3% as opposed to 3.6% in ex and never smokers (Table 2 top panel, Figure 2).

The daily incidence for wheeze over days 1-28 of the menstrual cycle showed a significant rhythm at $p \leq 0.001$ when analyzing all women and in each of the main subcategories (Table 2 – top panel; Figure 2). Overall, the highest daily incidence of wheezing occurred with two peri-ovulatory peaks, before and after mid-cycle (days 14-16 near ovulation), with a dramatic decrease during this putative ovulatory span, and was lower before and after menses (Figure 2A). The same two-peaked pattern with a peri-ovulatory dip was found for $\text{BMI} \geq 23 \text{ kg/m}^2$ (Figure 2B), those with diagnosed asthma or not (Figure 2C), and among smokers and non-

smokers (Figure 2D). For women with $\text{BMI} < 23 \text{ kg/m}^2$, a single peak occurred prior to mid-cycle (Figure 2B).

The overall prevalence of shortness of breath was 1.7%; 2.1% when higher BMI ($\geq 23 \text{ kg/m}^2$) and 1.2% when lower BMI; 6.8% when diagnosed asthma and 1.7% when not; 3.1% among smokers and 1.1% in non-smokers (Table 2 middle panel, Figure 3).

The daily incidence for shortness of breath over days 1-28 of the menstrual cycle showed a significant rhythm at $p \leq 0.001$ when analyzing all women and in each of the main subcategories (Table 2 – middle panel; Figure 3). The amplitude of the rhythmic oscillations was almost as large as the mean prevalence; this was found for all subgroups. Overall, the highest daily incidence of shortness of breath occurred with two peaks before and during mid-cycle (near putative ovulation on days 14-16), and was lower before and after menses (Fig 3 – panel A). The same two-peaked pattern in shortness of breath was found for the high-prevalence groups, those with $\text{BMI} \geq 23 \text{ kg/m}^2$ (panel B), diagnosed asthma (panel C), and smokers (panel D). A single peak occurred just prior to mid-cycle in the lower prevalence groups, those with $\text{BMI} < 23 \text{ kg/m}^2$ (panel B), non-asthmatics (panel C) and non-smokers (panel D).

The overall prevalence of cough was 6.0%; with small differences between those with higher or lower BMI (6.1 % vs 5.8 %), and larger differences according to asthma status (14.6% vs 7.8%) and smoking status (7.9% vs 5.1%) (Table 2 bottom panel, Figure 4).

The daily incidence for cough over days 1-28 of the menstrual cycle showed a significant rhythm for all women ($p = 0.005$), and in each of the main subcategories (Table 2 bottom panel; Figure 4). Overall, the highest daily incidence of cough occurred with peaks before and after mid-cycle (near putative ovulation when values dropped dramatically) and prior to menses onset (major peak), and was lower after menses (Fig 4 – panel A). A similar three-peaked pattern in cough was noted in non-asthmatics (panel C), and smokers/non-smokers (panel D), but a two-peaked pattern before and after mid-cycle was found for $\text{BMI} \geq 23 \text{ kg/m}^2$ (panel B), and asthmatics (panel C). Women with $\text{BMI} < 23 \text{ kg/m}^2$ showed the most dissimilar pattern with highest values for cough before and after menses and lowest values during and following mid-cycle (panel B).

DISCUSSION

We found that respiratory symptoms varied significantly during the menstrual cycle in women from a general population with regular menstruations. There were large changes in symptom incidence through the cycle for all symptoms, with amplitudes as large as the mean incidence for shortness of breath. Wheeze and shortness of breath were characterized by prominent peaks located in the mid-luteal and mid-follicular phase, and a noticeable dip just prior to or during mid-cycle; however, the specific patterns differed between symptoms and between subgroups. Cough showed peaks before and after mid-cycle near putative ovulation and prior to menses onset, and was lower after menses. In subgroups with low incidence of symptoms

there was a tendency towards a pre- or periovulatory peak. The cyclical pattern varied according to BMI, supporting a metabolic component in airways symptoms. There were some differences in patterns by smoking status, in accordance with known hormonal influences of smoking. Pronounced cyclical variations were present in the subgroup of women with diagnosed asthma, suggesting a potential for individualized chronotherapy. These multi-oscillatory findings are based on chronobiological methodology for analysis of % incidence on each day of the menstrual cycle, rather than sampling restricted to specific sub-spans within the cycle.

It is novel that respiratory symptoms in a general population vary rhythmically during the menstrual cycle. However, this finding is consistent with a number of studies with different designs and methodology. There is evidence of menstrual cycle variation in BHR(21), lung function(9), gas diffusion capacity(9, 22) and FENO(12, 26). Among asthmatics, the literature describes menstrual cycle variations in respiratory symptoms (10-12), peak expiratory flow rate (15, 17-19, 35), total IgE(23), asthma exacerbations (11, 36), and hospital admissions (13, 14). Menstrual cycle variations in respiratory symptoms in a general population seem biologically plausible, as factors like edema(20), smooth muscle contractility and inflammatory mediators(25) varies during the menstrual cycle .

Differences in menstrual cycle patterns of respiratory symptoms according to BMI and smoking have not been reported previously, to the authors' knowledge. The observation of differences according to BMI is plausible, given the close interplay between hormonal and metabolic factors, and supports previous literature showing such interplay specifically in respiratory health outcomes(3, 5-7). The use of chronobiological methodology as used in the present study is novel.

The menstrual cycle pattern in the present analysis was characterized by prominent peaks in the mid-luteal and mid-follicular phase and a noticeable dip near mid-cycle. However, the pattern varied somewhat between the respiratory symptoms and differed between subgroups. Farha et al(9) showed higher FEV1 and higher DLCO around menstruation and lower levels around mid-cycle; this was similar among asthmatics and healthy controls. These findings are in accordance with the patterns presented in our study: phases of higher lung function and gas diffusion capacity in that study coincide roughly with periods of less respiratory symptoms in the present study. On the other hand, Dratva et al(21) found increased prevalence of BHR perimenstrually and a small peak in BHR around ovulation in a general population; we did generally not identify peaks in symptoms in the perimenstrual phase, and both peaks and troughs around ovulation, dependent on symptom and subgroup. Increased FENO as a marker of eosinophil airway inflammation was in one study positively related to progesterone (26), a pattern not easily comparable with our findings. A number of studies investigate pre-menstrual asthma (20, 37). Our study did not address specifically the phenotype of pre-menstrual asthma, but could not confirm particularly high incidences of respiratory symptoms in this general population prior to or during the time of menses, with the exception of cough in low symptomatic subgroups.

There are several possible explanations for differences in the literature concerning location of peaks during the menstrual cycle, among which methodological issues are very relevant. Different respiratory outcomes may not be comparable. Differences in study populations may be important, as indicated by differences according to asthma, BMI and smoking as described in the present analysis. Most studies are based on analysis of pre-defined sub spans, where the choice of cut-points may influence the results substantially; for instance, an imaginary regular cyclical variation with two peaks would reveal no differences between groups if analyzed in four equal categories. Several studies are based on reports of perceived worsening of symptoms during specific phases in the menstrual cycle, which introduce a possibility for differential reporting bias.

The course and timing of menstruation is steered through a complex endocrine interplay between hormones. The observed patterns in our study are most likely a result of such complex hormonal processes, and it does not seem plausible that one sex hormone should explain the variation in respiratory symptoms during the menstrual cycle. Both direct effects of sex steroid hormones on the airways and indirect effects of sex hormones for instance on inflammatory processes might influence respiratory symptoms, as might various other endocrine and paracrine hormones directly or indirectly involved in regulation of the menstrual cycle. Estrogens are known to have receptors in lung tissue(38), while progesterone acts centrally as well through an estrogen- (E2) dependent progesterone receptor-mediated mechanism to stimulate respiration(39, 40). Cyclical changes in respiratory symptoms could further be mediated through insulin resistance and inflammation, since insulin resistance(27) as well as CRP(24) has been shown to vary with the menstrual cycle. A role for such mechanisms is supported by our finding of differences in cyclical patterns according to BMI. Angiogenesis in the lung is another factor changing with the menstrual cycle (41) that might be suspected to contribute to cyclical patterns in respiratory symptoms. Considering the anti-estrogenic effects of smoking, differences in menstrual cycle patterns of airways symptoms by smoking status, as demonstrated in the present study, seem plausible. However, the differences between observed patterns in smokers and non-smokers cannot easily be attributed to one hormone.

The observed differences in cyclical patterns for wheeze, waking with shortness of breath and waking with cough might reflect slight differences in underlying physiological aspects. Wheeze and shortness of breath showed relatively similar patterns. The relative magnitude of the cyclical variation was considerably larger for shortness of breath, with amplitude as large as the mean incidence. In all subgroups, shortness of breath incidence was strikingly low in the late luteal phase, the time when progesterone peaks. The dip in incidence of wheeze on days 14-16 coincides with putative ovulation and peaks in estrogen, LH and FSH. Cough showed distinct and more complicated patterns as compared to wheeze and shortness of breath, which seems plausible given the broader range of triggering factors, also including upper airways and remote triggers.

Presence of airways symptoms in subjects without a doctor's diagnosis of asthma is well known and not fully understood. Unrecognized asthma as well as other diseases are likely to account for a considerable proportion of these symptoms. However, one may speculate

whether experiencing some degree of respiratory symptoms at specific times during the menstrual cycle possibly could be physiological.

The use of chronobiological methodology is novel in this setting and an important strength of the present analysis. Day in cycle was calculated from day of last menses onset for each woman, and analyses were based on all days of the menstrual cycle rather than specific subsections. This methodology is sensitive for detecting variations in respiratory symptoms continuously over the menstrual cycle that could otherwise be hidden if analyzing predefined sub-spans. Another strength of our study derives from the questionnaire recording symptoms over the immediate last three days in one section, and the date of last menses onset in another section. Thus, no bias was introduced by asking women to relate respiratory symptoms to specific parts of their menstrual cycle. Further, analyses being based on general population samples from several countries, allows for generalization of results to a broad population. The large sample size made relevant subgroup analyses possible.

A weakness of our study is the reliance on questionnaire data for assessment of the day in the menstrual cycle. The later in the cycle since the previous menses, the less accurate the date of its onset might be remembered. However, this phenomenon should introduce a non-differential bias and therefore attenuate our results and not strengthen them. Another difficulty in placing a woman accurately in her cycle is the finding that, among women reporting regular cycles, during one year approximately 20% have at least one cycle shorter than 21 days and 30% will have one or more cycles longer than 35 days (42). This should also introduce a non-differential bias and dilute associations. These sources of non-differential error suggest that the actual menstrual cycle variation in respiratory symptoms may be very large.

Conclusions and implications

Respiratory symptoms varied significantly during the menstrual cycle and the rhythmic oscillations were large and consistent. This was found in a cohort of almost 4000 women analyzed with chronobiological methodology without pre-defined cut-points, and based on independently-reported symptoms and day of menstrual cycle, rather than perceived associations during cycle sub-spans. Cyclicity in respiratory symptoms was present also in the population without diagnosed asthma. Patterns varied between subgroups, thus, modifying factors needs to be considered in menstrual cycle variations in respiratory health. Differences according to body mass index contribute to the evidence of metabolic – hormonal interplay in respiratory health. The findings suggest substantial hormonal influences in interplay with metabolic factors on airways physiology and on pathophysiological processes in respiratory diseases like asthma.

Pronounced cyclical variations among asthmatics but differences in patterns according to various characteristics, suggests that adjustment of asthma medication to the menstrual cycle may prove feasible and efficient, but must be adapted on an individual basis. We recommend that physicians advise asthmatic women to record their disease activity during several

menstrual cycles, e.g., with the asthma control test and/or a peak flow meter, and attempt to adapt asthma medication according to individual pattern in symptoms. Adjustment of asthma medication to the menstrual cycle may potentially improve the efficacy of asthma treatment and reduce disability and health costs related to asthma in women.

For Review Only

Figure Legends

Figure 1 Generalized scheme of the hormonal and body temperature changes during a typical menstrual cycle (used by permission; adapted from Fig 7.19 in Koukkari and Sothorn, 2006(29)). Day1 = first day of menses onset.

Figure 2 Chronograms showing % incidence of wheezing on menstrual cycle days 1-28 as recorded in the RHINE survey by 3926 premenopausal women. Percent incidence/day smoothed over 4-day intervals and analyzed for rhythm by the least-squares fit of a composite 4-component cosine. Fitted cosine (shown) significant at $p \leq 0.001$ for each grouping, with peak incidence (oØ) indicated by arrows. All numerical results listed in Table 2 – top panel.

Figure 3 Chronograms showing % incidence of shortness of breath on menstrual cycle days 1-28 as recorded in the RHINE survey by 3926 premenopausal women. Percent incidence/day smoothed over 4-day intervals and analyzed for rhythm by the least-squares fit of a composite 4-component cosine (see Methods). Fitted cosine (shown) significant at $p < 0.001$ for each grouping, with peak incidence (oØ) indicated by arrows. All numerical results listed in Table 2 – middle panel.

Figure 4 Chronograms showing % incidence of cough on menstrual cycle days 1-28 as recorded in the RHINE survey by 3926 premenopausal women. Percent incidence/day smoothed over 4-day intervals and analyzed for rhythm by the least-squares fit of a composite 4-component cosine (see Methods). Four-component fitted cosine (shown) significant at $p = 0.005$ for all subjects, $p = 0.03$ for asthmatics, and $p < 0.001$ for all other groupings, with peak incidence (oØ) indicated by arrows. All numerical results listed in Table 2 – bottom panel.

TABLE 1. CHARACTERISTICS OF WOMEN WITH REGULAR MENSTRUAL CYCLES AND CYCLE LENGTH UP TO 28 DAYS PARTICIPATING IN THE RHINE POPULATION BASED STUDY

	N responded	
Age (mean±SD), yr	3926	38.9±6.3 (range: 25-54)
BMI<23.0 kg/m ²	3875	49.9% (1933/1942)
Asthma	3868	7.7% (298/3570)
Current smoker	3871	28.5% (1104/2767)
Wheeze in the last 3 days	3884	5.6% (219/3665)
Shortness of breath in the last 3 days	3887	1.8% (70/3817)
Cough in the last 3 days	3884	6.0% (234/3650)

Definition of abbreviations: BMI = body mass index; SD = standard deviation.

TABLE 2. STATISTICAL EVALUATION BY SINGLE COSINOR MODELING OF MENSTRUAL CYCLE VARIATIONS FOR WHEEZE, SHORTNESS OF BREATH AND COUGH

		Rhythm parameters*					
Wheeze		%R [†]	P Value [‡]	Mesor± S.E [§]	Amp± S.E	Orthophase day (oØ) ^{**}	Bathypphase day (bØ) ^{**}
All		80.1	<0.001	5.48±0.12	1.40±0.20	19.1	27.2
BMI	<23	79.5	<0.001	4.35±0.14	1.63±0.21	12.9	24.8
	≥23	91.7	<0.001	6.57±0.13	2.83±0.20	18.9	0.5
Asthma	No	85.0	<0.001	5.34±0.12	1.92±0.20	11.7	27.8
	Yes	74.5	0.001	22.19±0.82	8.49±1.06	21.5	27.5
Smoking	Never+ex	87.3	<0.001	3.58±0.10	1.67±0.15	12.0	27.8
	Current	87.7	<0.001	10.31±0.29	4.94±0.34	19.1	23.6
Shortness of Breath		%R [†]	P Value [‡]	Mesor± S.E [§]	Amp± S.E	oØ ^{**}	bØ ^{**}
All		92.1	<0.001	1.70±0.07	1.16±0.10	14.2	23.8
BMI	<23	77.5	<0.001	1.23±0.11	1.18±0.16	12.6	22.7
	≥23	93.8	<0.001	2.07±0.07	1.70±0.12	16.8	24.7
Asthma	No	91.0	<0.001	1.71±0.08	1.31±0.12	13.6	1.9
	Yes	85.9	<0.001	6.83±0.42	6.43±0.61	7.4	24.9
Smoking	Never+ex	88.0	<0.001	1.12±0.08	1.17±0.13	13.2	22.8
	Current	94.6	<0.001	3.09±0.10	2.65±0.15	6.8	24.8
Cough		%R [†]	P Value [‡]	Mesor± S.E [§]	Amp± S.E	oØ ^{**}	bØ ^{**}
All		69.4	0.005	6.02±0.10	1.03±0.11	25.0	1.4
BMI	<23	92.2	<0.001	5.82±0.13	2.28±0.18	25.9	18.5
	≥23	89.9	<0.001	6.05±0.15	2.86±0.20	18.1	1.2
Asthma	No	89.9	<0.001	7.78±0.13	2.09±0.15	25.2	20.9
	Yes	59.8	0.030	14.58±0.45	4.76±0.72	16.5	26.9
Smoking	Never+ex	75.9	<0.001	5.05±0.13	1.44±0.18	10.3	19.9
	Current	78.3	<0.001	7.93±0.22	2.90±0.25	19.1	1.5

Explanation of terms:

* Rhythm parameters for composite 4 harmonic (28d+14d+9.33d+7d) cosine.

† %R (percent rhythm) = % of variability around flat line average [mean] reduced by cosine model.

‡ P Value= for rejecting the zero-amplitude assumption. Determined by an F-test of variance accounted for by the fit of the single or compound period waveform versus the variance accounted for by a straight line approximation of the arithmetic mean. Rhythm detection was considered statistically significant if $p \leq 0.05$ for each fitted period in the cosine model separately and overall, and borderline significant if $p \leq 0.10 > 0.05$.

§ Mesor (Midline Estimating Statistic of Rhythm) = 28d adjusted mean (middle of cosine).

¶ Amp = amplitude of cosine model: half the distance from the peak and trough of the best-fitting curve.

** Phases ($\emptyset$): Orthophase ($o\emptyset$) = cycle day with highest point of multiple component cosine; Bathyphase ($b\emptyset$) = cycle day with lowest point of multiple component cosine. Phase units = day of menstrual cycle from Day 1 of menses onset.

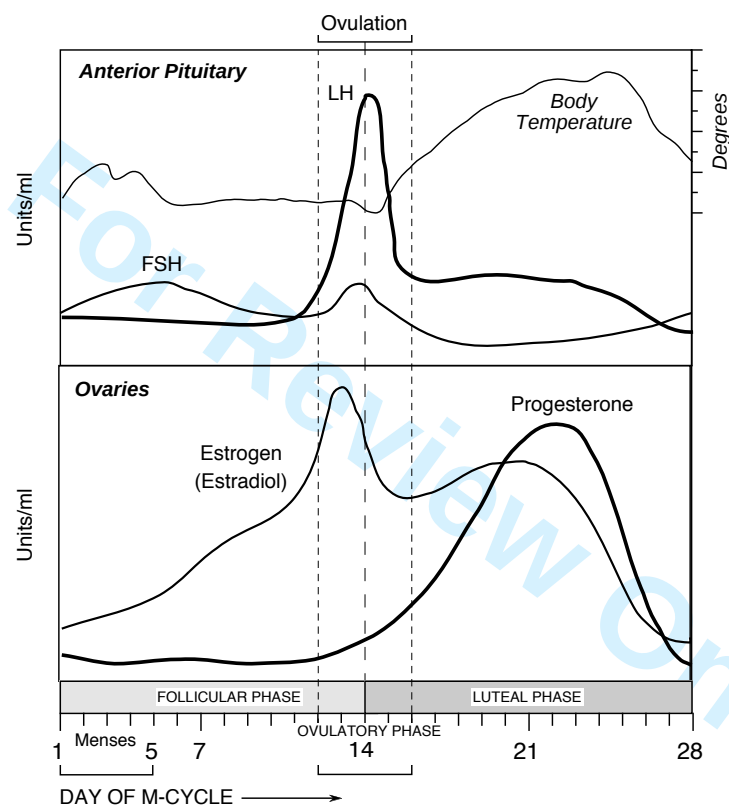
For Review Only

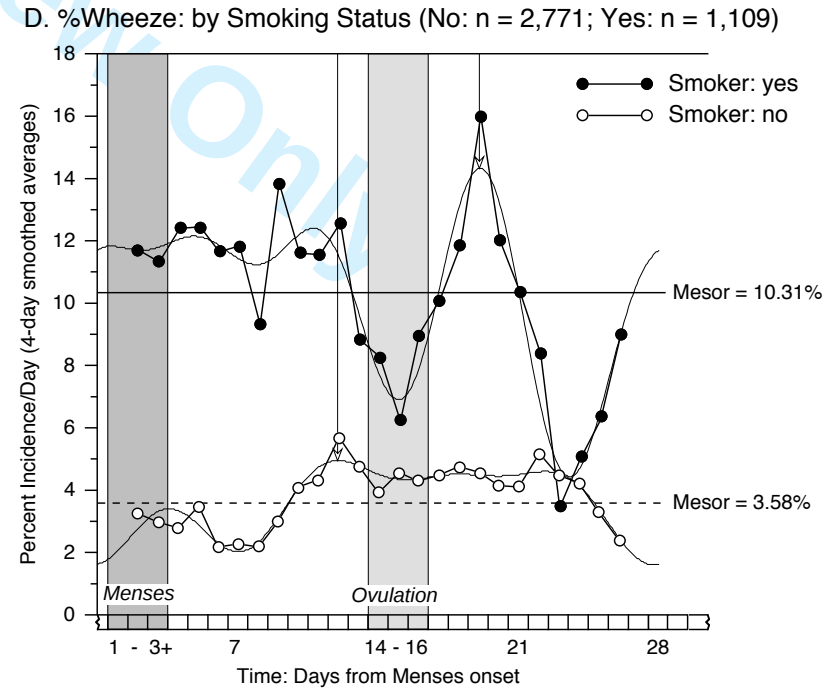
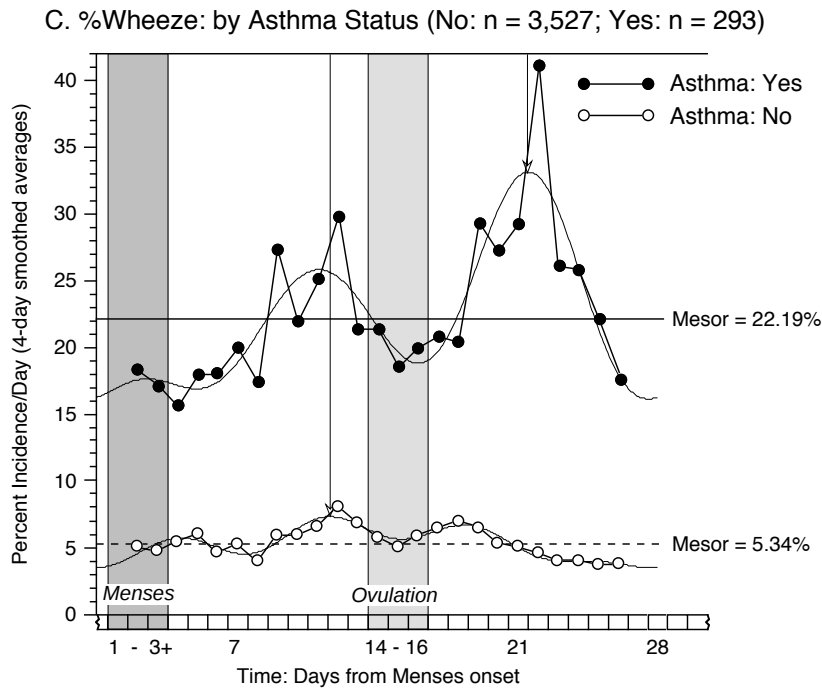
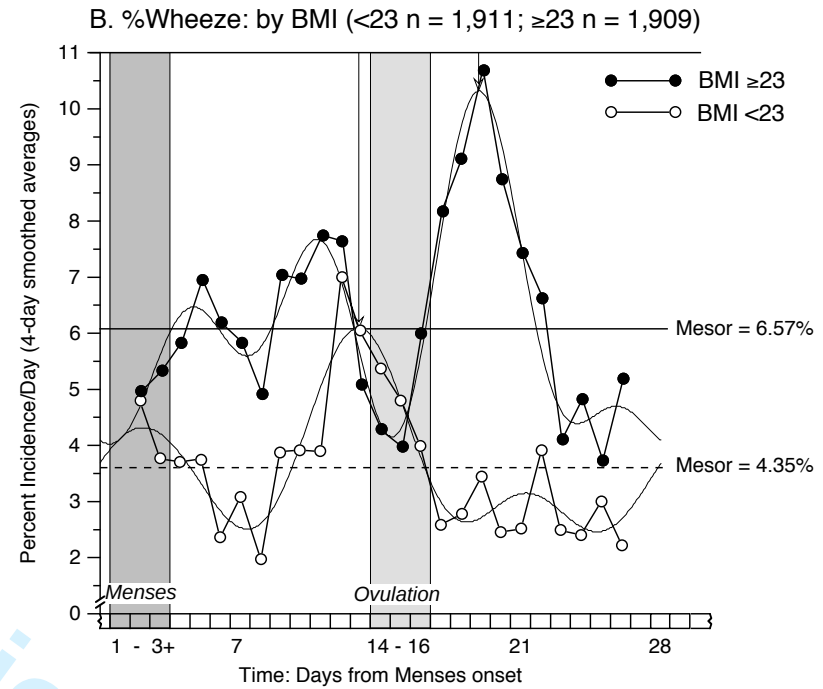
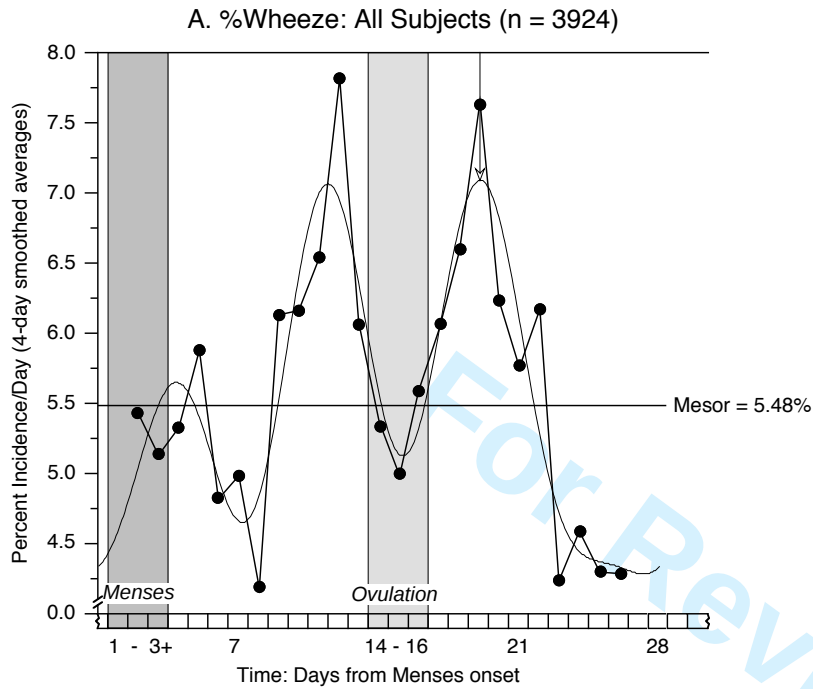
References

1. Melgert BN, Postma DS. All men are created equal?: New leads in explaining sex differences in adult asthma. *Proc Am Thorac Soc* 2009;6:724-727.
2. Macsalı F, Real FG, Plana E, Sunyer J, Anto J, Dratva J, Janson C, Jarvis D, Omenaas ER, Zemp E, Wjst M, Leynaert B, Svanes C. Early age at menarche, lung function, and adult asthma. *Am J Respir Crit Care Med* 2011;183:8-14.
3. Macsalı F, Real FG, Omenaas ER, Bjorge L, Janson C, Franklin K, Svanes C. Oral contraception, body mass index, and asthma: A cross-sectional nordic-baltic population survey. *J Allergy Clin Immunol* 2009;123:391-397.
4. Svanes C, Real FG, Gislason T, Jansson C, Jogi R, Norrman E, Nystrom L, Toren K, Omenaas E. Association of asthma and hay fever with irregular menstruation. *Thorax* 2005;60:445-450.
5. Real FG, Svanes C, Omenaas ER, Anto JM, Plana E, Janson C, Jarvis D, Zemp E, Wjst M, Leynaert B, Sunyer J. Menstrual irregularity and asthma and lung function. *J Allergy Clin Immunol* 2007;120:557-564.
6. Real FG, Svanes C, Omenaas ER, Anto JM, Plana E, Jarvis D, Janson C, Neukirch F, Zemp E, Dratva J, Wjst M, Svanes K, Leynaert B, Sunyer J. Lung function, respiratory symptoms, and the menopausal transition. *J Allergy Clin Immunol* 2008;121:72-80 e73.
7. Real FG, Svanes C, Bjornsson EH, Franklin KA, Gislason D, Gislason T, Gulsvik A, Janson C, Jogi R, Kiserud T, Norback D, Nystrom L, Toren K, Wentzel-Larsen T, Omenaas ER. Hormone replacement therapy, body mass index, and asthma in peri-menopausal women: A cross-sectional survey. *Thorax* 2005.
8. Macsalı F, Svanes C, Bjorge L, Omenaas ER, Real FG. Respiratory health in women: From menarche to menopause. *Expert review of respiratory medicine* 2012;6:187-202.
9. Farha S, Asosingh K, Laskowski D, Hammel J, Dweik RA, Wiedemann HP, Erzurum SC. Effects of the menstrual cycle on lung function variables in women with asthma. *Am J Respir Crit Care Med* 2009;180:304-310.
10. Eliasson O, Scherzer HH, DeGraff AC, Jr. Morbidity in asthma in relation to the menstrual cycle. *J Allergy Clin Immunol* 1986;77:87-94.
11. Suzuki K, Hasegawa T, Sakagami T, Koya T, Toyabe S, Akazawa K, Arakawa M, Gejyo F, Suzuki E, Niigata Asthma Treatment Study G. Analysis of perimenstrual asthma based on questionnaire surveys in japan. *Allergol Int* 2007;56:249-255.
12. Oguzulgen IK, Turktaş H, Erbas D. Airway inflammation in premenstrual asthma. *J Asthma* 2002;39:517-522.
13. Brenner BE, Holmes TM, Mazal B, Camargo CA, Jr. Relation between phase of the menstrual cycle and asthma presentations in the emergency department. *Thorax* 2005;60:806-809.
14. Zimmerman JL, Woodruff PG, Clark S, Camargo CA. Relation between phase of menstrual cycle and emergency department visits for acute asthma. *Am J Respir Crit Care Med* 2000;162:512-515.
15. Chandler MH, Schuldheisz S, Phillips BA, Muse KN. Premenstrual asthma: The effect of estrogen on symptoms, pulmonary function, and beta 2-receptors. *Pharmacotherapy* 1997;17:224-234.
16. Gibbs CJ, Coutts II, Lock R, Finnegan OC, White RJ. Premenstrual exacerbation of asthma. *Thorax* 1984;39:833-836.
17. Agarwal AK, Shah A. Menstrual-linked asthma. *J Asthma* 1997;34:539-545.
18. Hanley SP. Asthma variation with menstruation. *Br J Dis Chest* 1981;75:306-308.
19. Magadle R, Berar-Yanay N, Weiner P. Long-acting bronchodilators in premenstrual exacerbation of asthma. *Respir Med* 2001;95:740-743.
20. Pereira-Vega A, Sanchez JL, Gil FL, Maldonado JA, Bravo JM, Ignacio JM, Vazquez R, Alvarez F, Romero P, Sanchez I. Premenstrual asthma and symptoms related to premenstrual syndrome. *J Asthma*;47:835-840.

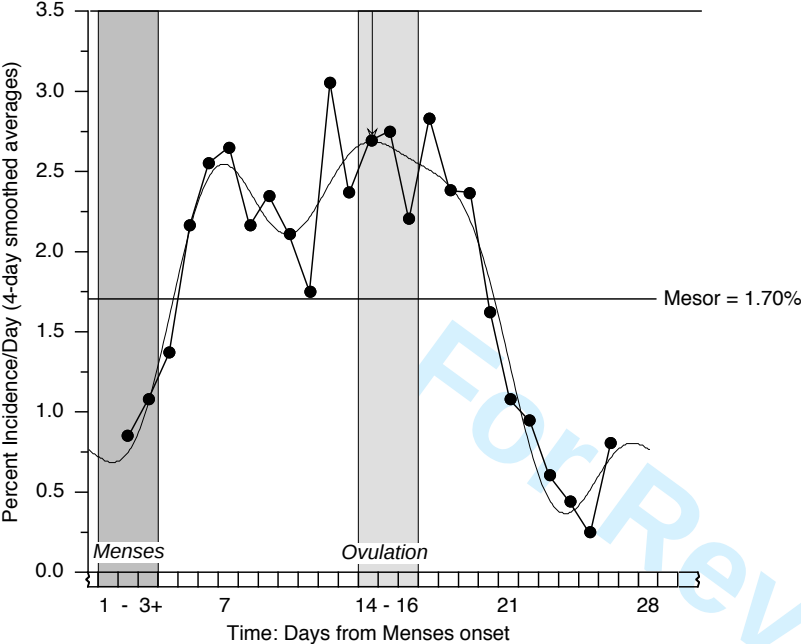
21. Dratva J, Schindler C, Curjuric I, Stolz D, Macsali F, Gomez FR, Zemp E, Team S. Perimenstrual increase in bronchial hyperreactivity in premenopausal women: Results from the population-based sapaldia 2 cohort. *J Allergy Clin Immunol* 2010;125:823-829.
22. Sansores RH, Abboud RT, Kennell C, Haynes N. The effect of menstruation on the pulmonary carbon monoxide diffusing capacity. *Am J Respir Crit Care Med* 1995;152:381-384.
23. Pereira-Vega A, Sanchez JL, Maldonado JA, Borrero F, Rico IV, Vazquez R, Alvarez F, Ignacio JM, Romero P, Gil FL. Premenstrual asthma and atopy markers. *Ann Allergy Asthma Immunol*;105:218-222.
24. Gaskins AJ, Wilchesky M, Mumford SL, Whitcomb BW, Browne RW, Wactawski-Wende J, Perkins NJ, Schisterman EF. Endogenous reproductive hormones and c-reactive protein across the menstrual cycle: The biocycle study. *Am J Epidemiol* 2012;175:423-431.
25. Faas M, Bouman A, Moesa H, Heineman MJ, de Leij L, Schuiling G. The immune response during the luteal phase of the ovarian cycle: A th2-type response? *Fertil Steril* 2000;74:1008-1013.
26. Mandhane PJ, Hanna SE, Inman MD, Duncan JM, Greene JM, Wang HY, Sears MR. Changes in exhaled nitric oxide related to estrogen and progesterone during the menstrual cycle. *Chest* 2009.
27. Yeung EH, Zhang C, Mumford SL, Ye A, Trevisan M, Chen L, Browne RW, Wactawski-Wende J, Schisterman EF. Longitudinal study of insulin resistance and sex hormones over the menstrual cycle: The biocycle study. *J Clin Endocrinol Metab*;95:5435-5442.
28. Soldin OP, Makambi KH, Soldin SJ, O'Mara DM. Steroid hormone levels associated with passive and active smoking. *Steroids* 2011;76:653-659.
29. Koukkari W, Sothorn RB. Introducing biological rhythms. Springer, New York; 2006.
30. WHO. Obesity: Preventing and managing the global epidemic. . 2000.
31. Real FG. Hormonal status in women and asthma, allergy and lung function : Results from population-based studies concerning menstrual irregularity, menopausal transition, hormonal replacement therapy and body mass index. Bergen, Norway: University of Bergen; 2008.
32. Macsali F. Menstrual cycle and respiratory symptoms in a general nordic-baltic population European Respiratory Society 2010. Barcelona; 2010.
33. Nelson W, Tong YL, Lee JK, Halberg F. Methods for cosinor-rhythmometry. *Chronobiologia* 1979;6:305-323.
34. Mojon A, Fernandez JR, Hermida RC. Chronolab: An interactive software package for chronobiologic time series analysis written for the macintosh computer. *Chronobiol Int* 1992;9:403-412.
35. Gibbs CJ, Cou t, , II, Lock R, Finnegan OC, White RJ. Premenstrual exacerbation of asthma. *Thorax* 1984;39:833-836.
36. Mirdal GM, Petersson B, Weeke B, Vibits A. Asthma and menstruation: The relationship between psychological and bronchial hyperreactivity. *Br J Med Psychol* 1998;71 (Pt 1):47-55.
37. Pereira Vega A, Sanchez Ramos JL, Maldonado Perez JA, Alvarez Gutierrez FJ, Ignacio Garcia JM, Vazquez Oliva R, Romero Palacios P, Bravo Nieto JM, Sanchez Rodriguez I, Gil Munoz F. Variability in the prevalence of premenstrual asthma. *Eur Respir J*;35:980-986.
38. Jain R, Ray JM, Pan JH, Brody SL. Sex hormone-dependent regulation of cilia beat frequency in airway epithelium. *Am J Respir Cell Mol Biol*.
39. Bayliss DA, Millhorn DE, Gallman EA, Cidlowski JA. Progesterone stimulates respiration through a central nervous system steroid receptor-mediated mechanism in cat. *Proc Natl Acad Sci U S A* 1987;84:7788-7792.
40. Bayliss DA, Millhorn DE. Central neural mechanisms of progesterone action: Application to the respiratory system. *J Appl Physiol* 1992;73:393-404.
41. Farha S, Asosingh K, Laskowski D, Licina L, Sekiguchi H, Losordo DW, Dweik RA, Wiedemann HP, Erzurum SC. Pulmonary gas transfer related to markers of angiogenesis during the menstrual cycle. *J Appl Physiol* 2007;103:1789-1795.
42. Munster K, Schmidt L, Helm P. Length and variation in the menstrual cycle--a cross-sectional study from a danish county. *Br J Obstet Gynaecol* 1992;99:422-429.

For Review Only

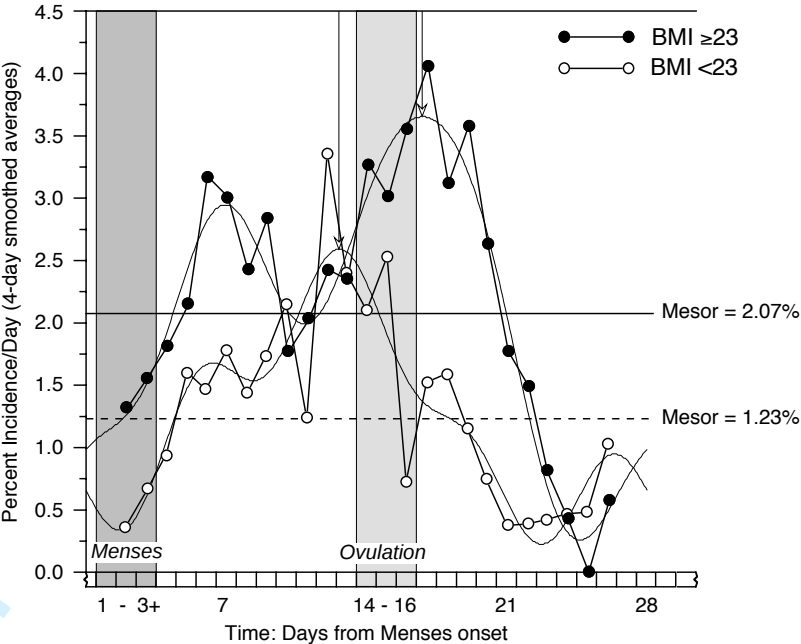




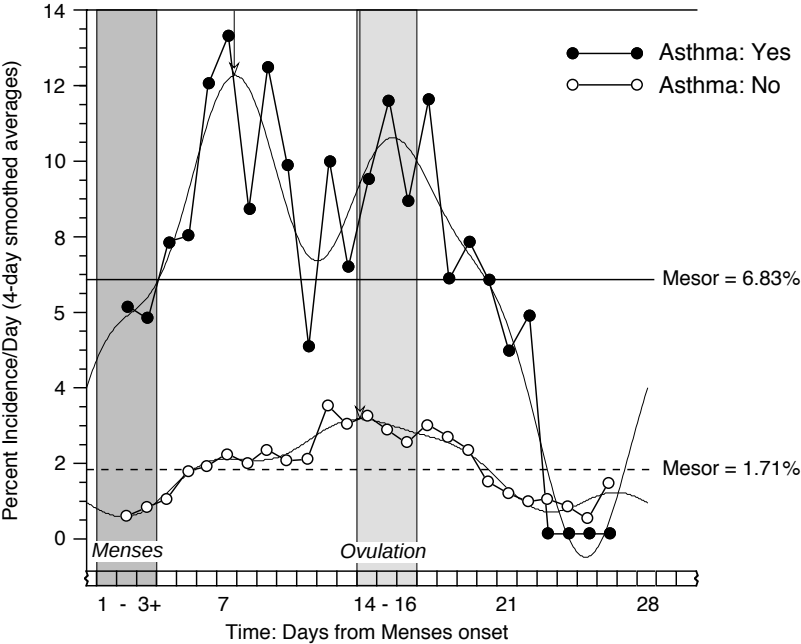
A. %Shortness of Breath: All Subjects



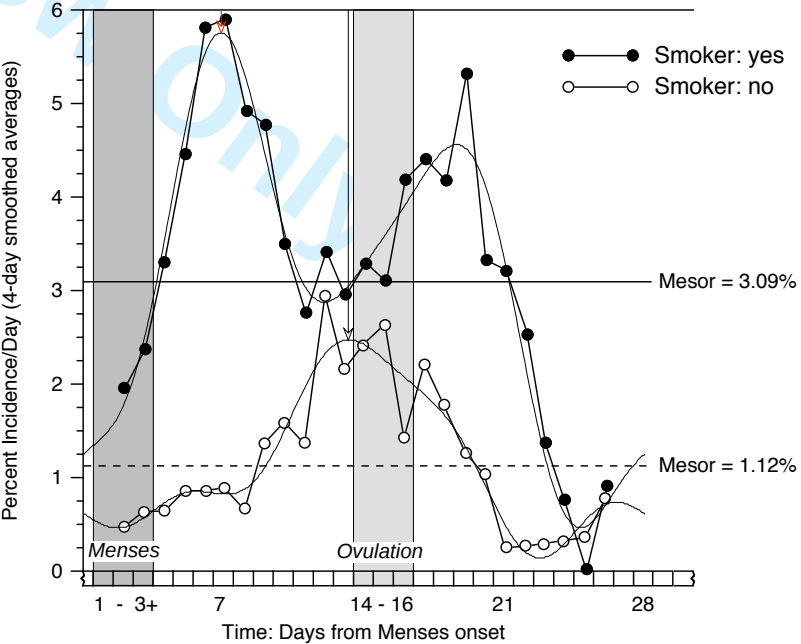
B. %Shortness of Breath: by BMI



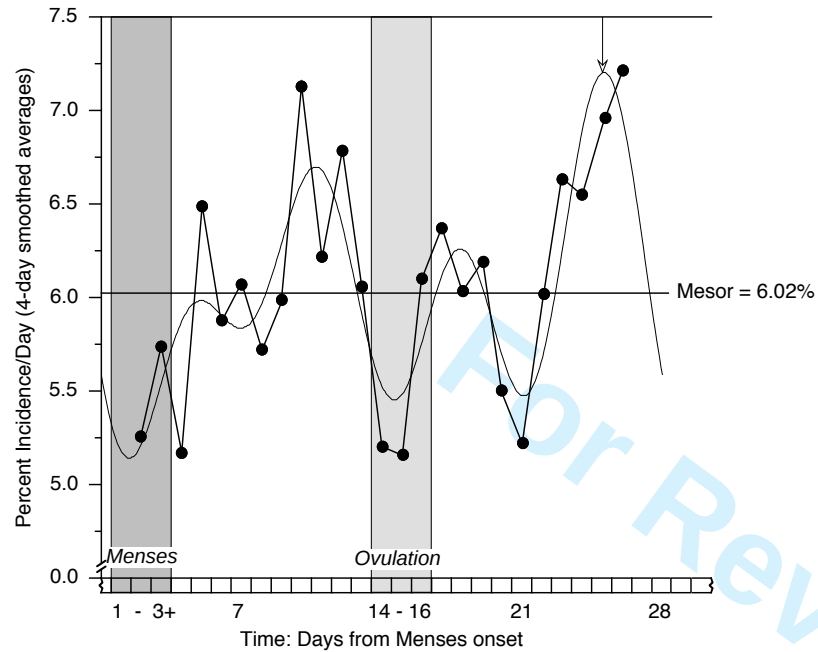
C. %Shortness of Breath: by Asthma Status



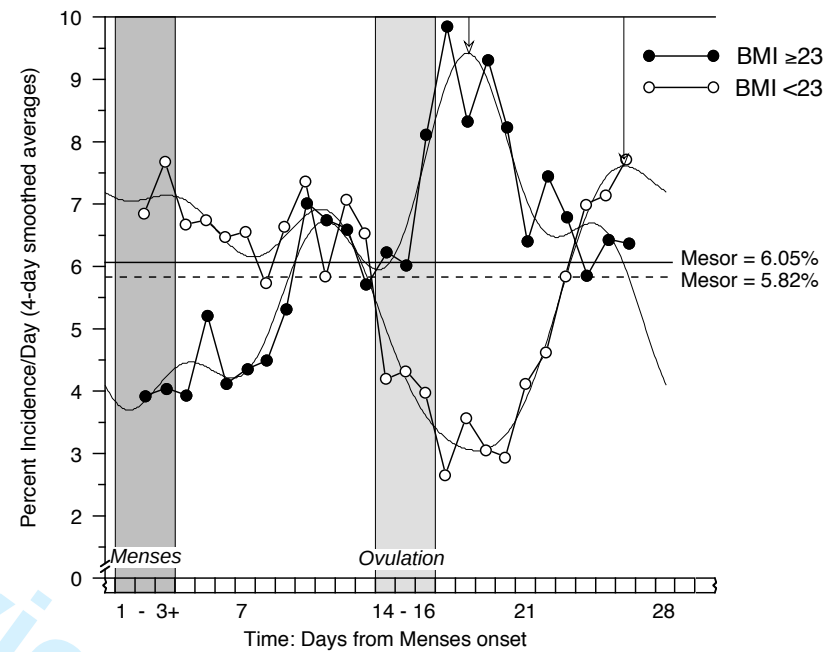
D. %Shortness of Breath: by Smoking Status



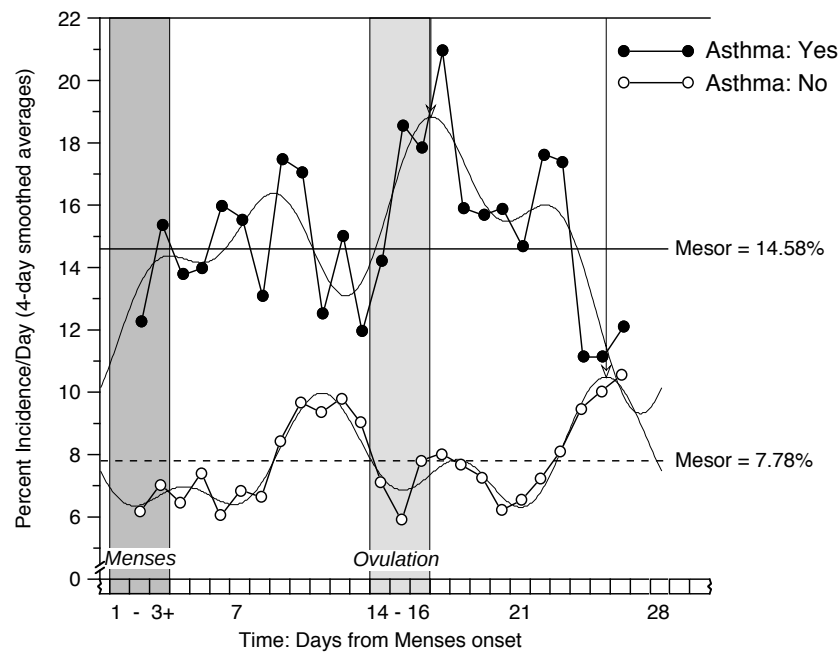
A. %Cough: All Subjects



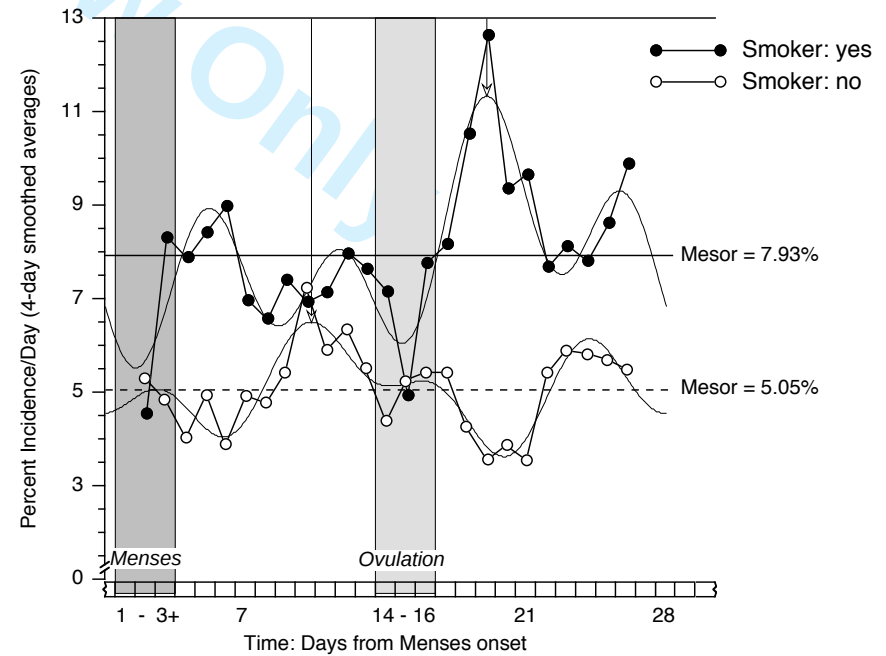
B. %Cough: by BMI



C. %Cough: by Asthma Status



D. %Cough: by Smoking Status



Respiratory Health in Northern Europe (RHINE; www.rhine.nu) is a follow-up study of subjects from seven Northern European centres who participated in the European Community Respiratory Health Survey (ECRHS I stage I, www.ecrhs.org) in 1990-1994. In ECRHS I stage I, men and women aged 20 to 44 years were randomly selected from population registers within specific boundaries of each participating centre. The population included in RHINE were responders from Reykjavik in Iceland, Bergen in Norway, Umeå, Uppsala and Gothenburg in Sweden, Aarhus in Denmark and Tartu in Estonia (n=21,802, response rate 83.7%). The eligible subjects (excluding 264 deaths) were sent a postal questionnaire at follow-up in 1999-2001. In total, 16191 subjects answered the questionnaire, including 8592 women (response rate 77%) born between 1945 and 1973. Written consent was obtained from all participants, and the local ethics committees approved the study.

The set of questions answered by the women included age, height and weight, if they were asthmatic ("Have you ever had asthma diagnosed by a doctor"), had allergies ("Do you have hay fever or nasal allergies?"), or smoked ("Do you smoke?" 'never', 'yes, but quit', 'yes, still smoke'), and three questions about respiratory symptoms during the last three days ("Have you had wheezing or whistling in your chest at any time in the last three days?", "Have you been woken by an attack of shortness of breath at any time in the last three days?", "Have you been woken by an attack of coughing, at any time, in the last three days?").

Women were also asked to record the date of the first day of their last menses onset. The day in their current menstrual cycle was calculated from this date to the date of answering the questionnaire, with day 1 = first day of current menses onset. Body mass index (BMI) was computed as each woman's weight in kilograms divided by the square of her height in meters (kg/m^2).

In order to analyze a hormonally well-defined group of women with regular natural menstrual cycles, the following groups of women were excluded: ages >55 years (n = 138), pregnant (n = 260), using HRT (n = 634), using oral contraception (n = 1,023), irregular menstruation (n = 1360), postmenopausal (n = 158), and first day of the last menstruation occurring after filling out the questionnaire (n = 101). Women with cycle lengths longer than 35 days were excluded (n=762). Results derived from up to 4,156 women were thus available for analyses.

Since metabolic status is known to interact with hormonal status, we compared women in the lower range vs. the upper range of BMI for differences by dividing the group according to a median BMI of 23 (n = 2033 for BMI <23 and 2069 for BMI $\geq$ 23). Because of the imbalance

in distribution of women with high or low BMI (range = 15-73), we did not subdivide the dataset further or by WHO categories. Perimenstrual exacerbation of respiratory symptoms has been demonstrated mostly among asthmatics, so in order to compare results we stratified all women according to asthma status. Smoking has an effect on both respiratory symptoms and menstrual cycle, and therefore analyses were also stratified for smoking status. Final subgroup totals for a category may not equal the overall total N since some women failed to answer one or more of the questions and therefore could be assigned to one category (i.e., BMI) and not another (i.e., smoking or asthma).

After sorting questionnaire data by BMI, asthma status and smoking status, each subgroup was further sorted by menstrual cycle day. The percentage of women reporting each of the three respiratory symptoms (wheeze, shortness of breath, cough) on each day of the menstrual cycle was calculated by dividing the N of positive responses by the total women reporting on each day 1-35. Due to an abrupt drop in the number of women with menstrual cycles longer than 28 days, we chose to look for menstrual cycle periodicities in respiratory symptoms using only data from women with cycle lengths of up to and including 28 days, thereby restricting the analyses to 3926 women. We also calculated 2-, 3- and 4-day moving averages in order to smooth the data, since there were noticeably large daily fluctuations between the number of women on some days, possibly due to a woman being a day or more off in remembering the onset of her last menses, as well as any uncertainty caused by the 3-day interval to report the incidence of any symptom.

Statistical analysis

Analysis of each time series (with 1-day values assigned to midday [12:00h] on each day 1-28, and 2-, 3-, and 4-day smoothed values assigned to the midpoints of their respective averaging intervals) for menstrual periodicity was accomplished by the single cosinor procedure (20) using percent incidence of each variable on days 1-28 of the menstrual cycle. The single cosinor method is a data modelling procedure involving the least-squares fitting of cosine curves with period(s) that are expected to characterize the dataset. This involved the approximation of each time series data by the least-squares linear regression fitting of a single component (28 days = 672 h) or multiple component (28 days plus 1, 2 or 3 harmonic periods) cosine waveform using the Chronolab statistical package(21). A p-value for the rejection of the zero-amplitude assumption was determined by an F-test of the variance

accounted for by the fit of the single or compound period waveform versus the variance accounted for by a straight line approximation of the arithmetic mean. Rhythm detection was considered statistically significant if $p \leq 0.05$ for each fitted period in the cosine model separately and overall and borderline significant if $p \leq 0.10 > 0.05$.

Rhythm characteristics determined from the best-fitting cosine include: the 'mesor' (M, the middle of the cosine, representing an adjusted 28-day average if unequidistant sampling); 'amplitude' (A, half the distance from the peak and trough of the best-fitting curve, with 2A indicating the predictable range of change); and 'phase' of the cosine ($\emptyset$, in days after 00:00h on day 1 of M). The peak of the fitted cosine, representing the calculated average time of high values in the data, is termed the 'acrophase (a $\emptyset$)' (*acro* = peak) for a single-component cosine (i.e., 28d) and 'orthophase (o $\emptyset$)' (*ortho* = true) for a multiple-component cosine, while the 'bathyphase (b $\emptyset$)' (= trough time) indicates lowest values of a single or multiple-component cosine waveform.

The analyses are presented as figures with numerical parameters of rhythm estimates in tables or text. The methodology did not allow for analyses of interaction, thus stratified analyses are presented for subgroups.

Fig.: Prevalence (%) of women answering the questionnaire for each day in a 28 days cycle

