Sex differences in monocyte expression of IL-6: role of autonomic mechanisms

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O'Connor M-F, Motivala SJ, Valladares EM, Olmstead R, Irwin MR. Sex differences in monocyte expression of IL-6: role of autonomic mechanisms. *Am J Physiol Regul Integr Comp Physiol* 293: R145–R151, 2007. First published April 11, 2007; doi:10.1152/ajpregu.00752.2006.—Sex differences in the prevalence of inflammatory disorders exist, perhaps due to sex differences in cellular mechanisms that contribute to proinflammatory cytokine activity. This study analyzed sex differences of monocyte intracellular expression of IL-6 and its associations with reproductive hormones and autonomic mechanisms in 14 matched pairs of men and women ($n = 28$). Monocyte intracellular IL-6 production was repeatedly assessed over two circadian periods. Sympathetic balance was estimated by heart rate variability and the ratio of power in the low-frequency (LF) to high-frequency (HF); vagal tone was indexed by the power of HF component. As compared to men, women showed greater monocyte expression of IL-6 across the circadian period. In addition, women showed lower sympathetic balance (LF/HF ratio), and greater levels of vagal tone (HF power). In women, but not men, sympathovagal balance was negatively associated with monocyte IL-6 expression, whereas vagal tone was positively associated with production of this cytokine. Levels of reproductive hormones were not related to monocyte IL-6 expression. The marked increase in monocyte expression of interleukin-6 in women has implications for understanding sex differences in risk of inflammatory disorders. Additionally, these data suggest that sex differences in sympathovagal balance or vagal tone may be a pathway to explain sex differences in IL-6 expression. Interventions that target autonomic mechanisms might constitute new strategies to constrain IL-6 production with impacts on inflammatory disease risk in women.

vagal; heart rate variability; inflammatory disorders; proinflammatory cytokines; sympathetic

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THE PREVALENCE OF MOST AUTOIMMUNE diseases is higher in women as compared to men (39). This greater inflammatory risk profile in women is thought to be due to sex differences in activity of proinflammatory cytokines such as IL-6, which is associated with a wide spectrum of inflammatory and autoimmune disorders (25). However, there is a striking paucity of information about sex differences in the expression of IL-6 in healthy middle-aged men and women. One study reported that young women have lower stimulated production of proinflammatory cytokines in response to the bacterial ligand LPS as compared to men (10), although the primary aim of this study focused on age-related differences in inflammatory cytokine production and not sex differences. Moreover, conclusions about sex differences were further constrained by the absence of relevant comparative information for a number of clinical variables that are known to influence IL-6 production, including ethnicity, physical activity, and levels of reproductive hormones. Evaluation of in vivo estradiol levels is particularly salient in relation to IL-6, given in vitro data that estrogen increases the synthesis of proinflammatory cytokines in cultured, macrophage-like cells (11).

Autonomic mechanisms have a role in the regulation of IL-6 production (16); hence, sex differences in autonomic activity might also contribute to differences in proinflammatory cytokine production. Increases in sympathetic activity along with β-adrenergic receptor activation suppress stimulated production of proinflammatory cytokines (12, 21, 38). Substantial evidence indicates that autonomic nervous system activity differs between men and women, in which men show higher levels of sympathetic balance than women (3, 19, 22, 35). In humans, a noninvasive method for assessing autonomic activity involves spectral analysis of heart rate variability; spectral power in the high-frequency (HF) band measures vagal tone, and the ratio of power in the low-frequency (LF) band to power in the high-frequency band (i.e., LF/HF ratio) indicates sympathetic balance. As compared to women, men show increases in the ratio of LF/HF (i.e., sympathetic dominance), and decreases in HF power or vagal tone (3, 19, 22, 35). To our knowledge, no study has evaluated whether sex differences in sympathovagal balance and/or vagal tone are associated with production of IL-6 in men as compared to women.

This study examined sex differences in IL-6 production by evaluating monocyte intracellular IL-6 production following ligation of the Toll-like receptor 4 (TLR4) with LPS. Monocytes, which make up about 5% of circulating leukocytes, are a major contributor to proinflammatory cytokine production in peripheral blood. TLRs mediate innate immune responses to common pathogens (14), and aberrant increases of TLR activity have been linked to inflammatory diseases such as rheumatoid arthritis (1) and Crohn's disease (1). Given evidence that proinflammatory cytokines show a marked circadian variation (8, 32, 37), differences between men and women were examined over two 24-h periods. Finally, we evaluated two potential mechanisms for sex differences in monocyte expression of IL-6 by measuring in vivo levels of reproductive hormones and by assessing variations in sympathetic and parasympathetic nervous system activity, as estimated by heart rate variability. As compared to men, women were hypothesized to have higher levels of stimulated production of IL-6, and lower levels of

sympathetic balance, as assessed by heart rate variability. Increases of sympathovagal balance were hypothesized to be associated with decreases in the stimulated production of IL-6.

METHODS

Subjects. The subjects included 28 healthy volunteers (14 men and 14 women) between the ages of 25 and 59 years ($36.2 + 8.7$ yr, means $\pm$ SD), who gave signed, informed consent to participate in the research protocol before undergoing screening tests. The protocol was approved by the Institutional Review Board of University of California at Los Angeles (UCLA). Inclusion in the study required that subjects be healthy by medical screening interview and physical examination; none had a history of an inflammatory disorder, cancer, or chronic or active infections. None of the subjects were obese; all had a body mass index <30 kg/m^2 ($24.6 + 4.8$ kg/m^2, means $\pm$ SD). Inclusion further required complete blood cell counts and chemistry tests to be within normal limits and for serology status of HIV and hepatitis C to be negative. A structured clinical interview for DSM-IV diagnoses determined that all participants fulfilled criteria for "never mentally ill." None of the subjects smoked. Physical activity was measured by self-report and converted into calories per day (6), then divided by current weight to create metabolic equivalents. Sleep diaries were completed for two or more weeks before study entry and showed a stable sleep-wake pattern defined by a lights-out time between 2200 and 2330 and awakening between 0600 and 0730.

Protocol. Subjects spent 2 days (24-h periods) in the National Institutes of Health (NIH) General Clinical Research Center (GCRC) at the UCLA David Geffen School of Medicine. After adaptation to the GCRC and the sleep laboratory with screening for sleep apnea and nocturnal myoclonus, subjects underwent 2 days of baseline testing. To be reported separately, subjects were instrumented for polysomonography recordings during each of the nights in the laboratory. During testing, uninterrupted sleep occurred between 2300 and 0700.

For a two-day period, blood samples were obtained at 0800, 1200, 1600, 2000, 2300, 0200 and 0500 during each day (*baseline 1*; *baseline 2*) via an indwelling venous forearm catheter for assessment of intracellular proinflammatory cytokine expression in monocyte populations. Hence, a total of 14 samples was obtained for each subject.

Heart rate variability assessment. The participant's ECG signal was sampled at a frequency rate of 200 Hz, and an interpolation algorithm was used to optimize temporal accuracy of R wave peak detection (5). The signal was then converted into an RR interval signal and spectral analyzed using a 12-point autoregressive algorithm (Somnologica, Flaga hf, Medical Devices, Reykjavik, Iceland) in accordance with recommended guidelines to generate estimates of HF and LF power (Electrophysiology Task 17). For all scoring of heart rate variability data, the rater(s) were blind to sex of the participant.

Two spectral variability measures were examined: LF power defined as the total spectral power in the 0.04 to 0.15 Hz frequency band and HF power defined as the total spectral power in 0.15 to 0.4 Hz frequency band. The HF band in normalized units (HF nu) is a measure of vagal tone. The LF/HF ratio was calculated by dividing the LF power component by the HF power component and is a measure of sympathovagal balance.

Heart rate variability was measured under two conditions: *1*) during an awake period, prior to sleep, and *2*) during sleep, across the entire nocturnal period. For the assessment of heart rate variability during an awake period, ECG recording was obtained, while participants were supine in bed with eyes closed for 10 min of recording beginning at 2230. For assessment of heart rate variability during the night, ECG recordings were obtained along with all-night polysomnography, from 2300 to 0700. The ECG signal was analyzed using 5-min segments across the entire nocturnal period; each segment used was free of ECG artifacts.

Intracellular monocyte IL-6 assay. Monocyte intracellular production of IL-6 in response to LPS stimulation of whole blood was assessed by flow cytometry using peridinin chlorophyll protein (PerCP)-labeled CD14 mAb and phycoerythrin (PE)-labeled anti-IL-6 Ab, as previously described (13, 29). In brief, heparin-treated blood (1 ml) was mixed with 100 pg/ml of LPS (Sigma, St. Louis, MO) plus 10 μg/ml brefeldin A (Sigma, St. Louis, MO) and incubated for 4 h at 37°C in a platform mixer followed by an overnight incubation at 4°C. Red blood cells were then lysed in FACS lysing solution (BD Biosciences, San Jose, CA), the remaining cells were permeabilized in FACS permeabilizing buffer (BD Biosciences, San Jose, CA), and fluorescence-conjugated antibodies were added for 30 min at room temperature in the dark. Cells were then washed and resuspended in 1% paraformaldehyde for flow cytometry. Three-color flow cytometric analysis was performed on a Coulter Elite flow cytometer using the Coulter Elite software. Forward and side scatter were used to gate on the target population (on the population consisting of monocytes and granulocytes). For the monocyte population, the percentage of cytokine-secreting (PE positive) cells among CD14-PerCP-positive population was determined by counting about 12,000 CD14+ events. Unstimulated sample analysis was used to determine quadrant coordinates for stimulated sample analysis. Net stimulated cytokine-positive events were obtained by subtracting unstimulated percentages from stimulated percentages within constant numbers of monocytes. Results for IL-6 cytokine-positive monocytes were expressed as percentages of the total CD14+HLA-DR+ cells.

Hormonal measures. Reproductive hormones were compared between men and women without restricting assessments to one point in the menstrual cycle in women. Hormonal evaluations of estradiol, dehydroepiandrosterone (DHEA), and progesterone were performed on plasma samples taken at 1200, 2000, 2300, and 0500. Coated-tube radioimmunoassay kits were utilized. The specific kits used were DSL-4300 ACTIVE estradiol coated-tube radioimmunoassay kit for estradiol, DSL-5000 ACTIVE 17 alphaOH progesterone coated-tube radioimmunoassay kit for 17 alphaOH progesterone and DSL-8900 DHEA radioimmunoassay kit for DHEA.

Statistical analyses. All data were entered and analyzed in SPSS (SPSS 13.0 for Windows, Chicago: SPSS). The distributions of monocyte expression of IL-6, LF/HF ratio, HF nu, and reproductive hormone variables all demonstrated normality. Differences between men and women on continuous variables, including demographics, IL-6 expression, heart rate variability measures, and reproductive hormones were tested by ANOVA. Differences on categorical variables were evaluated with Pearson χ^2-tests. Mixed models were used to test sex differences in repeated measures of IL-6 across the circadian periods, and repeated-measures ANOVA was used to test sex differences in reproductive hormones across these periods. Additionally, secondary analyses examined the role of reproductive hormones in accounting for possible sex differences; separate regression analyses were done by examining IL-6 expression, HF, and LF/HF ratio as outcome variables and using sex and reproductive hormone levels as predictors. A background demographic variable was included in these analyses, if there was a trend or a significant difference in the background variable between men and women and the background variable was related to monocyte production of IL-6 or heart rate variability measures. Regression analyses were also used to test the associations between heart rate variability measures and monocyte expression of IL-6. Separate regression analyses were done examining IL-6 expression as an outcome variable and using heart rate variability measures, sex, and reproductive hormones as predictors. An interaction term (sex by HF, or sex by LF/HF ratio) was included in these analyses to determine whether the relationships between sympathovagal balance and IL-6 were different in men and women.

RESULTS

Subject characteristics. This study was designed to match men and women as closely as possible to reduce confounding factors in testing the specified hypotheses. Men and women were similar in age, education, body mass index (BMI), metabolic equivalents (Table 1), and ethnicity ($x^2 = 0.62$, df $= 1$, $P < 0.43$). Because there was a trend for a sex difference in metabolic equivalents, it was tested in all subsequent regression analyses below and was not a significant covariate in any of the models. The two groups differed in estradiol, with women showing higher levels (Table 1).

Monocyte-stimulated IL-6 expression. Stimulated monocyte expression of IL-6 was profiled across two circadian periods in men and women. Using 14 independent samples of IL-6 expression, there was a main effect of sex [$F(1,13) = 4.30$, $P < 0.001$], in which women showed higher levels as compared to men (Fig. 1). In addition, both groups showed a nocturnal increase of IL-6. There was also a main effect of time [$F(1,13) = 43.26$, $P < 0.001$], but no sex by time interaction [$F(1,13) = 0.65$, $P \leq 0.81$], indicating both men and women show a similar nocturnal increase in IL-6 production, and the circadian pattern did not differ between men and women. When the 14 measures of IL-6 were aggregated, women showed greater IL-6 production as compared to men, supporting our first hypothesis [12.3 ± 6.1 vs. 6.9 ± 4.4; $F(1,26) = 7.16$, $P < 0.01$].

Heart rate variability. Consistent with our prior findings (36), spectral analyses of heart rate variability showed significant sex differences for the ratio of LF/HF and for normalized HF power. For the ratio of LF/HF during a wake period prior to sleep, women showed lower levels as compared to men [$F(1, 26) = 7.8$, $P \leq 0.01$; Fig. 2A], which indicates a shift in sympathovagal balance toward less sympathetic dominance in women. Results using heart rate variability averaged across the entire nocturnal period from 2300 to 0700 yielded similar results, with women showing lower LF/HF ratio as compared to men [$F(1,26) = 9.4$, $P < 0.01$; Fig. 2A].

For normalized HF power during a wake period prior to sleep, women showed higher levels as compared to men [$F(1,26) = 10.4$, $P < 0.01$; Fig. 2B], which indicates higher vagal tone in women. As above, results using heart rate variability averaged across the entire nocturnal period from 2300 to 0700 yielded similar results, with women showing high levels of normalized HF as compared to men [$F(1,26) = 9.8$, $P < 0.01$].

Secondary analyses of sex differences controlling for reproductive hormones. Given group differences in circulating levels of estradiol and evidence that this hormone can influence cellular production of IL-6 (28), both estradiol and progester-
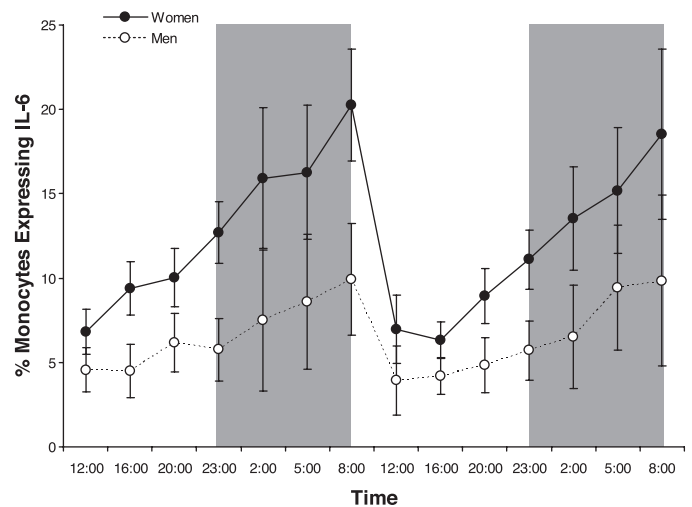

Fig. 1. Percentage of stimulated monocytes expressing IL-6 at each time point across two circadian periods for men (○) and women (●). Shaded area reflects the nocturnal period.

one were examined for any contributing variance. These reproductive hormones were profiled across two circadian periods in men and women. Using eight independent samples of estradiol per subject, there was a main effect of sex [$F(2,18) = 5.88$, $P < 0.03$], but no effect of time [$F(2,18) = 0.15$, $P = 0.86$], and no sex by time interaction [$F(2,18) = 0.26$, $P = 0.77$]. Because the circadian pattern did not differ between men and women, data were averaged across the 8 time points into an aggregate estradiol variable (Table 1). In a similar manner, using eight independent samples of progesterone per subject, there was no main effect of sex [$F(2,17) = 0.30$, $P = 0.59$], no main effect of time [$F(2,17) = 1.68$, $P = 0.20$], and no sex by time interaction [$F(2,17) = 0.38$, $P = 0.69$], and these data points were therefore also averaged into an aggregate progesterone variable (Table 1).

Analyses were then performed using a regression model to evaluate whether estradiol and/or progesterone contributed to sex differences in monocyte expression of IL-6. The model included the following predictor variables: sex (i.e., men vs. women) and aggregate levels of estradiol and progesterone across two circadian periods, given that the profiles of reproductive hormones were similar across men and women. There was a trend for a main sex effect (b coefficient $= 4.24$, $P = 0.10$), and neither progesterone (b coefficient $= 0.35$, $P = 0.32$) nor estradiol (b coefficient $= 0.07$, $P = 0.75$) was related to the monocyte expression of IL-6.

To examine the contribution of reproductive hormones on sex differences in sympathovagal balance (i.e., ratio of LF/HF

Table 1. *Demographic characteristics and reproductive hormone levels in male and female participants*

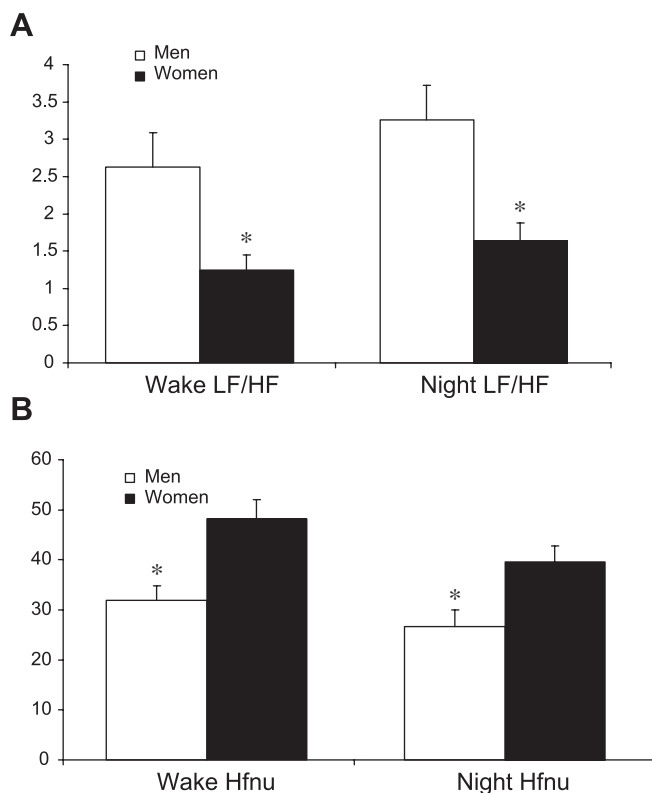|	Male (n = 14)		Female (n = 14)			
	Mean	SD	Mean	SD	F Value	P Value
Age	35.4	9.0	37.1	8.7	0.26	0.61
Education, yr	15.43	1.7	16.07	1.4	1.17	0.29
Body mass index	24.26	3.5	24.9	6.0	0.10	0.75
Metabolic equivalents	18.73	4.9	16.3	1.8	3.12	0.09
Progesterone	0.63	0.3	2.7	4.4	3.0	0.10
Estradiol	30.9	5.6	89.8	66.67	10.8	0.01

A

B

Fig. 2. Comparison of heart rate variability during a single waking recording and across the night, in male (□; $n = 14$) and female participants (■; $n = 14$). *A*: low frequency/high frequency (LF/HF) ratio. *B*: high-frequency power in normalized units. Data are depicted as means ± SE. *Significant differences at $P < 0.01$.

wake prior to sleep), a similar regression model was used. There was a significant sex effect (b coefficient = −1.3, $P = 0.04$) and neither progesterone (b coefficient = −0.05, $P = 0.55$) nor estradiol (b coefficient = 0.001, $P = .87$) was significant. Similar analyses were performed to evaluate the contribution of reproductive hormones on sex differences in vagal tone (i.e., normalized HF wake period prior to sleep). There was a significant sex effect (b coefficient = 15.17, $P < 0.02$); neither progesterone (b coefficient = 1.02, $P = .24$) nor estradiol (b coefficient = −0.02, $P = .76$) was related to normalized HF.

Relationship between sex and autonomic variables on stimulated production of IL-6. Further analyses were conducted to examine the independent contributions of sex and sympathovagal balance on monocyte expression of IL-6. Additionally, these analyses tested whether measures of sympathovagal balance had similar effects on monocyte expression of IL-6 in men and women. A regression model was used with the following predictor variables: sex, waking LF/HF ratio, interaction of sex by LF/HF ratio, estradiol, and progesterone. The overall model was significant [$F(5, 22) = 3.17$, $P < 0.03$, $R^2 = 0.42$], with a significant effect of sex (b coefficient = 11.49, $P < 0.01$), a significant effect of waking LF/HF ratio (b coefficient = 5.39, $P < 0.05$), and a significant interaction of sex by LF/HF ratio interaction (b coefficient = −4.48, $P < 0.05$). Neither estradiol (b coefficient = 0.001, $P = 0.96$) nor progesterone (b coefficient = 0.14, $P = 0.35$) was related to the monocyte expression of IL-6. Figure 3*A* illustrates the

interaction between sex and LF/HF ratio on stimulated monocyte expression of IL-6, in which a negative relationship between waking LF/HF ratio and IL-6 production was found in women, but not in men.

Further analyses examined the relationships between sex and vagal tone as measured by normalized HF power on monocyte expression of IL-6, with the regression model, including the following predictor variables: sex, waking-normalized HF, interaction of sex by normalized HF, estradiol, and progesterone. The overall model was significant [$F(5, 22) = 3.22$, $P < 0.03$, $R^2 = 0.42$], with a significant effect of the interaction of sex by normalized HF (b coefficient = 0.33, $P < 0.05$), but neither sex (b coefficient = −9.40, $P = 0.16$) nor waking normalized HF (b coefficient = −0.46, $P = 0.07$) were significant. Neither progesterone (b coefficient = 0.11, $P = 0.50$), nor estradiol (b coefficient = 0.005, $P = 0.83$) was related to the monocyte expression of IL-6. The significant
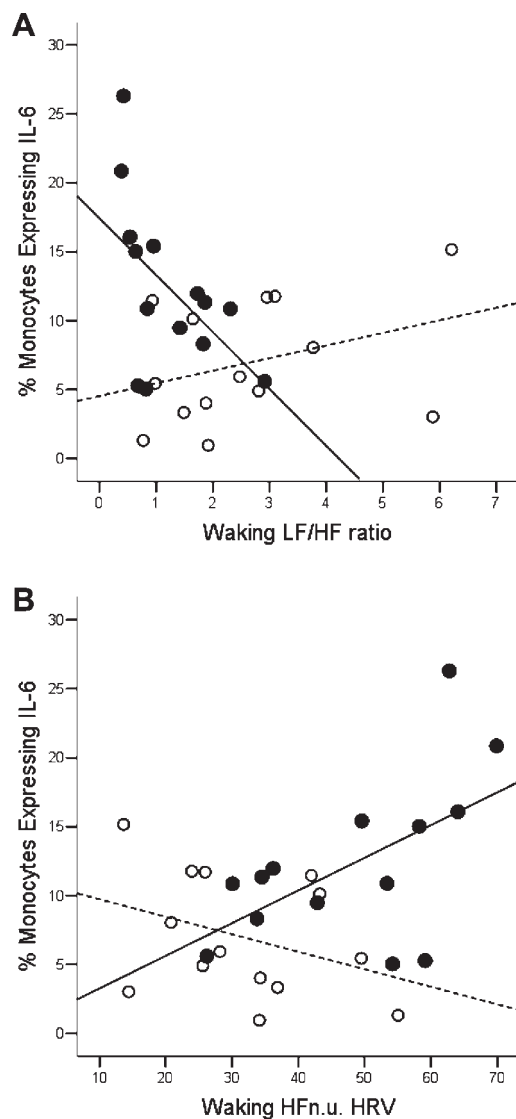
A

B

Fig. 3. *A*: scatterplot of correlations between waking LF/HF ratio and percent monocytes producing IL-6 grouped by men (○) and women (●) ($r = −0.53$, $P < 0.05$). *B*: scatterplot of correlations between waking HF normalized units and percent monocytes producing IL-6 grouped by men and women ($r = 0.35$, $P = 0.22$).

interaction between sex and normalized HF on IL-6 production indicates a differential relationship between vagal tone and monocyte expression of IL-6 in men and women. As illustrated in Fig. 3B, there was a positive relationship between vagal tone as measured by normalized HF and stimulated production of IL-6 in women, but no relationship was found in men. Similar results were obtained when the averaged nocturnal measures of heart rate variability (LF/HF ratio, HF) were used in the regression models.

DISCUSSION

The major findings of the present study demonstrate sex differences in monocyte expression of IL-6, as well as in measures of sympathovagal balance. As compared to men, women showed increases in LPS-stimulated monocyte intracellular production of IL-6 across the circadian period. In addition, as compared to men, women show decreases in sympathetic balance as assessed by the ratio of LF/HF power, along with increases in vagal tone as measured by HF power. Moreover, there are sex differences in the association of autonomic activity with monocyte production of IL-6. In women, sympathovagal balance was inversely related to IL-6 expression, whereas vagal tone was positively correlated with IL-6 production. In men, no relationships were found between autonomic activity and expression IL-6 expression. Interestingly, the interactions between sex and autonomic measures on IL-6 expression were independent of reproductive hormone levels.

The nocturnal rise of IL-6 production replicates and extends previous research that has demonstrated a circadian pattern in circulating levels of IL-6 levels in men (15, 20, 31), and in soluble IL-6 receptor (sIL-6R) in men (15). The immune system shows a dynamic variation over the course of a normal sleep-wake cycle, and these variations are driven by a combination of circadian and sleep-related factors. Exactly what these changes reflect is subject to debate. It is tempting to speculate that sleep promotes "restorative" functioning, including greater immunological activity with implication for immune competence, although these clinical implications remain an area of active inquiry.

Women showed enhanced cellular responsiveness to stimulation of the TLR4/CD14 signaling pathway ex vivo, as assessed by responses to LPS. Altered monocyte intracellular proinflammatory cytokine production, as measured by increases of TLR activity, has been linked to inflammatory diseases, such as rheumatoid arthritis (1) and Crohn's disease (1). Together, these data suggest that alterations in cytokine expression and TLR4/CD14 signaling between men and women might be a mechanism to explain sex differences in inflammatory disorders (39).

Sex differences in monocyte intracellular production of IL-6 were independent of physiological fluctuations of reproductive hormone levels. In contrast, pharmacological studies implicate sex steroids in altering IL-6 expression (27). For example, hormone replacement therapy in elderly women has been found to increase stimulated production of IL-6 in mixed peripheral blood mononuclear cell populations and to ameliorate age-related differences in this cytokine (11, 26, 27). One in vitro study found that estrogen increases the synthesis of proinflammatory cytokines in cultured, macrophage-like cells (28, 30), although other studies report that estrogen inhibits IL-6 gene expression (18, 23). Nevertheless in an animal model of autoimmune thyroiditis, for example, disease onset and course can be modulated by castration or administration of sex steroids (21).

This study found that increased sympathetic balance and/or vagal withdrawal were associated with lower monocyte IL-6 production in women. Substantial evidence in animals has found that sympathetic effector mechanisms suppress proinflammatory cytokine expression (12, 21, 38). Together, with these studies, the findings from the current study linking sympathovagal balance and IL-6 production in women suggest that the autonomic activity plays a role in modulating inflammatory cytokine expression. There is emerging evidence that vagal and sympathetic activity is important in autoimmune conditions; for example, insensitivity to β-adrenergic modulation might be involved in overshooting inflammation in multiple sclerosis and rheumatoid arthritis (34a). Taken together with the findings from the current study, it would suggest that the autonomic nervous system provides potential opportunities for the control of inflammatory cytokine expression which are more prevalent in women (for a review, see 16).

The functional and molecular basis for sex differences in monocyte IL-6 production is not yet known, although previous studies have suggested that alterations in cell surface TLR4 receptor levels may contribute to age-related differences in cytokine response to LPS (34). However, other studies have shown age-related alterations in LPS response that do not involve changes in TLR4 receptor levels, suggesting that signaling processes lying downstream of the receptor may also be altered (7). Effects of age on TLR4 signaling are not likely to explain the effects observed here because men and women were not different in age. It is also unclear whether the associations between autonomic activity and IL-6 production observed here have specific effects on the activation of TLR4 by LPS or whether other pathogen motif recognition pathways might also be altered (e.g., viral activation of TLR3 and TLR2). Direct analysis of TLR3 expression and signaling, and the distribution and activity of other TLRs, will provide important avenues for clarifying the basis for altered inflammatory signaling in women as compared to men and the extent to which autonomic activity exerts is effects. Such analyses will also help define specific molecular targets for interventions that ameliorate sex differences in risk for inflammatory disorders.

This study provides the first evidence that autonomic mechanisms have a differential influence on IL-6 production in women as compared to men. In prior studies, measures of heart rate variability were examined in relationship to proinflammatory cytokines in one sex (4, 24) or in a mixed sample of men and women, without consideration of men and women separately (9). Moreover, assessment of heart rate variability was primarily restricted to time domain methods, as opposed to spectral analyses performed in the present study. Findings in the present study underscore the importance of examining sex as a moderator of relationships between the autonomic nervous system and inflammatory cytokines.

There are several limitations to this study. While men and women were matched for many characteristics, such as age, ethnicity and BMI, the sample size is small, which precludes testing of multiple predictors in regression analyses. In addi-

tion, this study focused on healthy volunteers, and it is not known whether sex-related differences in IL-6 production, autonomic activity, and their relationships generalize to persons with an autoimmune disorder. The supine position of participants may be considered a limitation, because changes in activity levels may affect relative numbers of monocytes in the circulation. However, this study examined intracellular production of IL-6, not production from whole blood mononuclear cells. Intracellular production measurement controls for the number of monocytes.

Given the cross-sectional design of the present study, the causal relationship between IL-6 production and autonomic variables cannot be assessed. This prevents a clear conclusion as to whether vagal tone and sympathovagal balance contribute to IL-6 production or whether IL-6 production increases vagal tone and decreases sympathovagal balance. One study in rats demonstrates that intracerebroventricular injection of IL-1 (another proinflammatory cytokine) increases catecholamine output (33). Hence, we also regressed IL-6 production on measures of vagal tone and sympathovagal balance. Similar to the findings reported in the results of the present study, there was a negative association between IL-6 production and sympathovagal balance and a positive association between IL-6 production and vagal tone, in women but not men. Again, levels of reproductive hormones were not significant (data not shown).

Despite these limitations, these data provide novel evidence of sex differences in the cellular expression of IL-6, which is related to sympathetic and/or vagal activity. These data should motivate further investigations to define the differential effects of autonomic mechanisms on inflammatory cytokine production, with implications for autoimmune disorders in humans.

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