Chronic alteration of circadian rhythm is related to impaired lung function and immune response

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Abstract

Background: Alterations of the circadian rhythm negatively impact several aspects of the health, including the lung function. Chronic shiftwork scale classically induces alterations in the circadian rhythm. However, its effects on pulmonary immune response are unknown. Aims: To evaluate the impact of chronic alteration of circadian rhythm on pulmonary function and immune response. Methods: In this context, a 12h x 24h and 12h x 48h work scale in shiftwork scale policemen (n = 25; 38,73±6,92 years old) were compared with fixed work scale (8h/day) civil men (n = 25; 34,00±9,60 years old) who were evaluated for perceived stress, sleepiness, physical activity levels, anthropometric characteristics, sleepiness levels, lung function, pulmonary and systemic cellular and humoral immune response. Results: Policemen presented increased levels of perceived stress ($p<0.0008$), impaired sleepiness ($p<0.04$) and lung function as demonstrated by reduced FVC ($p<0.053$) and FEV1 ($p<0.043$) when compared to civil men. In addition, increased levels of exhaled nitric oxide ($p<0.037$) and of IL-2 ($p<0.0046$) in the breath condensate revealed that policemen presented chronic lung inflammation compared to civil men. Although the whole blood analysis did not showed any differences between the two groups concerning the number of leukocytes, the humoral response revealed that policemen presented increased levels of IL-2 ($p<0.002$) and lower levels of IL-10 ($p<0.001$), clearly displaying a clinical status of low grade inflammation. Conclusions: Chronic alteration of circadian rhythm in shiftwork scale policemen results in impaired lung function, beyond to impair pulmonary and systemic immune function.

What is already known about this topic?

Alterations of the circadian rhythm negatively impact not only the lung function but also lung mechanics.

Lung mechanics alterations more precisely represent structural alterations in the lungs.

Alterations of the circadian rhythm impairs systemic immune response.

Increased levels of exhaled nitric oxide indicate pulmonary inflammation.

What does this article add?

Alterations of the circadian rhythm negatively impact not only the lung function but also lung mechanics.

Alterations of the circadian rhythm impair humoral immune response, which can predispose to chronic diseases.

Alterations of the circadian rhythm impairs not only systemic immune response but also the pulmonary immune response.

Increased levels of exhaled nitric oxide may serve as a biomarker of lung function and mechanics impairment induced by alterations of the circadian rhythm.

Introduction

The circadian rhythm is characterized by biological oscillations occurring into a period of 24 hours, being responsible for a normal functioning of all organic systems, which may be influenced by variations in the light, temperature, sleeping timing and quality and different types of stress[1] Shiftwork scale is characterized by working time outside the standard hours of 7:00 am to 6:00 pm and it is believed to negatively affects circadian rhythm, resulting in impairment of different organs and physiological systems[2].

In this way, is widely accepted that alterations in the circadian rhythm can negatively affects the immune system[3,4] endocrine and metabolic response[5], cardiovascular response[5] and also the pulmonary response[6]. Specifically, on the immune-regulated pulmonary response, it has been demonstrated that circadian rhythm drives bronchoconstriction/bronchodilatation, airway resistance, respiratory symptoms, which in summary may leads to exacerbation of respiratory diseases, particularly to chronic respiratory diseases, such as asthma[4] and chronic obstructive pulmonary disease (COPD)[7]. However, concerning the immune-regulated pulmonary response, the effects of altered circadian rhythm on pulmonary cytokines, has been never investigated.

In fact, the alterations of circadian rhythm may lead to dysfunction of pulmonary but certainly of the systemic immune response[3,4,6]. In this context, it has been demonstrated that human monocytes obtained from individuals during the night, specifically during the sleep timing, release increased amounts of IL-12 (a pro-inflammatory cytokine) in response to endotoxin stimulation, while monocytes obtained during the day, responded to increased release of IL-10 (a immunoregulatory and anti-inflammatory cytokine)[8]. In the same way, establishing a causal nexus, some studies have demonstrated that the frequency and severity of exacerbations of COPD and asthma are increased specially during the night and in the early hours of the morning, which are hallmarks of the interference of circadian rhythm[4,7]. However, concerning the key role of humoral response, measured by analysis of cytokines, at the moment, no study has evaluated the possible alterations in the cytokine's levels in the lungs in response to chronic stress.

Thus, the present study investigated for the first time the effects of chronic alteration of circadian rhythm in shiftwork scale policemen compared to civil men working in a diurnal constant scale, on the pulmonary immune response and function, as well as on the cellular and humoral systemic immune response.

Methods

Ethical Approval and Study design

All proceedings performed in this study have been approved by local ethical committee from *Universidade Brasil* (registration number 3.020.830).

Fifty volunteers (25 sedentary civil individuals working into a normal diurnal fixed working day; 8 hours per day; [?]5 years; 34,00±9,60 years old) and (25 sedentary military policemen working in a shiftwork scale 12 x 24 hours and 12 x 48 hours; [?]5 years; 38,73+-6,92 years old) were recruited and took part in the study, after agreement and signature of the term of consent. To be included into the study, the policemen must have at least [?]5 years working as policemen in the presented working scale, while the civil men should not have worked at least for 5 years into a working scale that not in a fixed time (diurnal and for maximal 8 hours per day and between 36 to 44 hours per week).

All volunteers were classified as non-obese, civil (BMI = 26,30+-3,85) and policemen (BMI = 28,58+-5,28), non-hypertensive, non-diabetics, without dyslipidemia, no cardiovascular diseases, never smokers and without any previous or current respiratory diseases. In addition, a complete body composition analysis using multifrequency octopolar bioimpedance equipment, Bioscan 920 II S (Maltron Int, UK) was performed.

Evaluation of Perceived Stress

The levels of perceived stress were evaluated by questionnaire Perceived Stress Scale (PSS)[9].

Evaluation of Sleepiness

The levels of sleepiness were evaluated by Epworth Sleepiness Scale (ESS)[10].

Evaluation of Physical Activity / Inactivity Levels

Short form of International Physical Activity Questionnaire (IPAQ) was used to evaluate the level of physical activity[11].

Lung Function

Lung function was evaluated through spirometry pre and post 400mcg of salbutamol sulfate, by using the forced maneuver in the Masterscreen spirometer (Jaeger, Germany). The following parameters were analyzed [forced vital capacity (FVC), expired forced volume in the first second (FEV1), the relation FEV1/FVC, peak expiratory flow (PEF), MEF25%, MEF50%, MEF75%] [12].

Exhaled Nitric Oxide

The levels of nitric oxide in the exhaled air was measured by chemiluminescence by using the NOBreath monitor 2^{nd}Generation (Bedfont Scientific) in order to assess the lung inflammation[13].

Pulmonary Humoral Immune Response

Pulmonary immune response was evaluated by measurements of the levels of IL-2, IL-10, IL-17 and IFN-gamma in the breath condensate by DuoSet ELISA kits (R&D Systems; MN, USA). Breath condensate was collected for 10 minutes using RT Tube (Respiratory Research, TX, USA) according to the manufacturer's recommendations and immediately stored in liquid nitrogen and further transferred to -86degC freezer, until the measurements were done, using a microplate reader Spectramax I3 (Molecular Devices, CA, USA).

Systemic Inflammation and Immune Response

Five milliliters of venous blood were collected by using vacuum tubes containing EDTA K2 as anticoagulant. The whole blood analysis (white and red series) was performed using the automated system Sysmex 800i (Sysmex Europe GmbH, Germany). Immediately after whole blood analysis, the blood tubes were centrifuged at 1000g, for 7 minutes at 4degC. The serum was stored until the measurements of IL-2, IL-10, IL-17 and IFN-gamma were done by DuoSet ELISA kits (R&D Systems), using a microplate reader Spectramax I3 (Molecular Devices, CA, USA). In addition, the serum levels of desoxicortisol have been measured by chemiluminescence.

Statistical Analysis

The graphs were built, and the data were analyzed by using GraphPad Prism 5.0 software (California, USA). Normality of the data was evaluated by the Kolmogorov-Smirnov test. The data were submitted to an unpaired t test for a comparison between the groups. Significance values were adjusted to 5% ($p<0.05$).

Results

Chronic alteration of circadian rhythm impairs the levels of perceived stress and sleepiness

The Figure 1 showed that chronic alteration ([?] 5 years) of circadian rhythm induced by irregular and alternate work scale in policemen resulted in increased perceived stress (Figure 1A; $p<0.008$), when compared with civil men working in a diurnal 8 hours/day work scale. In addition, the evaluation of sleepiness by

Epworth sleepiness scale revealed that policemen working in an irregular and alternate work scale resulted in increased sleepiness when compared with civil men (Figure 1C; $p<0.04$). Together, these results point out that such irregular and alternate work scale in policemen can impairs two main parameters which modulates the circadian rhythm. For the other side, the evaluation of the levels of physical activity revealed no differences between civil and policemen, which are both sedentary (Figure 1B; $p>0.05$).

Impact of chronic alteration in the circadian rhythm on lung function

Figure 2 showed that policemen working in an irregular and alternated scale presented impaired lung function, as denoted by reduced FVC (% predicted) (Figure 2A; $p<0.0053$) and FEV1 (% predicted) (Figure 2B; $p<0.043$). However, other parameters of lung function, such as FEV1/FVC (% predicted) (Figure 2C; $p>0.05$), PEF (% predicted) (Figure 2D; $p>0.05$), MEF25% (% predicted) (Figure 2E; $p>0.05$), MEF50% (% predicted) (Figure 2F; $p>0.05$), MEF75% (% predicted) (Figure 2G; $p>0.05$) and IN VC (% predicted) (Figure 2H; $p>0.05$) present no differences when compared to civil men.

Chronic alteration in the circadian rhythm increases the levels of exhaled nitric oxide

Figure 3 showed that policemen working in an irregular and alternated scale presented increased levels of exhaled nitric oxide, a marker of chronic lung inflammation, as denoted by increases above 25 parts per billion when compared to civil men ($p<0.037$).

Altered circadian rhythm impairs lung immune response

Figure 4 presented the influence of altered circadian rhythm on the levels of cytokines in the breath condensate. It was observed that the levels of IL-2 were significantly higher in policemen comparing to civil men (Figure 4A; $p<0.004$). On the other hand, the results demonstrated that no statistical differences were observed for the levels of IL-10 (Figure 4B; $p>0.05$), IL-17 (Figure 4C; $p>0.05$) and IFN-gamma (Figure 4D; $p>0.05$).

Altered circadian rhythm induces low grade inflammation

Table 1 displayed the effects of altered circadian rhythm on the whole blood analysis (white and red blood components) and Figure 5 the levels of systemic (serum) cytokines and desoxicortisol. The results demonstrated that policemen presented a clinical status of low grade inflammation, as demonstrated by increased levels of pro-inflammatory cytokine IL-2 comparing to civil men (Figure 5A; $p<0.002$) and reduced levels of anti-inflammatory cytokine IL-10 (Figure 5B; $p<0.0001$). On the other hand, no differences were found related to cellular response (Table 1), as well as for other cytokines, such as IL-17 (Figure 5C; $p>0.05$) and IFN-gamma (Figure 5D; $p>0.05$). In addition, the analysis of serum desoxicortisol, showed no differences between civil and policemen (Figure 5E; $p>0.05$).

Discussion

The present study shows for the first time that chronic alterations of circadian rhythm related to stress in policemen impairs not only the perceived stress levels and sleepiness, but also the lung function and the pulmonary and the systemic immune response.

The association between chronic shiftwork with impaired perceived levels of stress is clearly stablished[14], while the impact of such association in different organs and systems are less understood. Therefore, the present study shows for the first time that policemen working in a shiftwork scale for at least 5 years, present increased levels of perceived stress, followed by impaired lung function and impaired systemic and pulmonary immune response.

Significant positive associations have been observed between stress indices and increased incidence of chronic diseases, such as cardiovascular and metabolic diseases[7,15], as well as systemic immune dysregulation[5]. Herein, the present study shows for the first time that compared with civil men working in a continuous 8 hours/day diurnal scale, without increased levels of perceived stress and sleepiness disturbance, policemen presented increased levels of serum IL-2. Increased levels of IL-2 play a major role in the growth and proliferation of many immune cells such NK and T cells and is known as a pro-inflammatory cytokine. The activation of T lymphocytes via interleukin IL-2 and IL-2 receptor plays an important role in pulmonary diseases such as asthma[16,17], in which airway obstruction is present at different levels. Furthermore, asthmatic patients have higher pulmonary levels of IL-2 compared to healthy subjects[18]. In addition, several studies have reported inverse association of pulmonary levels of IL-2 with the FEV1 in asthmatic individuals[17,18]. Our findings show that police officers have higher levels of lung IL-2 compared to civilians, which were followed by impaired lung function, an event never demonstrated before. Of note, we reinforce that the present study demonstrates that chronic shiftwork scale leads to increased levels of perceived stress and impaired sleepiness, which can be related to increased levels of pulmonary IL-2 associated with reduced lung function.

Interestingly, higher levels of pulmonary IL-2 in asthmatic individuals present inverse association with FEV1[17,18]. In this way, the present study shows that non-asthmatic police officers, beyond increased pulmonary IL-2 levels and reduced FEV1, also presented increased levels of exhaled nitric oxide, a molecule involved with airway inflammation and obstruction (reduced FEV1)[19]. In fact, the present study showed that police officers presented increased levels of exhaled nitric oxide, slightly above 25 parts per billion (ppb), which significate airway inflammation[20]. In addition, other sources of airway inflammation beyond allergen-induced asthma, such as nitrogen dioxide (a major traffic-related air pollutant)[21], lipopolysaccharide[9,22,23], and smoking[24] also can increase the levels of nitric oxide. Therefore, the present study showed that policemen working in a shiftwork scale clearly present airway inflammation, characterized by increased levels of exhaled nitric oxide, although the source of such airway inflammation could not be confirmed in this study.

On the other side, increased levels of blood IL-10, an immunomodulatory and anti-inflammatory cytokine has been associated increased and better FEV1/FVC values, which is also an important marker of airway obstruction[25]. In the present study, it was found similar response, since policemen working in a shiftwork scale presented reduced levels of IL-10 and reduced FEV1. These findings reinforce the findings of previous studies performed in asthmatics[25], demonstrating a possible association between lower levels of IL-10 with airway inflammation and airway obstruction. In fact, this concept that low levels of IL-10 can be associated with airway obstruction is strengthened by the present study, since that such phenomena were also found in non-asthmatic patients. In addition, reduced levels of IL-10 has been associated also with increased pulmonary inflammation induced by *Mycoplasma pneumoniae* [26], obstructive sleep apnea[27], and poor progression of COPD patients[28], demonstrating that perhaps, police officers could be more susceptible to develop pneumonias, obstructive sleep apnea and even COPD. However, the present study has not evaluated such hypothesis and cannot confirm that policemen could be more susceptible to develop pneumonias, obstructive sleep apnea and COPD.

Conclusions

Alterations in chronic circadian rhythm in policemen worse the lung function beyond to impair pulmonary and systemic immune response.

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Figure Legends

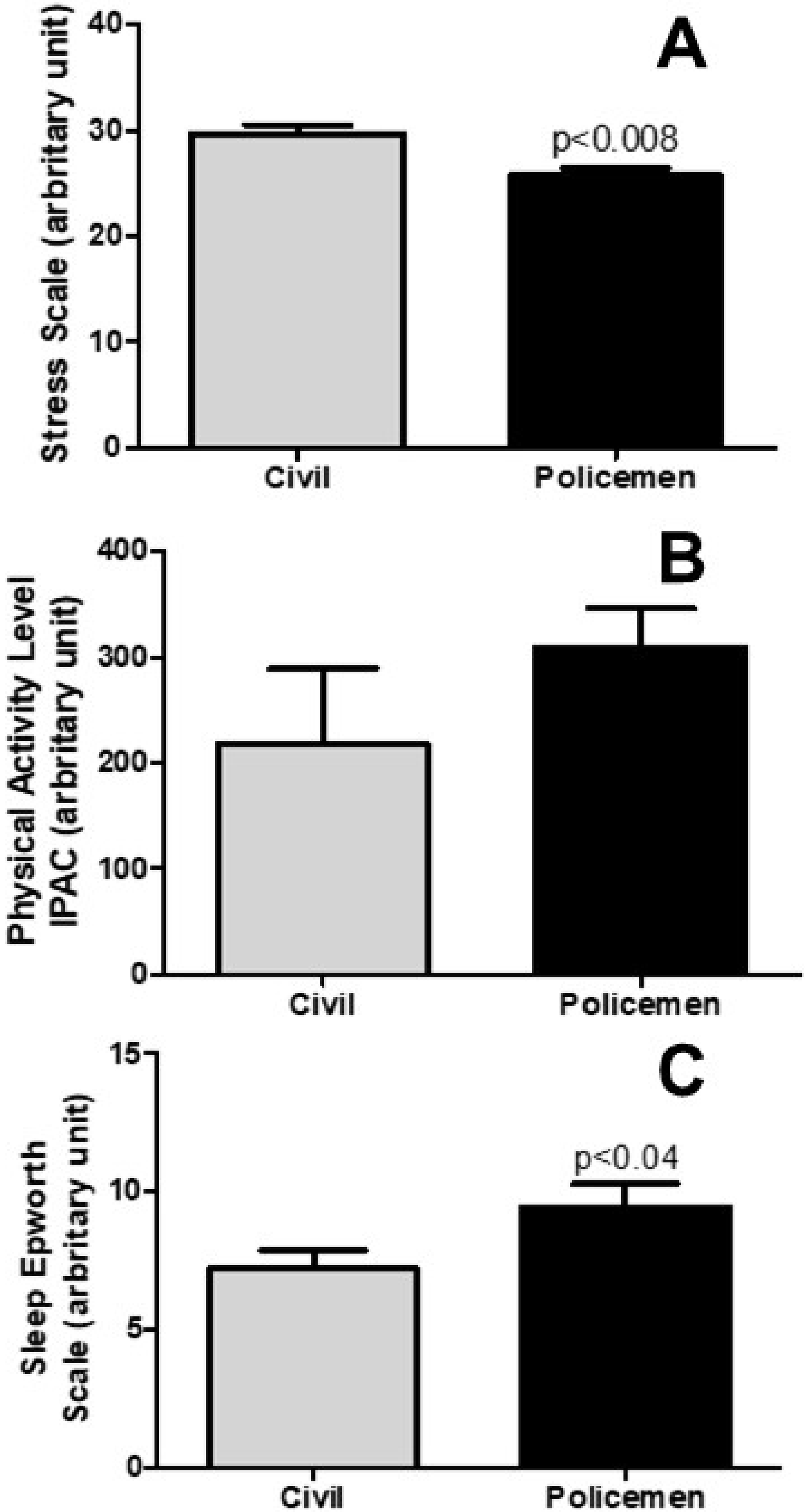
Figure 1 – Figure 1A shows the stress scale. Figure 1B shows the physical activity levels. Figure 1C shows the levels of sleepiness by Epworth sleepiness scale. The results were expressed as mean +- SD.

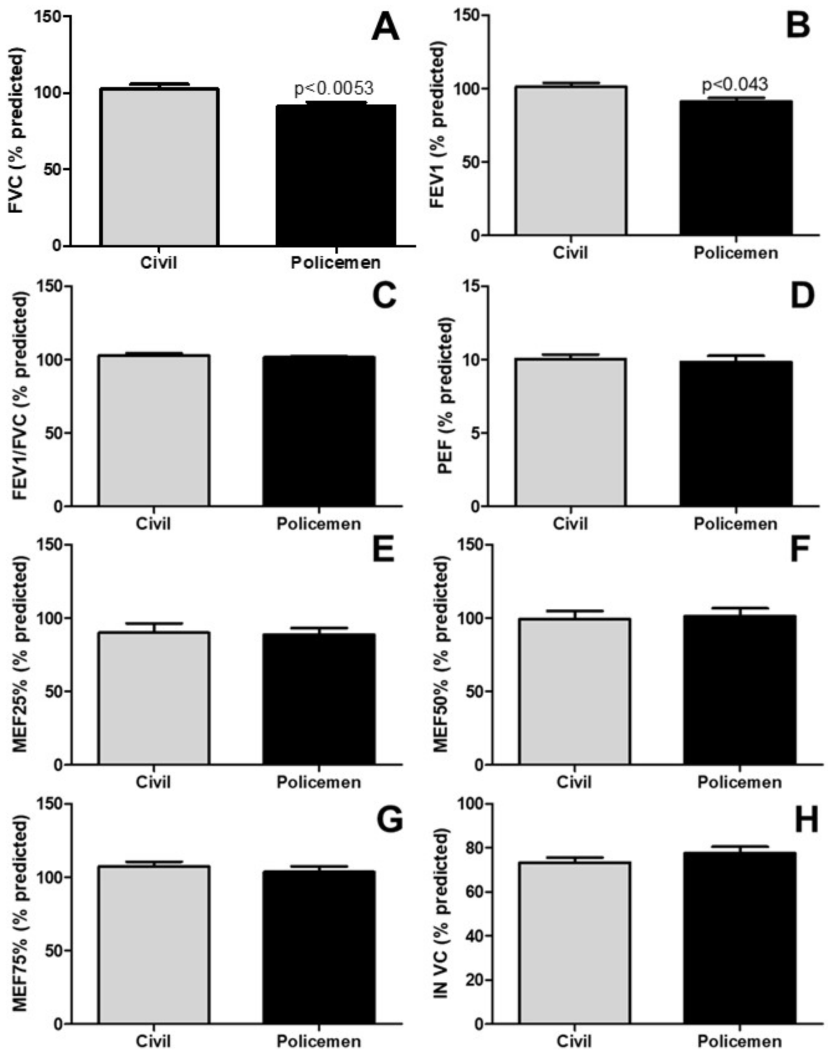
Figure 2 – Figure 2A shows the values of forced vital capacity (FVC). Figure 2B shows the values of forced expiratory volume in the first second (FEV1). Figure 2C shows the values of FEV1/FVC. Figure 2D shows the values for peak expiratory flow (PEF). Figure 2E shows the values for mean expiratory flow at 25% of FVC curve. Figure 2F shows the values for mean expiratory flow at 50% of FVC curve. Figure 2G shows the values for mean expiratory flow at 75% of FVC curve. Figure 2G shows the values of inspiratory vital capacity. The results were expressed as mean +- SD.

Figure 3 – Figure 3 shows the levels of exhaled nitric oxide. Results are expressed in parts per billion (ppb). The results were expressed as mean +- SD.

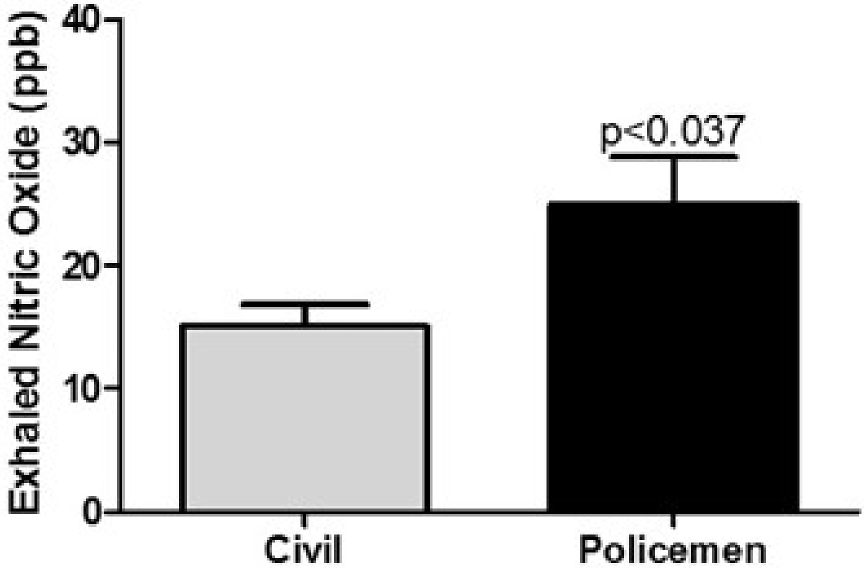
Figure 4 – Figure 4 shows the levels of cytokines in the breath condensate. Figure 4A shows the levels of IL-2. Figure 4B shows the levels of IL-10. Figure 4C shows the levels of IL-17. Figure 4D shows the levels of IFN-gamma. The results were expressed as mean +- SD.

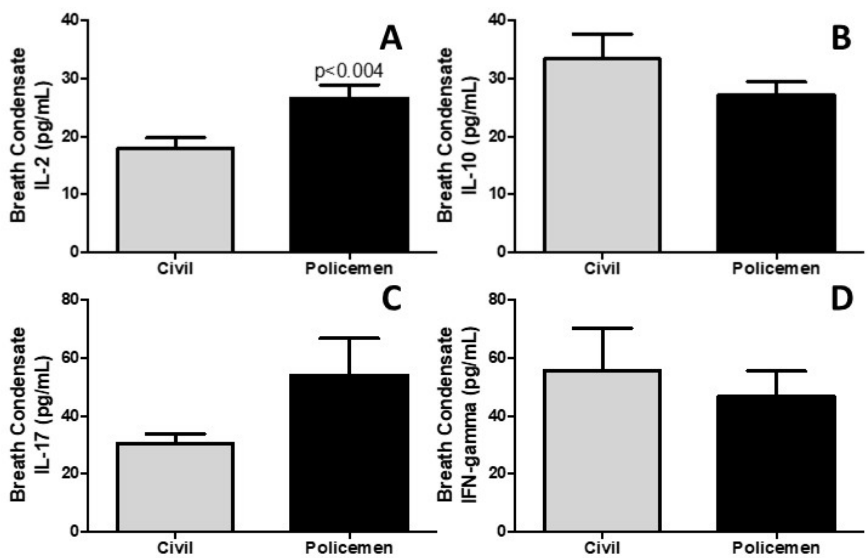
Figure 5 – Figure 5 shows the levels of cytokines and desoxicortisol in the serum. Figure 5A shows the levels of IL-2. Figure 5B shows the levels of IL-10. Figure 5C shows the levels of IL-17. Figure 5D shows the levels of IFN-gamma. Figure 5E shows the levels of desoxicortisol. The results were expressed as mean +- SD.

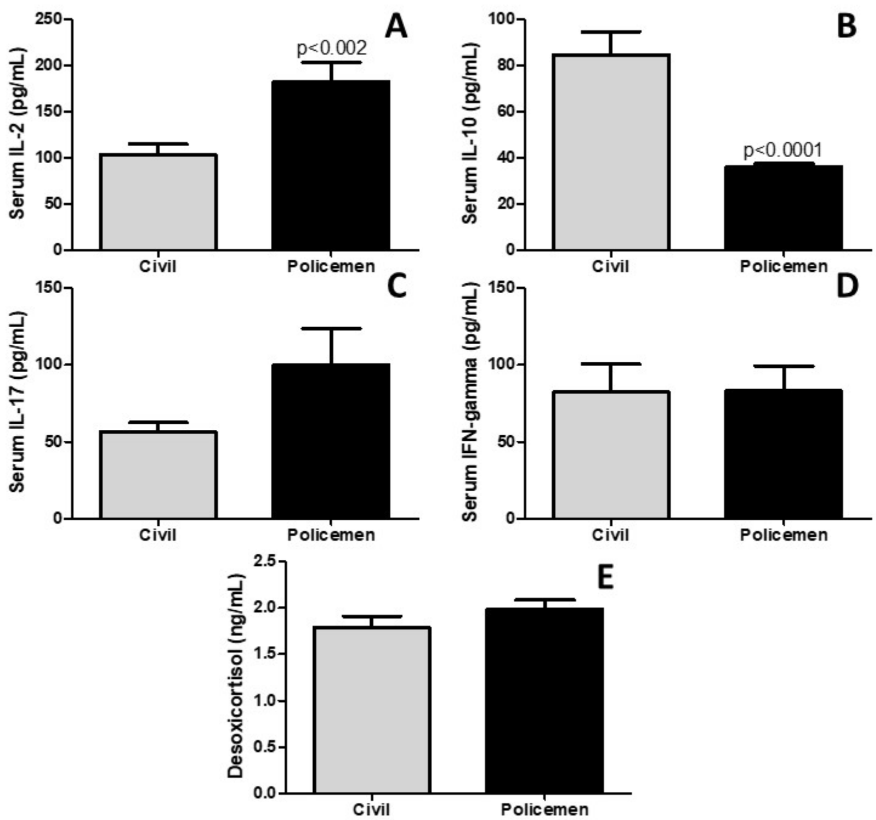

A
Stress Scale (arbitrary unit)
40
30
20
10
0
p<0.008
Civil
Policemen
B
Physical Activity Level
IPAC (arbitrary unit)
400
300
200
100
0
Civil
Policemen
C
Sleep Epworth
Scale (arbitrary unit)
15
10
5
0
p<0.04
Civil
Policemen



A
FVC (% predicted)
150
100
50
0
p<0.0053
Civil
Policemen
B
FEV1 (% predicted)
150
100
50
0
p<0.043
Civil
Policemen
C
FEV1/FVC (% predicted)
150
100
50
0
Civil
Policemen
D
PEF (% predicted)
15
10
5
0
Civil
Policemen
E
MEF25% (% predicted)
150
100
50
0
Civil
Policemen
F
MEF50% (% predicted)
150
100
50
0
Civil
Policemen
G
MEF75% (% predicted)
150
100
50
0
Civil
Policemen
H
IN VC (% predicted)
100
80
60
40
20
0
Civil
Policemen


Exhaled Nitric Oxide (ppb)
40
30
20
10
0
p<0.037
Civil
Policemen


A
Breath Condensate IL-2 (pg/mL)
p<0.004
B
Breath Condensate IL-10 (pg/mL)
C
Breath Condensate IL-17 (pg/mL)
D
Breath Condensate IFN-gamma (pg/mL)
Civil
Policemen



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`Table 1.docx` available at https://authorea.com/users/310767/articles/441524-chronic-alteration-of-circadian-rhythm-is-related-to-impaired-lung-function-and-immune-response