# Circadian Variations in Proinflammatory Cytokine Concentrations in Acute Myocardial Infarction

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**Introduction and objectives.** The concentration of certain proinflammatory cytokines has been found to be elevated in patients with acute coronary syndrome. Many studies have shown that coronary ischemic accidents do not show a uniform distribution throughout the day, but instead exhibit rhythmic variations. The objective of this study is to determine whether there is a circadian pattern of variation in the concentrations of proinflammatory cytokines in patients with acute myocardial infarction.

**Patients and method.** The sample included 40 patients with acute myocardial infarction and 40 controls. Levels of interleukin 6 and 1β were determined in the first 24 hours after the acute coronary ischemic episode. Blood samples were extracted at 3:00 a.m. (period of darkness) and at 10:00 a.m. (period of daylight).

**Results.** Both groups were similar in age, sex distribution, and coronary risk factors. Interleukin 6 levels showed a significant variation between daylight and nighttime concentrations in patients with acute myocardial infarction and controls (41.93 ± 5.90/100.39 ± 13.60 vs 25.76 ± 4.45/52.67 ± 7.73 pg/ml). However, interleukin 6 concentrations were higher in the acute myocardial infarction group than in the control group. Interleukin 1β concentrations did not vary between daylight and darkness.

**Conclusions.** In both the control group and acute myocardial infarction group, interleukin 6 concentrations varied between daylight and darkness. Patients with acute myocardial infarction shown a higher concentration of interleukin 6 secondary to the physiological response to tissue damage. Circadian variations can affect the measurements obtained for different physiological and biochemical parameters.

**Key words:** *Interleukins. Myocardial infarction. Inflammation. Circadian rhythm. Basic research.*

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**Ritmo luz/oscuridad de las citocinas proinflamatorias en el infarto agudo de miocardio**

**Introducción y objetivos.** Determinadas citocinas proinflamatorias se han encontrado elevadas en pacientes con síndrome coronario agudo. En algunos estudios ha podido comprobarse que la distribución de los accidentes isquémicos coronarios a lo largo del día no es uniforme, sino que experimenta variaciones rítmicas. El objetivo de este estudio es determinar si existe un ritmo de luz/oscuridad de las citocinas proinflamatorias en los pacientes con infarto agudo de miocardio.

**Pacientes y método.** Se incluyeron 40 pacientes con infarto agudo de miocardio y 40 controles. Se determinaron los valores de interleucina 6 y 1β en las primeras 24 h del episodio isquémico coronario agudo. Las muestras de sangre se extrajeron a las 03.00 (período oscuridad) y a las 10.00 h (período luz).

**Resultados.** Ambos grupos fueron similares en edad, sexo y factores de riesgo coronario. Las concentraciones de la interleucina 6 demostraron un ritmo luz/oscuridad significativo, tanto en los pacientes con infarto agudo de miocardio como en los controles (41,93 ± 5,90/100,39 ± 13,60 frente a 25,76 ± 4,45/52,67 ± 7,73 pg/ml). Además, la interleucina 6 fue significativamente mayor en los pacientes con infarto agudo de miocardio que en los controles. La interleucina 1β no demostró un ritmo luz/oscuridad.

**Conclusiones.** Tanto en el grupo control como en el de infarto agudo de miocardio, la interleucina 6 demostró un ritmo de luz/oscuridad. Los pacientes con infarto agudo de miocardio presentan concentraciones de interleucina 6 más altas, secundarias a una respuesta fisiológica a la lesión tisular. El ritmo luz/oscuridad puede afectar a diferentes parámetros fisiológicos y bioquímicos.

**Palabras clave:** *Interleucina. Infarto de miocardio. Inflamación. Ritmo circadiano. Investigación básica.*

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ABBREVIATIONS

AMI: acute myocardial infarction.
ACS: acute coronary syndrome.

## INTRODUCTION

In recent years it has been observed that inflammation is a key mechanism in atherogenesis and the rapid propagation of coronary artery disaese.[1] Atherosclerosis is a complex process involving different types of cells and numerous families of cytokines and growth factors.[2] Cytokines are signaling peptides, chemical mediators produced when tissues are damaged which cause the inflammatory response.[3] The majority are multifunctional molecules with different actions in the different cells on which they act.[4]

It is now known that the occurrence of coronary syndromes during the day is not uniform; rather, they occur with rhythmic variation. It has clearly been shown that the initial phases of acute myocardial infarction (AMI) occur more frequently during the early hours of the morning. Many attempts have been made to discover the causes of this circadian rhythm, and to understand its clinical and therapeutic implications.[5-6]

Daily modifications have been detected in humoral factors such as an increase in platelet aggregability during the early hours of the morning,[7] a reduction in the activity of t-PA,[8] and an increase in catecholamines,[9] all of which could encourage the formation of blood clots. A clear link – in which influence is reciprocal – exists between inflammation and thrombosis.[10] Endothelial cells stimulated by cytokines produce coagulatory substances, and activated inflammatory cells synthesize molecules that modulate the thrombotic cascade. Increased proinflammatory cytokine levels have been seen in patients with acute coronary syndrome (ACS).[11] At the present time, however, few data are available on the circadian rhythm of these molecules in such patients.

The aim of this work was to study the circadian rhythm of proinflammatory cytokines by monitoring levels of interleukin 6 (IL-6) and 1β in AMI patients and healthy controls.

## PATIENTS AND METHODS

### Patients

Between May and December 2001, 75 patients with AMI were treated in the intensive care unit of the Hospital Universitario de Canarias (The Canary Islands University Hospital). Forty patients (53%) presented with ACS, showing prolonged elevation of the ST segment. These patients received reperfusion treatment. A group of 40 healthy subjects of similar age and sex ratio was also included. The study was approved by the Scientific Ethics Committee of the University. Subjects were studied under strictly controlled environmental conditions in the cardiological intensive care unit. The light/darkness ratio established for this unit is 14 h light (1745 ± 33 lux) and 10 h dark (1.33 ± 0.3 lux). Lights are switched on at 07:00 hours and switched off at 21:00 hours.

Before inclusion, all subjects were evaluated by anamnesis, physical exploration, chest x-ray and blood analyses. The exclusion criteria were:[12] specific and non-specific infections, autoimmune diseases, collagen disease, malignancy, drug addiction, radiotherapy treatment, acute or chronic renal insufficiency, liver disease, immunosuppressant treatment, and chemotherapy. In particular, only non diabetic subjects were admitted to the study.

### Clinical information

The 40 patients (23 men and 17 women, mean age 59 ± 3.4 years) were diagnosed with AMI as defined by the following criteria:[13-14] a characteristic increase and then progressive fall in tropoinin or an increase and more rapid fall in CK-MB mass (biological markers of myocardial necrosis), accompanied by at least one of the following: *a)* symptoms of ischemia; *b)* the appearance of new necrosis Q waves in the electrocardiogram; *c)* changes in the electrocardiogram suggestive of ischemia (a raised or depressed ST segment); and *d)* coronary intervention (e.g., coronary angioplasty).

### Controls

Of the 40 healthy controls, 22 were men and 18 were women. Their mean age was 53 ± 4.6 years. All were asymptomatic, had no history of disease, and all had normal physical examination results.

### Experimental protocol

Once admitted to the cardiological intensive care unit, all patients were catheterized in the forearm to

TABLE 1. **Baseline characteristics of the study group**

| | Patients (n=40) | Controls (n=40) | *P* |
|---|---|---|---|
| Smokers | 44.0 | 4.0 | NS |
| High blood pressure | 33.3 | 22.0 | NS |
| Hypercholesterolemia | 32.0 | 14.0 | NS |
| Hypertriglyceridemia | 4.0 | 1.0 | NS |

Data in percentages.
NS indicates not significant.

TABLE 2. **Analytical parameters**

| | Patients (n=40) | Controls (n=40) | *P* |
|---|---|---|---|
| Total cholesterol, mg/dL | 196.4 ± 8.4 | 202.3 ± 8.9 | NS |
| LDL, mg/dL | 115.0 ± 6.6 | 121.0 ± 8.2 | NS |
| HDL, mg/dL | 56.0 ± 2.2 | 52.0 ± 2.9 | NS |
| Triglycerides, mg/dL | 137.3 ± 16.2 | 156.6 ± 17.6 | NS |
| Glucose, mg/dL | 113.1 ± 2.3 | 100.0 ± 3.0 | .005 |
| CK-MB, U/L | 324.6 ± 53.0 | 19.2 ± 1.6 | <.001 |
| Troponin I, mg/dL | 6.0 ± 1.0 | 0.01 ± .002 | <.001 |

Values expressed as means ± SD.
NS indicates not significant.

draw blood samples extraction. Samples for determining IL-6 and 1β levels were extracted in the first 24 h after the onset of symptoms. The time from the onset of symptoms to nighttime extraction was 7.6 ± 1.2 h, and to daytime extraction was 13.6 ± 1.0 h. Blood for determining interleukin levels was extracted at 10:00 h (light period) and 03:00 h (dark period). Patients were sleeping when blood was extracted during the night. To help in this procedure, the nurse on duty used a dull red light (<100 lux) so that he/she could see the catheter opening. This light was used for as short a time as possible, at all times avoiding it falling on the patient's eyes.

Aliquots of serum from these blood samples were placed in different tubes and stored at –80 ºC until analysis.

All patients maintained bed rest for the duration of the study.

## Laboratory methods

Plasma glucose, total cholesterol, HDL-cholesterol and triglycerides were determined by enzyme colorimetric methods. LDL-cholesterol was calculated using the Friedewald formula.

To determine levels of creatin kinase MB isozyme (CK-MB), the activated CK-MB NAC immunological method was used, in which the CK-M units are inhibited by a specific antibody which has no interaction with the CK-B units. Troponin I was determined immunoenzymatically using a technique based on sandwich ELISA (Boehringer Mannheim). Serum interleukin levels were determined by ELISA using commercial kits and following the manufacturer's instructions (DRG International, Marburg, Germany).

## Statistical analysis

SPSS version 10.0 for Windows was used for all statistical analyses. Qualitative variables were expressed as percentages, quantitative variables as means ± SD. Proximity to normal distribution was evaluated using the Kolmogorov-Smirnov test. Qualitative variables were analyzed by the chi-squared test. The differences between means of quantitative variables with normal distribution were analyzed using the Student *t* test. Forward logistic regression was used to estimate the odds ratio (OR) of confounding variables (expressed at the 95% confidence level). Significance was set at $P<.05$.

TABLE 3. **Relationship between IL-6 and AMI after controlling for the main risk factors**

| | OR (95% CI) | *P* |
|---|---|---|
| IL-6*, pg/mL | 1.04 (1.01-1.06) | .01 |
| High blood pressure | 1.44 (0.35-5.88) | NS |
| Hypercholesterolemia | 5.88 (0.60-10.0) | NS |
| Smoking | 2.55 (0.55-6.66) | NS |

*Forward method for the estimation of the odds ratio.
CI indicates confidence interval; NS, not significant.

## RESULTS

Tables 1 and 2 show the patients' clinical and analytical characteristics. Table 1 shows that the risk factors for coronary artery disease in patients were not significantly different to those in controls. Sixty percent of patients received thrombolytic treatment with reteplase; 40% required primary angioplasty.

Table 2 shows the concentrations of glucose, total cholesterol and its different fractions, and triglycerides for both patients and controls. Except for glucose, no significant differences exist between them. With respect to markers of myocardial necrosis, significant differences were seen between patients and controls.

Table 3 shows the association between IL-6 values and AMI (controlling for the main independent risk factors).

Figure 1 shows there was no circadian rhythm for IL-1β, either in controls or patients. The mean values

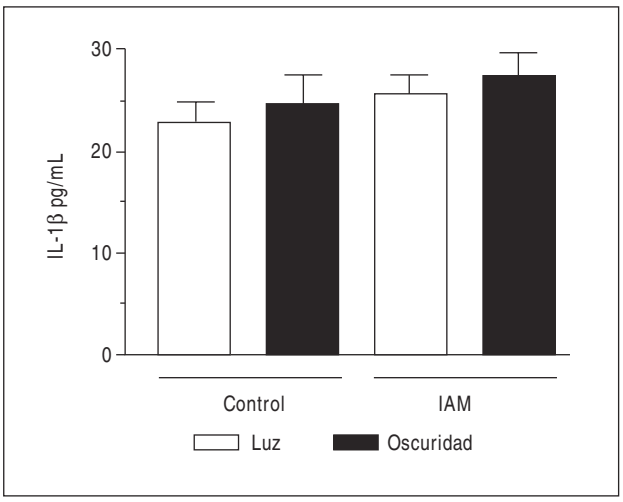


**Fig. 1.** Circadian rhythm of IL-1β.

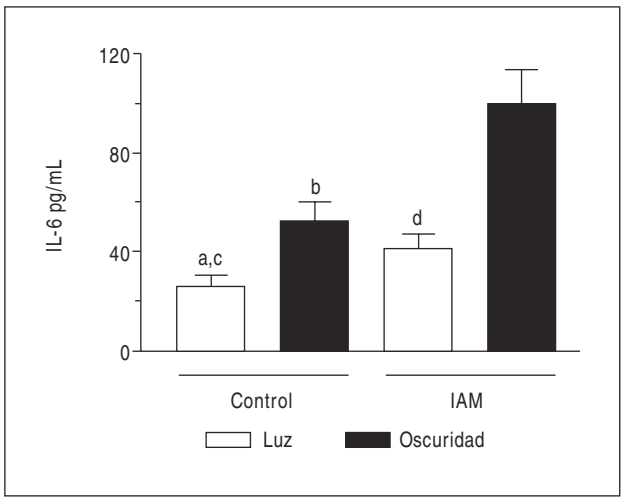


**Fig. 2.** Circadian rhythm of IL-6.
[a]*P*<.05 compared to IAM - light. [b]*P*<.007 compared to IAM - darkness. [c]*P*<.005 compared to controls - darkness. [d]*P*<.0003 compared to IAM - darkness.

for IL-1β for both the light and dark periods were slightly (but not significantly) greater in the patients. Mean IL-1β values during darkness were greater than those recorded during the light period, both in patients and controls, but no significant differences were seen.

Figure 2 shows that IL-6 has a circadian rhythm in both patients (*P*<.0003) and controls (*P*<.005). IL-6 concentration in the dark was higher in patients than in controls (*P*<.001). Differences in IL-6 light period concentrations between patients and controls were also significant (*P*<.05).

## DISCUSSION

A large number of studies have linked cells and molecules involved in the immune response to vascular lesions with atherosclerosis and atheromatosis. The lesion in the vascular wall motivates the adhesion of monocytes and T lymphocytes to the endothelial surface, as well as the release of IL-6 by both endothelial cells and leucocytes.[15] The results of the present study show IL-6 levels to be higher in patients with AMI, probably because of its release from myocytes and inflammatory cells activated by tissue necrosis.[16]

In the course of vascular disease, increased IL-6 levels reflect immune activation *in situ*. But recent evidence suggests that both the endothelium, stimulated by hyperglycemia, as well as adipose tissue, can produce this cytokine. Only non-diabetic subjects were allowed into the present study, so the significant differences seen between the glycemia levels of the two groups cannot explain the increase in IL-6.[17]

A circadian rhythm was seen for IL-6 in both the patients and controls. Several studies report that the secretion and activity of cytokines are under central neuroendocrine control (by the pineal gland via the circadian secretion of the hormone melatonin).[18]

No significant differences were seen between dark and light period levels of IL-1β, probably for two reasons: *a)* IL-6 is a cytokine with potent proinflammatory properties that regulates the expression of adhesion molecules and other cytokines such as IL-1β[19] (since IL-6 regulates IL-1β, the secretion of the latter is delayed); and *b)* IL-6 induces the migration and differentiation of activated macrophages — the main producers of IL-1β.[20]

The existence of a circadian rhythm in AMI patients suggests that the problem might, in some way, be associated with, or started by, physiological rhythms with peak activity at a certain time of day or night.[21] It is currently accepted that the breakage of an atherosclerotic plaque and the ensuing thrombosis underlies the majority of AMIs. Several characteristics appear to define the vulnerability of the plaques, including factors directly related to their physical characteristics and their tissue composition, as well as systemic factors that promote their breakage and facilitate thrombosis.

Schieffer et al[19] found that IL-6 was localized at the intersection between the dysfunctional endothelium covering the plaque and the surrounding endothelium — the most common site of plaque breakage. Mehta et al[22] observed that the inflammation of atherosclerotic plaques could be initiated, maintained and even increased by multiple factors such as activated lymphocytes

and macrophages, or increased IL-6, IL-1, interferon gamma and lipoprotein(a) concentrations. In the late phases of atherosclerosis, the release of hydrolytic enzymes and cytokines contributes to the degradation of the fibrous plug of the atherosclerotic plaque, which in turn contributes to its breakage. IL-6 has potent proinflammatory properties that contribute to triggering ACS by potentiating the synthesis of metalloproteases and the expression of LDL receptors in macrophages, as well as an increase in the capture of LDL-C and the secretion of chemotactic substances such as monocyte chemotactic protein 1.[23]

### Limitations of the study

Among the limitations of the present study is the small sample size. This is a case control study in which the baseline characteristics of the study groups were similar (convenience sample). This, plus the fact that the controls and patients showed similar risk factors for coronary artery disease, hampered the finding of significant differences with respect to IL-6.

No definitive conclusions can be drawn on the whether the circadian rhythm of IL-6, with its greater increase during the dark hours in patients, is a factor that promotes AMI. More studies are needed to clarify the mechanisms underlying the periodicity of heart attacks, which might result in better protection by offering appropriate pharmacological treatment at the times of greatest risk.

## CONCLUSIONS

The results of this study show that IL-6 has a circadian rhythm in both patients and controls, although the former have higher concentrations as a result of the physiological response to tissue damage. The study of the circadian rhythm of proinflammatory cytokines, although of no clinical importance, opens the door to new research into biological rhythms in Man.

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