

# Decreased Testosterone Levels Precede a Myocardial Infarction in Both Men and Women

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**The potential role of the hormone testosterone in the risk for myocardial infarction is investigated in this study of middle-aged men and women compared with a large random control sample from the general population. Radioimmunoassay was used to measure testosterone levels in hair, approximately 1 month and 3 months before an ST-elevation or non-ST-elevation acute myocardial infarction. Mean testosterone levels were measured for middle-aged men and women (n = 168) with diagnosed myocardial infarction (the acute myocardial infarction [AMI] cases). As controls, n = 3,150 randomly selected subjects from the general population of similar age were measured at 1 time point. No significant difference in testosterone levels in hair was found 3 months before AMI for men and women compared with the controls. However, 1 month before AMI, the testosterone levels were decreasing (p < 0.001) for both men (from 2.84 to 2.10 pg/mg) and women (from 1.43 to 1.10 pg/mg), indicating that a decrease in testosterone concentrations precedes a severe cardiac event. Conventional cardiovascular risk factors were tested as confounders but did not alter this tendency. The AMI cases were also compared with a randomly selected second control group from the general population (n = 205), for whom comparable segmental hair analyses were conducted. A tendency of some decreasing testosterone levels, also in the small control group, was only significant for men. This control group was a small sample, and there might be some natural biologic variation in testosterone levels over time. This study indicates that decreased testosterone levels may be among the pathophysiological processes preceding myocardial infarction and merits further investigation. © 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>) (Am J Cardiol 2022;00:1–5)**

After periods of increased stress load, when the cortisol levels are elevated, other hormones in the body, such as testosterone, are affected. Animal studies have shown that stress exposure with elevated levels of cortisol suppresses testosterone levels.<sup>1</sup> These animal studies in baboons and in birds have revealed that elevated stress with increasing cortisol levels suppresses testosterone levels.<sup>1–4</sup> The biomechanism that links testosterone to cortisol is also relevant for humans when stress exposure and cardiovascular diseases are analyzed. Pharmacologic studies have shown that testosterone levels are negatively affected by cortisol concentrations but also in an inverse relation because cortisol is suppressed by testosterone.<sup>5,6</sup> Human studies during the recent severe acute respiratory syndrome COVID-19 pandemic revealed that testosterone could be a key factor and that decreased serum

testosterone levels in men who were admitted to intensive care units indicated a poor prognosis.<sup>7,8</sup> Low male gender hormone testosterone levels are associated with increased risks for atherosclerosis, thrombosis, dyslipidemia, coronary artery disease, insulin resistance, and cardiovascular events.<sup>9–12</sup> Men with ischemic heart disease commonly have low serum testosterone concentrations and chronic vascular and systemic inflammation. Low serum testosterone concentrations have been claimed to predict increased cardiovascular risk.<sup>9,13,14</sup> This biomechanism of the stress response that links cortisol to testosterone is also relevant for humans when analyzing stress exposure and cardiovascular diseases. However, the causal role of testosterone in cardiovascular diseases is intriguing and warrants further investigation. Studies of testosterone levels before a severe cardiac event are scarce because such analysis requires that retrospective data are available by foresight or chance. Measuring testosterone and cortisol concentrations in hair enables retrospective analysis of hormone levels months before a specific event.<sup>15</sup> This study aimed to determine whether testosterone levels in hair approximately 3 months and 1 month before a myocardial infarction predict the event.

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Funding: This work was partly funded by the Swedish AFA Insurance, Stockholm (grant number Dnr: 160,340) and regional research grants from the County Council of Östergötland, Sweden.

See page 5 for disclosure information.

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## Methods

Hair testosterone concentrations (HTCs) were measured for middle-aged men and women (age range 44 to 65 years)

(n = 168) who were admitted to the hospital owing to either an ST-elevation or non-ST-elevation myocardial infarction.<sup>16</sup> The inclusion criterion of these cases was at least 3-cm hair length cut at discharge from the hospital. Every hair sample was divided into 2 pieces, reflecting 3 months and 1 month before the cardiac event.

A large control group of n = 3,150 patients aged 50 to 65 years (men; n = 1,149 and women; n = 2,001) were drawn from the SCAPIS (Swedish CARDioPulmonary bioImage Study), representing the general population in Sweden within the same geographical area as the cases.<sup>17</sup> HTC in the control group was measured at inclusion in the SCAPIS study.

A set of conventional cardiovascular risk factors, age, gender, smoking, educational level, ethnicity, previous acute myocardial infarction (AMI), stroke, hypertension, hyperlipidemia, diabetes, body mass index, systolic blood pressure, and diastolic blood pressure were also analyzed as potential confounding factors. A significant inverse relation for the male and female ratio was evident between the cases and controls. Men were predominant in the AMI cases and women in controls. The distribution of social and cardiovascular risk factors in the AMI cases and controls from the SCAPIS study is shown in Table 1. The conventional cardiovascular risk factors were significantly higher in the AMI cases than in the controls.

A second control group, representing a random minor selection of n = 103 men and n = 102 women from the SCAPIS study, had hair samples that were cut and analyzed in 2 segments, that is, 2 months apart, to emulate the timing of the hair segments in the AMI cases.

Hair was cut from the posterior vertex of the scalp close to the skin by trained staff. Hair samples of 3-cm length represented the cumulative, retrospective hormone exposure over approximately 3 months. HTC was analyzed

using a competitive radioimmunoassay, allowing measurement of testosterone in both men and women.<sup>16</sup> Hair samples weighing 5 to 10 mg were pulverized and extracted with methanol. A similar method has successfully been applied to measure cortisol levels in hair.<sup>15</sup>

The estimates of AMI-control difference in HTC came from linear regression, with a change from 3 months to 1 month as the outcome (Table 2). All statistical calculations were performed in SPSS version 27 and STATA version 17. Because of the non-normality of the distribution of HTC, simple contrasts of AMI cases with controls concerning HTC (Table 1) were measured using the Wilcoxon rank-sum test. In contrast, the within-person change in HTC was calculated using the Wilcoxon signed-rank test (Table 2). Contrasts adjusted for potentially confounding factors were created through linear regression with formal statistical inference using the nonparametric bootstrap with 2,000 bootstrap samples. The difference between AMI cases and controls concerning change in HTC is the primary analysis, and p < 0.025 is considered statistically significant because the hypothesis tests were conducted separately in men and women.

This study was carried out following the Code of Ethics of the World Medical Association (Declaration of Helsinki) and approved by the Regional Ethical Review Board in Linköping, Sweden (Dnr 2017/177-31, Dnr 2018/378-32). All participants provided informed consent.

## Results

Testosterone levels for a sample of n = 168 middle-aged patients (n = 116 men and n = 52 women) who suffered a myocardial infarction were included in this study. The testosterone levels were measured in hair cut into 2 segments, representing 3 months and 1 month before the cardiac event. A decreased HTC was shown 1 month before the cardiac event in the proximal hair segments, illustrated in Figure 1. Within the AMI cases, the testosterone levels decreased between 3 months and 1 month before the cardiac event by a median of -66.7 (interquartile range -160.6 to -31.2) p < 0.0001 (signed-rank test). Splitting the AMI cases into men and women, the median testosterone levels also decreased significantly (p < 0.0001) for men between 3 months and 1 month before the cardiac event, and this decrease in testosterone levels was also evident for women (p < 0.0001).

A comparison of the AMI cases with a large control group from the general population (the SCAPIS control group, n = 3,150) is shown in Table 2. The mean HTC for the AMI cases 3 months before the AMI was not different (p > 0.3) from the mean HTC of men and women in this large control group. However, the HTC for female and male patients with AMI was significantly lower (p < 0.001) 1 month before the AMI than in the SCAPIS controls, shown in Table 2.

Within-person changes in HTC are reported in Table 3. AMI cases revealed substantial negative changes in the 2 months leading up to their infarction, which was more prominent in men than in women (p = 0.004). However, this represents a similar percentage change owing to a higher baseline HTC in men than in women, with a 26%

Table 1  
Distribution of social factors and conventional cardiovascular risk factors among the AMI-cases and controls from the SCAPIS study

	AMI-cases n = 168		Controls n = 3150		p-Value
	%	n	%	n	
Male	69.0	116	36.5	1150	<0.001
Female	31.0	52	63.5	1999	
Low education (9 years)	26.2	44	8.6	264	
Medium education (12 years)	54.2	91	47.1	1451	<0.001
High education (university)	19.6	33	44.4	1368	
Ethnicity (born in Sweden)	85.7	144	93.9	2902	<0.001
Earlier myocardial infarction	25.0	38	1.2	37	<0.001
Earlier stroke	2.0	3	1.4	40	0.524
Hypertension diagnosis	35.0	55	19.4	596	<0.001
Hyperlipidemia diagnosis	23.2	36	9.5	292	<0.001
Diabetes diagnosis	4.5	7	4.1	127	0.848
Current smoker	31.9	53	9.1	285	<0.001
Age at inclusion; mean (std)	55.7 (5.6)		57.4 (4.4)		<0.001
BMI; mean (std)	27.5 (4.1)		26.9 (4.5)		0.059
Systolic blood pressure; mean (std)	143.6 (32.8)		132.0 (17.5)		<0.001
Diastolic blood pressure; mean (std)	98.4 (26.0)		83.1 (10.2)		<0.001

Table 2

Mean testosterone levels (pg/mg) for women and men who suffered a myocardial infarction (AMI cases) compared with a random sample of controls from the general population, the SCAPIS study

Women		
	Mean (SD)	p-Value*
AMI-cases		
3 month prior N = 52	1.43 (0.71)	0.36
AMI-cases		
1 month prior N = 52	1.10 (0.59)	<0.001
SCAPIS		
Controls N = 2001	1.67 (1.84)	
Men		
AMI-cases		
3 month prior N = 116	2.84 (1.47)	0.52
AMI-cases		
1 month prior N = 116	2.10 (0.90)	<0.001
SCAPIS		
Controls N = 1149	3.05 (5.11)	

\* AMI-cases compared with controls.

reduction from 3 months in men and a 23% reduction in women. A minor decrease over the same time interval was recorded for female controls, which did not reach statistical significance. Although the reduction in male controls was a little under half the corresponding magnitude in male cases, it did reach statistical significance ( $p < 0.001$ ).

Comparing the testosterone changes over 2 months between AMI cases and a random selection from the general population, the difference reached statistical significance for both genders, illustrated in Figure 2. The potential for confounding by conventional cardiovascular risk factors was considered, but only hypertension met the criteria for potential confounding in men and a hyperlipidemia diagnosis in women. All other traditional risk factors were uncorrelated to changes in testosterone. Controlling the difference between AMI cases and controls for hypertension diagnosis in men reduced the difference slightly. Still, it remained statistically significant (adjusted difference =  $-0.29$ , SE = 0.09,  $p = 0.001$ ), whereas controlling the difference in women for hyperlipidemia diagnosis resulted in an adjusted difference between changes of  $-0.25$ , SE = 0.12,  $p = 0.04$ .

## Discussion

The main findings in this study are that hair testosterone levels decrease significantly ( $p < 0.001$ ) approximately 1 month before a myocardial infarction, notably evident for both men and women. All AMI cases in this study have, in a previous study, exhibited significantly elevated cortisol levels 1 month before their cardiac event.<sup>15</sup> This is in line with a biologic mechanism first studied in baboons, showing that stress reduces testosterone levels.<sup>1,3</sup> Since the testosterone levels were significantly reduced in the same way for both men and women that was found in this study indicates a common biologic mechanism.

Unless by chance, biomarkers can never be measured before a severe cardiac event. However, measuring HTCs makes it possible to go back for several months, as in the year rings on a tree, which is a unique strength in this study.<sup>16</sup>

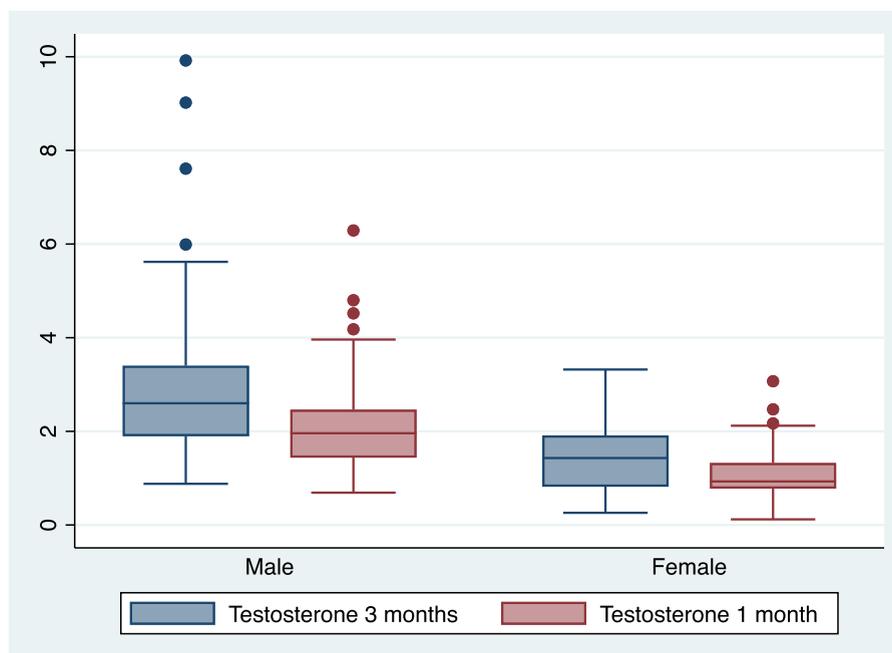


Figure 1. Testosterone levels for AMI cases 3 months and 1 month before a myocardial infarction.

Table 3

Comparison of change in testosterone levels (pg/mg) between AMI-cases and controls, stratified by gender (data also adjusted for conventional cardiovascular risk factors)

Group	Women			Men		
	3 months	1 month	change	3 months	1 month	change
<b>AMI-cases*</b>						
n = 116 men	1.43	1.10 (0.59)	-0.33 (0.60)	2.84 (1.47)	2.10 (0.90)	-0.74
n = 52 women	(0.71)		p<0.001			(1.09)
						p<0.001
<b>Controls*</b>						
n = 103 men	1.71	1.65 (0.89)	-0.07 (0.63)	2.71 (1.01)	2.37 (0.76)	-0.35
n = 102 women	(0.84)		p=0.06			(0.58)
						p<0.001
<b>Difference†</b>			-0.39 (0.12)			-0.27
			p=0.001			(0.10) p=0.01

\* Values are mean (SD) and p-value.

† Values are difference in mean change comparing AMI cases with controls (SE) and p-value.

Analysis of testosterone concentrations in small amounts of hair, particularly in women, with gold standard methods such as liquid chromatography tandem mass spectrometry, are associated with methodologic challenges regarding detection limits. The radioimmunoassay used in the present study has an adequate detection limit for hair testosterone measurement in both men and women.<sup>16</sup> Because scalp hair grows approximately 1 cm/month, a 3-cm long hair sample will theoretically represent the average hormone exposure during the previous 3 months.<sup>18</sup>

Because there were many participants in the SCAPIS study, no definitive criteria were set for hair length.<sup>17</sup> Thus, the hair samples of the control groups in this study were of varying lengths. Consequently, possible variations in hormone concentrations among hair segments have not been evaluated in the large control group. The very possibility that there could be a decrease in the HTC over time in the

control group prompted us to include a smaller control group (from the SCAPIS study) with participants who had sufficient hair length to allow an analysis of the same hair segments (3 cm of hair length divided into a proximal and a distal half). These were analyzed in the same manner as for the patients in the AMI group. A tendency of some decreasing testosterone levels, also in the small control group, was only significant for men. This control group was a small sample, and there might be some natural biologic variation in testosterone levels over time depending on morbidity, season, daylight exposure, life event exposures, and so on. However, this analysis of a second small control group enabled us to differentiate between the spontaneous decrease in testosterone concentrations in the control group and the more substantial and statistically significant decrease in testosterone concentrations in the myocardial infarction group.

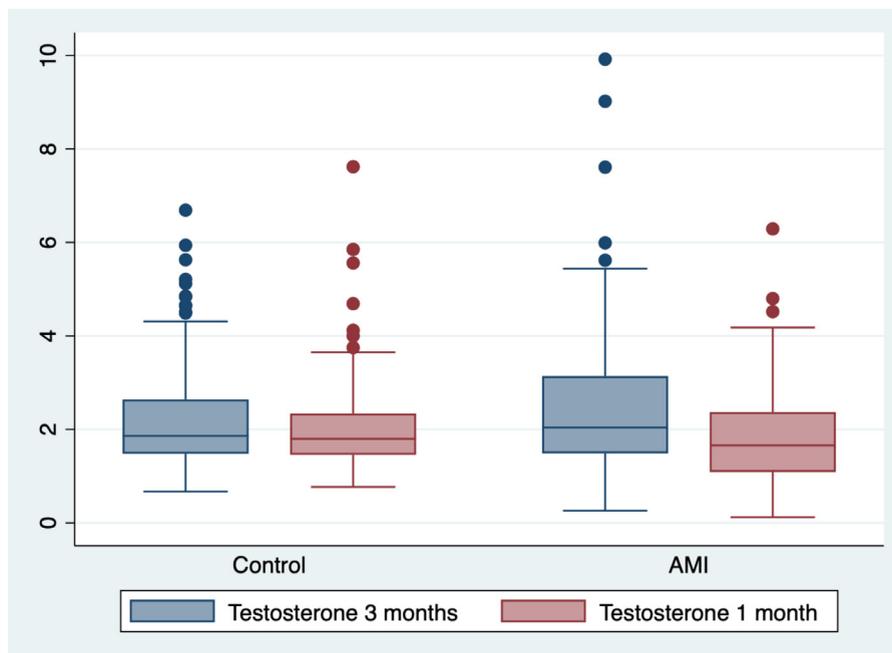


Figure 2. Box plots of testosterone levels for controls at 2 random time points 2 months apart, compared with AMI cases 3 months and 1 month, respectively, before a myocardial infarction.

The effects of testosterone levels on cardiovascular morbidity and mortality have been the subject of extensive debate. The findings of this study indicate that patients who suffer a myocardial infarction appear to have lower testosterone levels the month before a severe cardiac event.

In conclusion, this finding is in accordance with a stress-response mechanism that has previously only been shown in animal studies. Decreased testosterone levels may be a part of the stress-related pathophysiologic process preceding a myocardial infarction.

### Author Contributions

Åshild Faresjö was responsible for the conceptualization, methods, writing the original draft-preparation, writing-reviewing, and editing. Julia Preinberg undertook conceptualization, methods, writing the original draft-preparation, writing-reviewing, and editing. Mike Jones conducted the formal analysis, writing-reviewing, and editing. Andrea Labena undertook writing-reviewing and editing. Elvar Theodorsson was responsible for conceptualization, methods, writing the original draft-preparation, writing-reviewing, editing, and supervision. Tomas Faresjö was responsible for the conceptualization, methods, writing the original draft-preparation, writing-reviewing, and editing.

### Disclosures

The authors have no conflicts of interest to disclose.

### Data availability

Data are available on request to Linköping University Electronic Press <https://ep.liu.se>.

### Acknowledgment

The authors are grateful for data accessibility and support from the Linköping site of the SCAPIS study led by C. J. Östgren, PhD, also the vice PI of the National SCAPIS study in Sweden. The authors thank research engineer Helena Quist, who performed all the laboratory analyses of testosterone.

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