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News & Views

Could NLRP3-inflammasome be a cardiovascular risk biomarkers in Acute Myocardial Infarction Patients?

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ABSTRACT

Conventional cardiovascular risk factors (CVRF) are accepted to identify asymptomatic individuals with high risk of acute myocardial infarction (AMI). However, AMI affects many patients previously classified at low risk. New biomarkers are needed to improve risk prediction. We propose to evaluate the NLRP3-inflammasome complex as a potential CVR indicator in healthy males and post-AMI patients and compare both groups by known CVRFs. We included 109 men with no history of cardiovascular disease (controls) and 150 AMI patients attending a cardiac rehabilitation program. AMI patients had higher mean of BMI and waist circumference than the controls. However, high percentages of the controls had a high BMI and a waist circumference >95 cm. The controls also had higher systolic blood pressure (P>0.001), total and LDL-cholesterol, dietary nutrient and calorific intake. Fuster BEWAT Score (FBS) correlated more closely than Framingham risk score (FRS) with most CVRF, groups. However, only the FBS showed a correlation with inflammasome cytokine IL-1β. Several conventional CVRFs were significantly better in AMI patients; however, this group also had higher mRNA expression of the inflammasome gene *NLRP3* and lower expression of the autophagy gene *MAP-LC3*. The controls had high levels of CVRF, probably reflecting unhealthy lifestyle. FBS reflects the efficiency of strategies to induce lifestyle changes such as cardiac rehabilitation programs, and could provide a sensitive evaluation cardiovascular risk. These results lead to the hypothesis that NLRP3-inflammasome and associated IL-1β release have potential as CVR biomarkers, particularly in post-AMI patients with otherwise low risk scores.

Key words: NLRP3-inflammasome complex, cardiovascular risk factors, Framingham Risk Score, Fuster BEWAT Score**.**

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death worldwide, with prevalence high in industrialized and low- and middle-income countries (2). Between 1999 and 2004, only ~8% (1 in 12) of US adults had a low burden of cardiovascular risk factors, and similar levels of cardiovascular risk are reported in Spain (2). There is thus vast untapped potential for the prevention of atherosclerosis and CVD (3), prompting the promotion of lifestyle-modification strategies as a first-line approach. However, it is important to carefully evaluate how modifying specific parameters influences cardiovascular risk.

Current guidelines identify 4 main primary risk factors for cardiovascular health (CVH): hypertension (blood pressure ≥140/90 or medication with blood-pressure-lowering drugs), overweight or obesity (BMI ≥25), smoking, and physical inactivity (≤150 min/week) (3). However, a number of secondary risk factors also correlate closely with cardiovascular risk, such as waist circumference and diabetes mellitus, and there is moderate evidence for and influence of lipid profile (3). All of these risk factors are strongly influenced by diet, which like other lifestyle factors can be modified to either increase or reduce cardiovascular risk; for example, daily intake of fruit and vegetables lowers the risk of death from all causes, particularly CVD (3). Physical activity/exercise training, a healthy diet, weight loss, smoking cessation, and control of blood pressure (BP) and blood lipids and glucose are general strategies for cardiac rehabilitation programs. Based on these parameters (BP, exercise, weight, diet, and tobacco) the Fuster BEWAT Score (FBS) (2) has been designed as a tool for evaluating changes in healthy behaviors in relation to CVH.

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Prominent among other possible cardiovascular risk indicators are markers of inflammation, and IL-1β in particular has been linked to several steps in the development of atherosclerosis, heart disease, and type II diabetes [Li]. The cytokines IL-1β and IL-18 are activated by the inflammasome complex, a multiprotein complex composed of an intracellular sensor, typically a Nod-like receptor (NLR), the precursor procaspase-1, and the adaptor ASC (apoptosis-associated speck-like protein containing a CARD) (5).

In the present study, we compared cardiovascular risk factors and diet between men undergoing cardiac rehabilitation after acute myocardial infarction (AMI) and a control group of men with no history of CVD. The cardiovascular risk profile of both participant groups was assessed by Framingham 10 year risk score (FRS) and the FBS.

RESULTS

Anthropometric parameters are listed in Table 1. AMI patients had a higher mean BMI, (30.1±4.5 kg/m^2) than the control group (28.2 ± 4.1 kg/m^2), with a medium effect size (Cohen's d, 0.48). Similarly, the AMI group had a higher mean waist circumference (107.1 ± 13.2 cm versus 102.6 ± 11.9 cm), with a low effect size (Cohen's d, 0.35). However, a high percentage of the control group had a high BMI (overweight 48%, obesity 28.4%). Interestingly, the control group had higher systolic BP (144.8 ± 23.6 mmHg versus 117.3 ± 16 mmHg) and diastolic BP (83.7 ± 11.7 mmHg versus 71.8 ± 9 mmHg), with high effect sizes. This difference was reflected in 55% of control group members having systolic BP >140 mmHg, compared with 42% of the AMI group. Moreover, FRS-predicted CVR in the control group (13.5 ± 8.9%) was higher than in the AMI group (9.5 ± 6.9%).

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Cardiovascular risk is linked to several biochemical parameters. No significant between-group differences were found for glucose, triglycerides, or VLDL-cholesterol. In contrast, levels of total cholesterol and LDL-cholesterol were higher in the control group (195.5 ± 36.6 mg/dL and 130.2 ± 34.5 mg/dL) than the AMI group (150.6 ± 42 mg/dL and 88.4 ± 30.9 mg/dL), with a high effect size (Table 2). Control subjects also had higher levels of HDL-cholesterol (47.4 ± 13 mg/dL versus 40.2 ± 10.2 mg/dL), with a moderate effect size (Table 2). These differences translated into higher cardiovascular risk estimates for the control group, with 27% of control group members having total cholesterol >220 mg/dL, versus 4% for the AMI group; similarly, 30% of control group individuals had LDL-cholesterol >150md/dL, versus 3% of the AMI group.

FBS was significantly higher in the AMI group than in the control group, both for overall score (AMI group, 8.73 ± 1.86 versus control group, 5.67 ± 2.64) and for 4 of the 5 individual score variables (Table 3). The control group had a higher score than the AMI group only for weight. Between-group differences were associated with high effect size for overall FBS and for 3 of the individual variables, whereas effect size was moderate for diet and weight (Table 3).

Estimated dietary intake of calories and micronutrients is summarized in Table S1. The control group showed an overall higher intake than the AMI group. For example, intake of calories, lipids, and cholesterol was significantly higher in the control group, with differences generally associated with a moderate effect size, whereas higher estimated intake of saturated fats and carbohydrates in the control group was associated with a high effect size. The control group also had a higher estimated intake of protein, monounsaturated fat, and polyunsaturated fat, but with a low effect size. Between-group differences in the estimated intake of other mineral and vitamin nutrients were small and associated with a small effect size (Tables S2 and S3). The most notable difference

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was sodium intake, which was higher in the control group (2734.8 ± 946.9 mg) than in the AMI group (2463.2 ± 1009.1 mg) (Table S2).

The pathophysiology of coronary artery disease has been linked to lipid peroxidation and other indicators of oxidative stress, autophagy impairment, and inflammatory markers such as IL-1β (4-6). The AMI group showed higher levels of oxidative stress and IL-1β, with a high effect size (Table 2). Despite the more favorable values in the AMI group for several blood biochemical markers, likely due to the rehabilitation program, oxidative stress and inflammation levels remained high during the observational period of this study. Consistent with this, the AMI group showed higher levels of *NLRP3* gene expression and lower levels of the autophagy-related gene *MAP-LC3* than the control group (Figure 1), suggesting an implication of both genes in the pathophysiology of coronary artery disease.

The potential of FBS to predict cardiovascular risk status was evaluated by calculating Pearson's correlation coefficient between total FBS and FRS. The 2 scores showed a significant negative correlation in both the control group and the AMI group (r= -0.364, $P< 0.001$ and r= -0.246, $P< 0.005$, respectively). The potential of the FBS and FRS to predict cardiovascular risk was assessed by calculating Pearson's correlation coefficient for anthropometric and blood biochemical data from the control and AMI groups. Significantly negative correlations were observed between FBS and LDL-cholesterol (for the control group only), weight, BMI, all anthropometric measures, systolic and diastolic BP, and the inflammasome cytokine IL-1β. In almost all cases, correlations between FRS and anthropometric and blood biochemical parameters were less significant (Table 4).

DISCUSSION

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The goal of this study was to evaluate novel cardiac risk factors in men with no history of cardiovascular disease and others with a history of acute myocardial infarction. AMI is usually associated with unhealthy habits, and observational epidemiology data show an association between cardiovascular events and the risk factor burden, leading to an emphasis on risk assessment as the basis of measures to prevent the onset of coronary artery disease. Probably as a consequence of unhealthy lifestyles and poor nutritional habits among the general population, the control group shared several known risk factors with the AMI group. For example, there was no between-group difference in body weight; indeed, the control group had higher proportions of individuals with overweight and high numbers with a waist circumference >95 cm although the level of obesity was lower than in the AMI group. The control group also had higher average systolic BP and a higher percentage of individuals with systolic BP above 140 mmHg. However, this observation should be interpreted with caution because the AMI patients were receiving antihypertensive medication. Risk factors for systemic hypertension include obesity, excessive sodium intake, and alcohol consumption (9). An unhealthy diet accompanied by high sodium ingestion can increase BMI and hypertension, and restriction of daily sodium intake to 2400 mg is generally viewed as beneficial (9), with the American Heart Association recommending a daily intake of no more than 1500 mg. In the present study, the control group had a significantly higher sodium intake than the AMI group, possibly reflecting the effectiveness of dietary recommendations made to the AMI group as part of the cardiac rehabilitation program. Similarly, the control group had higher intakes of saturated fat and cholesterol. Limiting saturated fat intake is a standard feature of nutritional recommendations because excess dietary fat and calories can increase blood concentrations of LDL-cholesterol (3). The higher blood concentrations of total cholesterol and LDL-cholesterol in the control group likely

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reflect the effectiveness of antilipid medication in the AMI group. Overall, estimated macronutrient consumption was higher in the control group. Within a fairly socioeconomically homogeneous population, our study thus identifies a clear tendency toward a hazardous intake of carbohydrates, proteins, and especially fat, associated with an elevated cardiovascular risk. This trend can be reversed by a greater focus on health promotion and cardiovascular disease prevention in the general population. To maximize their effectiveness, such interventions should begin in primary schools, with recommendations including moderate intake and better selection of macronutrients, adoption of low risk diets, and encouragement of healthy lifestyle habits.

Dietary intake, BMI, waist circumference, blood lipid levels, and blood pressure are established indicators of cardiovascular risk. However, dietary intake is difficult to evaluate. Other habits demonstrated to improve CVH include regular physical exercise, smoking cessation, and regular consumption of fruit and vegetables (3,9). We evaluated cardiovascular risk in the study populations by the noninvasive Framingham and Fuster BEWAT scores. The FBS is easier to apply and is more easily understood by the general population (2). The control group had a higher mean FRS and a lower FBS (punctuation is inversely proportional to the risk) than the AMI group, which should be understood as a reduced CVH. Furthermore, FBS generally showed a more significant correlation than FRS with the principal cardiovascular risk factors: LDL-cholesterol, body weight, BMI and other anthropometric measures, and blood pressure.

FBS also showed more significant correlation with the proinflammatory cytokine IL-1β, which is implicated in several CVDs (5). Inflammation has been proposed as cardiovascular risk factor, and high-sensitivity C-reactive protein (hsCRP) and IL-6 have been proposed as important risk biomarkers (7). IL-1β is strongly implicated in CVD and monoclonal antibodies targeting IL-1β have shown cardioprotective potential

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(5,7). IL-1β is one of two cytokines, together with IL-18, known to be activated by the inflammasome. The inflammasome is a protein complex composed of an intracellular sensor—typically a Nod-like receptor (NLR), the precursor procaspase-1, and the adaptor ASC. Inflammasome activation leads to the maturation of caspase-1 and the processing of its substrates, IL-1β and IL-18. The inflammasome has been implicated in CVD, and blockade of inflammasome-derived IL-1β has beneficial effects on cardiac function (5,7). The many factors implicated in inflammasome activation include high dietary levels of cholesterol, saturated fatty acid and obesity (5,7). The inclusion of obesity in FBS, along with a more quantitative compilation of other measures such as systolic blood pressure and smoking status (3) suggests that FBS could be a more accurate predictor of cardiovascular risk. Interestingly, despite the controlled diet of the AMI group, these participants had higher IL-1β concentrations than the control group, revealing a systemic inflammation that was not ameliorated by surgery and normalization of CVD biomarkers. The higher estimated intake of cholesterol and saturated fat among the control group participants anticipates a risk situation which could lead to inflammasome activation and associated IL-1β release. Consistent with the IL-1β data, the AMI group also showed increased gene expression of *NLRP3*.

The NLRP3 inflammasome is inhibited by autophagy, a biological process involving lysosomes that preserves the balance between synthesis, degradation and recycling of cellular components and which is therefore essential for cell function and survival. This observation led to interest in the potential of autophagy-inducing treatments. The mechanisms by which autophagy regulates inflammasome activation are still unknown; however, it is well known that autophagy limits IL-1β production and release (6). Autophagy is an important mechanism in the maintenance of cardiac homeostasis, and accumulating evidence suggests that reduced autophagy is associated with heart failure

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(6). In agreement with this, lower *MAP-LC3* gene expression in the AMI group indicated reduced levels of autophagy, providing a possible explanation for the high levels of the NLRP3-inflammasome and IL-1β. Together these data show that the rehabilitation program did not improve inflammasome complex activation and thus did not fully reduce cardiovascular risk in AMI patients, in line with a previous report in which exercise did not reduce inflammatory markers after AMI. Furthermore, *NLRP3* expression predicts the severity of coronary atherosclerosis and inflammasome inhibition has been shown to improve post-AMI cardiac remodeling (1). Finally, our study supports the implication of the IL-1β by NLRP3-inflammasome complex activation as a preventive target. In this sense, the large scale Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS) (8) will address to evaluate whether IL-1β inhibition with SC canakinumab every 3 months as compared to placebo can reduce recurrent cardiovascular event rates in stable coronary artery disease patients who remain at high inflammatory risk. Likewise, the inhibition of NLRP3 by MCC950 a has been shown in a very recent study to reduces infarct size and preserves cardiac function, so the inhibition of an active inflammasome could be a therapeutic potential in AMI patients

LIMITATIONS

Despite the statistical significance of the results and the high number of patients and control participants, the study is limited by the small sample size. This limitation may be resolved in the future by the inclusion of patients and control participants from other centers. Furthermore, the control group is a representative population age-matched with the AMI group, and with similar socioeconomic status and a low percentage of unemployed individuals. The study did not include women because of the low incidence of CVD in women and in order to exclude gender-specific risk factors.

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CONCLUSION

Our study demonstrates the presence of a high number of cardiovascular risk factors in the general asymptomatic population, probably the result of an unhealthy lifestyle. The study results corroborate the usefulness of the FBS for the evaluation of cardiovascular risk without invasive measurements, and indicate that this score could reflect the efficiency of strategies aimed at inducing lifestyle changes, such as cardiac rehabilitation programs. The study also shows how the FBS correlates with traditional risk factors and implicates the inflammasome as a valuable biomarker of CVD risk in post-AMI patients with low scores on traditional risk measures. Our results further suggest a molecular basis for this association and indicate that the inflammasome is a potential therapeutic target for the prevention of CVD.

INNOVATION

Inflammasome has been indicated as being in the pathophysiology of CVDs and metabolic diseases with a high cardiovascular component such as obesity and type II diabetes and it has been suggested that oxidative stress and autophagy impairment could be among the factor responsible for NLRP3-inflammasome activation. This is an interesting issue that warrants consideration to design new experiments in order to gain further insight into its potential therapeutic applications, given that inflammasome activation is a modifiable factor. The results described in this article may serve as a new way to AMI patient management and a biomarker to predict the risk repetition. Furthermore, non-healthy life style can to induce a NLRP3-inflammasome dependent cardiovascular risk factor.

Notes

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Study participants: The control group consisted of 109 men older than 35 years with no history of cardiovascular disease. All control participants were recruited from among the staff at the University of Seville and their families. The control group was compared with 150 male patients who had previously suffered a heart attack and were attending the Virgen Macarena University Hospital, Seville, Spain (AMI group). All AMI group participants were included on a cardiac rehabilitation program run according to Sociedad Española de Cardiología recommendations. The rehabilitation program consisting of 1) physical training, 2) psychological support, and 3) control of risk factors, including help with lifestyle choices, diet, BP control, stopping smoking, and dyslipidemia. The study protocol and procedures were previously approved by the Seville University Ethics Committee and all participants gave written informed consent.

Outcome measures

Patients were assigned to the AMI group 6 months after AMI. The primary outcome measure was the FRS, calculated as the summation of all Framingham risk-related factors: age, sex, LDL-C or total cholesterol, HDL-C, BP, and smoking status. We also calculated the mean difference in FBS between the control and AMI groups to monitor progress toward health in the AMI patients (2). The FBS categorizes the 5 individual variables (BP, exercise, body weight, alimentation, and tobacco smoking) on a 15-point simple ordinal scale to evaluate healthy habits and non-laboratory-based CVH factors. For each participant, each variable is graded (0, 1, 2, or 3) according to its approximation to international guidelines for cardiovascular risks and habits, with 3 being the optimal value (2).

General and anthropometric data: For all participants, data collected included age, height, weight, body mass index, abdominal waist circumference, hip circumference,

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wrist circumference, elbow circumference, bicipital fold, tricipital fold, subescapular fold, supraliac fold, BP, and pulse. Smoking status was recorded as smoker or non-smoker.

Biochemical parameters: Serum levels of glucose, total cholesterol, triglycerides, HDL, LDL, and VLDL were assayed by routine analytical methods.

IL-1β: Serum levels of IL-1β were assayed in duplicate using commercial ELISA kits (Biosource).

Lipid peroxidation: Lipid peroxidation in mononuclear blood cells was estimated from the accumulation of lipoperoxides using a commercial kit (Cayman Chemical, Ann Arbor, Michigan, USA). Thiobarbituric acid reactive substances (TBARS) were assessed from the levels of malondialdehyde (nmol malondialdehyde/mg protein).

Real-time quantitative PCR (qPCR): The expression of the *NLRP3* and *MAP-LC3* genes was analyzed by SYBR Green qPCR of mRNA extracted from blood mononuclear cells. Total cellular RNA was purified from the cells by the Trisure method (Bioline, London, UK). RNA concentration was determined spectrophotometrically. Contaminating genomic DNA was eliminated by incubation of 1 μg of total RNA from each sample with gDNA wipeout buffer for 5 min at 42 °C (Quantitect Reverse Transcription Kit, Qiagen, Hilden, Germany). RNA samples were subsequently reverse transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). PCR amplifications were conducted with primers targeting *NLRP3* (NM_004895.4), *MAPLC3* (NM_022818), and beta-actin, used as an internal control. Thermal cycling conditions used were denaturation at 95 °C for 20 s, 40 cycles of priming at 54 °C for 20 s, and elongation at 72 °C for 20 s. All reactions were performed in duplicate, and reaction mixes without RNA were used as negative

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controls in each run. Absence of contaminating genomic DNA was confirmed by setting up control reactions with RNA that had not been reverse transcribed. Fold changes in the expression of genes of interest were calculated using the ΔΔCt method.

Dietary assessment: Dietary intake was monitored over a 1-week period in which all food intakes was weighed and details of all meals recorded. In particular, three recollection days were registered at the day of recruitment by a dietitian. Another four days (including one weekend day) were registered by the patient, starting on the first day after incorporation in the study, with further supervision by the dietitian. Dietary content of macronutrients and selected micronutrients was calculated using the computer program Nutriber® and the Spain Food Composition Table.

Statistical analysis

Statistical analyses were performed with SPSS 23 and G * Power 3.1.9.2. Statistical significance of differences was assigned at $p < 0.05$ and statistical power at $1-\beta=0.80$. Samples and outcome variables were assessed by the use of descriptive statistical variables such as frequency analysis, minimum and maximum values, means, and standard deviations. Pairwise between-group comparisons were made by Student's *t*-test for normally distributed data and the Mann-Whitney *U* test for non-normally distributed data; normality was assessed with the Kolmogorov-Smirnov test. For the Student´s t-test, the homoscedasticity assumption was evaluated by Levene's test after the Lilliefors correction. Differences between the mean outcome variable scores in the AMI and control groups were assessed by *post hoc* analysis of the family of *t*-tests, including the *t*-test for independent samples and the Mann-Whitney *U* test, in G * Power 3.1.9.2. The following parameters for each outcome variable were introduced in the analysis: two tailed, normal distribution, probability alpha of 0.05, control group size, AMI group

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size, control group mean value, and AMI group mean value. To adjust for the different sizes of the control and AMI groups, common standard deviation was calculated using the following formula, where n is sample size and s is the standard deviation:

$$s_{pooled} = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}}$$

This analysis provides Cohen's d as a measure of effect size. Values of 0.2, 0.5 and 0.8 respectively indicate small, medium, and large effect sizes. Correlation between results was assessed by calculating Pearson's correlation coefficient.

Competing Interests

The authors declare that they have no competing interests.

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Abbreviations list:

AMI: acute myocardial infarction

ASC: apoptosis-associated speck-like protein containing a CARD

BMI: body mass index

BP: blood pressure

CVD: Cardiovascular disease

CVH: Cardiovascular health

CVR: Cardiovascular risk

CVRF: Conventional cardiovascular risk factors

FBS: Fuster BEWAT Score

FRS: Framingham risk score

NLR: Nod-like receptor

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Figure legend

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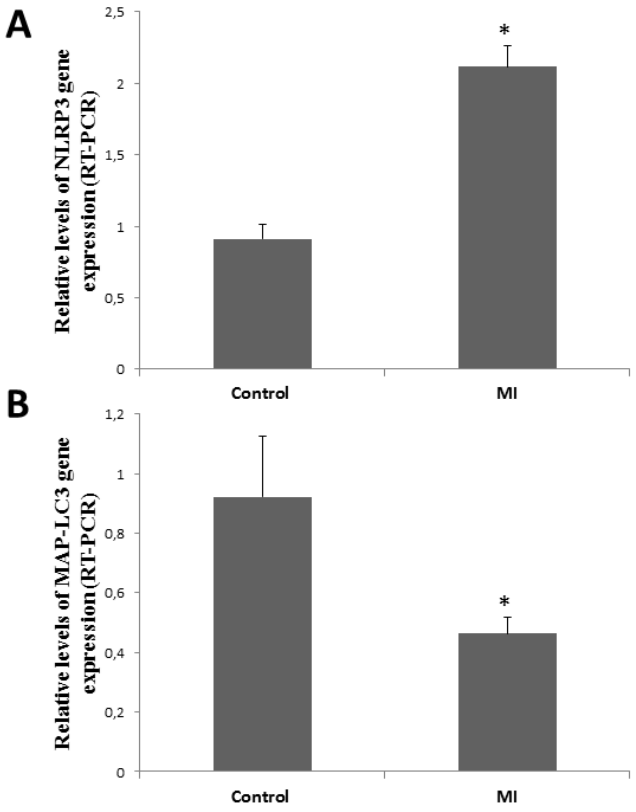

Figure 1. Gene expression profiles in blood mononuclear cells (BMC) from AMI patients and the control group. Quantitative PCR determination of the relative gene expression of *NLRP3* (A) and *MAP-LC3* (B). Data are means±SD of 3 independent experiments. *P < 0.001 between patients and controls.

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Tables

Table 1. Anthropometrical parameters in male control participants and AMI patients.

	Control group N=109	AMI group N=150	U/t*	p	Cohen´s d
Age, years	52.5 (11.8)	56.1 (10.2)	-	-	-
30-40	16.5%	10.6%	-	-	-
41-50	23.8%	20%	-	-	-
51-60	32.1%	38%	-	-	-
61-70	20.2%	22%	-	-	-
≥70	8%	9.3%	-	-	-
Educational level					
Low	7.1%	8.1%	-	-	-
Medium	51.3%	48.4%	-	-	-
High	41.6%	43.5%	-	-	-
Background hypertension	35%	55%	-	-	-
Treatment hypertension	31%	41.3%	-	-	-
Treatment hypercholesterolemia	7.5%	25.8%	-	-	-
Smokers	71%	85%	-	-	-
Height, cm	174.5 (6.8)	168.9 (6.4)	4341	0.000	0.84
Weight, kg	85.9 (14.3)	86.1 (14.1)	7755	0.637	0.01
BMI, kg/m^2	28.2 (4.1)	30.1 (4.5)	5632	0.000	0.48
Overweight, BMI 25.0–29.9 kg/m^2	48%	40%	-	-	-
Obesity BMI 30 kg/m^2	28.4%	50%	-	-	-
Waist circumference, cm	102.6 (11.9)	107.1 (13.2)	6344	0.006	0.35
>95 cm waist circumference	77.4%	86%	-	-	-
Hip circumference, cm	105.6 (8.1)	106.9 (8.2)	7079	0.136	0.16
Wrist circumference, cm	17.7 (1.8)	17.6 (1.2)	7529	0.470	0.10
Elbow circumference, cm	27.4 (2.2)	27.2 (2.3)	7678	0.642	0.06
Bicipital fold, mm	12.3 (10.1)	11.2 (5)	7875	0.898	0.15
Tricipital fold, mm	13.4 (5.9)	12.3 (5.8)	7623	0.575	0.06
Subescapular fold, mm	23.6 (8)	25.3 (7.5)	-1.699*	0.09	0.22
Supraliac fold, mm	21.5 (7.5)	19.4 (7.1)	6651	0.026	0.28
Systolic blood pressure, mmHg	144.8 (23.6)	117.3 (16)	2245	0.000	1.49
Systolic blood pressure, >140 mmHg	55%	42%	-	-	-
Diastolic blood pressure, mmHg	83.7 (11.7)	71.8 (9)	2869	0.000	1.53
Pulse	65.7 (9.5)	75.2 (13.4)	3782	0.000	0.77
FRS, %	13.5 (8.9)	9.5 (6.9)	4237	0.001	0.39
>20%	18.6%	12.4%	-	-	-

Values are expressed as mean (S.D.) or %. BMI, body mass index. *U*, Mann–Whitney *U* test; *t*= Student´s *t* test; *p= p values; d, Cohen´s d.*

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Tables

Table 2. Biochemical parameters in male control participants and AMI patients.

	Recommended value	Control group (N=109)	AMI group (N= 150)	U/t*	p	Cohen´s d
Glucose, mg/dL	70-100	106.3 (21.7)	114.6 (49.4)	6443	0.238	0.21
***Glucose, (>100* mg/dL)**		47%	57%	-	-	-
Total Cholesterol, mg/dL	150-220	195.5 (36.6)	150.6 (42)	8.894*	0.000	1.12
***Cholesterol, (>220* mg/dL)**		27%	4%	-	-	-
Triglycerides, mg/dL	70-170	143.8 (90.3)	135.4 (115.6)	7329	0.188	0.17
HDL, mg/dL	>35	47.4 (13)	40.2 (10.2)	3980	0.000	0.63
LDL, mg/dL	<150	130.2 (34.5)	88.4 (30.9)	2234	0.000	1.28
LDL, (>150* mg/dL*)		30%	3%	-	-	-
VLDL, mg/dL	5-40	28.1 (19.5)	24.7 (11.3)	4865	0.656	0.21
Lipid peroxidation, nmol/mg		26.9 (46.2)	110.9 (108)	3848	0.000	0.95
IL-1β, pg/mL		33.8 (34.3)	105.3 (67.3)	3085	0.000	1.26

Values are expressed as mean (S.D.) or %. IL-1β, Interleukin 1β; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; U, Mann–Whitney U test; t, Student´s *t* test; p, p value***; d, Cohen´s d.***

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Tables

Table 3: FBS in male control participants and AMI patients.

	Score range	Control group (N= 109)	AMI group (N= 150)	U	p	d
FBS	0-15	5.67 (2.64)	8.73 (1.86)	2491	0.000	1.50
Blood pressure	0-3	0.78 (1.01)	1.99 (1.06)	3257	0.000	1.15
Exercise	0-3	1.05 (0.9)	1.77 (0.44)	4019	0.000	1.10
Weight	0-3	1.15 (1.04)	0.71 (0.89)	5843	0.000	0.46
Alimentation	0-3	1.35 (0.79)	1.75 (0.76)	5866	0.000	0.51
Tobacco	0-3	1.46 (0.95)	2.51 (0.5)	3184	0.000	1.45

Values are expressed as mean (S.D.). FBS, Fuster BEWAT Score; *U*, Mann–Whitney *U* test; *t*, Student´s *t* test; *p, p values; d, Cohen´s d statistic*.

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Table 4. Relationship between FBS/FRS and anthropometric/biochemical parameters in male control participants and AMI patient (Pearson correlation coefficient).

	FBS Control	FBS AMI	FRS Control	FRS AMI
Glucose	-0.004	-0.122	0.169	**0.285****
Total Cholesterol	-0.137	-0.071	0.157	**0.238***
Triglycerides	-0.145	-0.083	0.202	0.118
HDL	0.180	0.109	**-0.209***	-0.178
LDL	-0.209*	-0.055	**0.286***	**0.246****
VLDL	-0.118	-0.084	0.181	**0.234***
Lipid peroxidation	-0.100	0.105	0.001	0.013
IL-1β	**-0.280****	**-0.277***	-0.010	0.049
Height	-0.007	0.045	-0.095	-0.324
Weight	**-0.544*****	**-0.451*****	0.118	**-0.198***
BMI	**-0.618*****	**-0.531*****	**0.241***	-0.031
Waist circumference	**-0.613*****	**-0.364*****	**0.319****	0.151
Hip circumference	**-0.494*****	**-0.434*****	0.196	0.005
Wrist circumference	**-0.253****	**-0.215***	0.014	-0.078
Elbow circumference	**-0.269****	**-0.319*****	-0.162	**-0.204***
Bicipital fold	**-0.334*****	**-0.324*****	0.032	-0.104
Tricipital fold	**-0.284****	**-0.222***	0.076	**-0.211***
Subescapular fold	**-0.545*****	**-0.431*****	0.110	-0.081
Supraliac fold	**-0.266***	**-0.296*****	0.090	-0.030
Systolic blood pressure	**-0.397*****	**-0.491*****	**0.60*****	**0.378*****
Diastolic blood pressure	**-0.287***	**-0.271****	**0.379*****	-0.011
Pulse	-0.081	0.119	0.072	-0.078

*P<0.05, ** P<0.005, ***P<0.001. FBS, Fuster BEWAT Score; FRS, Framingham Risk Score

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Supplementary data

Table 1. Estimated daily energy and macronutrients intakes in male control participants and AMI patients.

	Control group (N= 109)	AMI group (N= 150)	U/t*	p	Cohen´s d
Energy, kcal/d	2464.3 (483.4)	2181.7 (386.4)	4.963*	.000	.67
Protein,g/d	110.2 (24.5)	104.1 (35.2)	5259	.007	.22
Lipids, g/d	105.7 (24.3)	90.3 (21.5)	4249	.000	.68
SFA, g/d	26.1 (8.6)	19.3 (4.3)	3534	.000	.92
MUFA, g/d	49.1 (12.2)	44.9 (11.7)	2.615*	.009	.35
PUFA, g/d	14.2 (5.2)	12.2 (4.9)	4987	.001	.37
Cholesterol, g/d	352.4 (122.2)	275.8 (104.2)	4198	.000	.69
Carbohydrates, g/d	312.3 (51.4)	235.9 (44.4)	1600	.000	1.62
Fiber, g/d	25.5 (6.8)	25.4 (7.3)	.099*	.921	.01

Values are expressed as mean (S.D.). FSA, saturated fat; MUFA, monounsaturated fat; PUFA, polyunsaturated fat *U*, Mann–Whitney *U* test; *t*, Student´s *t* test; *p, p values; d, Cohen´s d.*

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Table 2. Estimated daily minerals intakes in male control participants and AMI patients.

	Control group (N= 109)	AMI group (N= 150)	U/t*	p	Cohen´s *d*
Na, mg/d	2734.8 (946.9)	2463.2 (1009.1)	5382	.013	.27
K, mg/d	3842.7 (960.7)	3746.1 (927.1)	.764*	.445	.10
Ca, mg/d	1119.2 (378.2)	1050.4 (420.3)	5765	.081	.17
Mg, mg/d	374.8 (98.5)	369.5 (81.6)	.425*	.672	.06
P, mg/d	1631.9 (401.1)	1628.2 (397.8)	6650	.965	.00
Fe, mg/d	27.5 (16.1)	23.6 (15.5)	5130	.003	.24
Cu, mg/d	4 (4.1)	6.1 (5.5)	5209	.005	.41
Zn, mg/d	16.3 (11.9)	12 (9)	4668	.000	.42
Cl, mg/d	2585.5 (1228.9)	2341.5 (1214.4)	5691	.509	.20
Mn, mg/d	19.5 (30.4)	13 (16)	6285	.457	.29
Se, µg/d	98.3 (34.1)	94.9 (28.7)	6219	.383	.10
I, µg/d	164.3 (127.6)	153.5 (142.2)	.582	.561	.07

Values are expressed as mean (S.D.). *U*, Mann–Whitney *U* test; *t*, Student´s *t* test; *p, p values; d, Cohen´s d.*

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Table 3. Estimated daily vitamins intakes in male control participants and AMI patients.

	Control group (N= 109)	AMI group (N= 150)	*U/t**	*p*	Cohen´s *d*
Thiamine, mg/d	2.5 (3.2)	1.9 (1.4)	5729	.07	.26
Riboflavin, mg/d	2 (0.6)	1.9 (0.7)	6194	.357	.11
Pyridoxine, mg/d	2.5 (0.6)	2.4 (0.9)	6038	.222	.04
Cyanocobalamin, µg/d	10.5 (8.2)	9.4 (8.5)	5922	.149	.12
Folate, µg/d	383.5 (133.7)	383.5 (136)	6540	.799	.00
Niacin, mg/d	36.1 (23.7)	30.7 (9.2)	5615	.042	.34
Ascorbic Acid, mg/d	189 (94.5)	195.7 (94.7)	6428	.638	.07
Pantothenic acid, mg/d	5.4 (1.5)	5.1 (1.4)	1,158*	.130	.20
Biotin, mg/d	9.3 (5.7)	10.8 (6.5)	5784	.088	.25
Retinol, µg/d	1096.5 (796.9)	976.2 (515.8)	6451	.671	.19
Cholecalciferol, µg/d	8 (6)	8.5 (9.6)	6477	.707	.07
Tocopherol, mg/d	15.2 (5.5)	14.1 (6.3)	5670	.054	.18

Values are expressed as mean (S.D.). *U*, Mann–Whitney *U* test; *t*, Student´s *t* test; p, *p values; d, Cohen´s d.*

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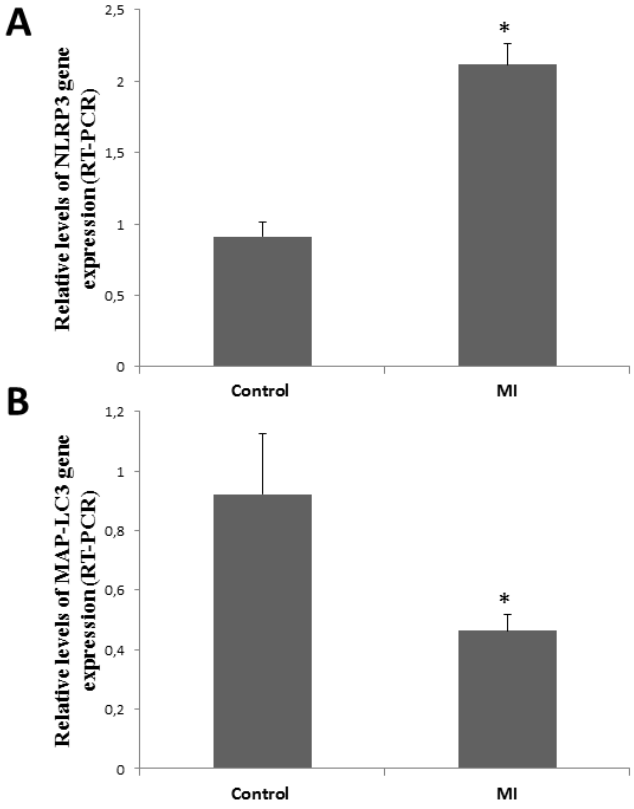

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