



## Randomized Control Trials

# Melatonin administration lowers biomarkers of oxidative stress and cardio-metabolic risk in type 2 diabetic patients with coronary heart disease: A randomized, double-blind, placebo-controlled trial

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## ARTICLE INFO

## Article history:

Received 7 October 2017

Accepted 5 December 2017

## Keywords:

Melatonin supplementation

Metabolic status

Type 2 diabetes mellitus

Coronary heart disease

Oxidative stress

Inflammation

## SUMMARY

**Background & aims:** Melatonin may benefit diabetic people with coronary heart disease (CHD) through its beneficial effects on biomarkers of oxidative stress and cardio-metabolic risk. This investigation evaluated the effects of melatonin administration on metabolic status in diabetic patients with CHD.

**Methods:** This randomized, double-blind, placebo-controlled trial was conducted and involved 60 diabetic patients with CHD. Subjects were randomly allocated into two groups to receive either 10 mg melatonin (2 melatonin capsules, 5 mg each) ( $n = 30$ ) or placebo ( $n = 30$ ) once a day for 12 weeks.

**Results:** Compared with the placebo, melatonin supplementation resulted in significant increases in plasma glutathione (GSH) ( $+64.7 \pm 105.7$  vs.  $-11.1 \pm 137.6$   $\mu\text{mol/L}$ ,  $P = 0.02$ ) and nitric oxide (NO) ( $+0.9 \pm 4.7$  vs.  $-3.3 \pm 9.6$   $\mu\text{mol/L}$ ,  $P = 0.03$ ), and significant decreases in malondialdehyde (MDA) ( $-0.2 \pm 0.3$  vs.  $+0.1 \pm 0.5$   $\mu\text{mol/L}$ ,  $P = 0.007$ ), protein carbonyl (PCO) ( $-0.12 \pm 0.08$  vs.  $+0.03 \pm 0.07$  mmol/mg protein,  $P < 0.001$ ) and serum high sensitivity C-reactive protein (hs-CRP) levels ( $-1463.3 \pm 2153.8$  vs.  $+122.9 \pm 1230.4$  ng/mL,  $P = 0.001$ ). In addition, taking melatonin, compared with the placebo, significantly reduced fasting plasma glucose ( $-29.4 \pm 49.0$  vs.  $-5.5 \pm 32.4$  mg/dL,  $P = 0.03$ ), serum insulin concentrations ( $-2.2 \pm 4.1$  vs.  $+0.7 \pm 4.2$   $\mu\text{IU/mL}$ ,  $P = 0.008$ ), homeostasis model of assessment-estimated insulin resistance ( $-1.0 \pm 2.2$  vs.  $+0.01 \pm 1.6$ ,  $P = 0.04$ ), total-/HDL-cholesterol ratio ( $-0.18 \pm 0.38$  vs.  $+0.03 \pm 0.35$ ,  $P = 0.02$ ) and systolic ( $-4.3 \pm 9.6$  vs.  $+1.0 \pm 7.5$  mmHg,  $P = 0.01$ ) and diastolic blood pressure ( $-2.8 \pm 7.3$  vs.  $+0.1 \pm 3.6$  mmHg,  $P = 0.04$ ). Melatonin treatment also significantly increased quantitative insulin sensitivity check index ( $+0.006 \pm 0.01$  vs.  $-0.004 \pm 0.01$ ,  $P = 0.01$ ) and serum HDL-cholesterol ( $+2.6 \pm 5.5$  vs.  $-0.01 \pm 4.4$  mg/dL,  $P = 0.04$ ). Supplementation with melatonin had no significant effect on other metabolic parameters.

**Conclusions:** Overall, melatonin intake for 12 weeks to diabetic patients with CHD had beneficial effects on plasma GSH, NO, MDA, PCO, serum hs-CRP levels, glycemic control, HDL-cholesterol, total-/HDL-cholesterol ratio, blood pressures and parameters of mental health. Registered under [ClinicalTrials.gov](http://www.clinicaltrials.gov) Identifier no. <http://www.irct.ir>: IRCT2017051333941N1.

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## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a main risk factor for Coronary heart disease (CHD) [1]. Multiple studies demonstrated CHD is

responsible for more than 80% of death in subjects with T2DM, and unfortunately, the actual number of subjects with T2DM is estimated to double by the year 2030, exhibiting an elevation in mortality rate during the coming years [2]. Increased parameters of oxidative stress and inflammation as well as rises in the risk of CHD, T2DM and metabolic syndrome (MetS) are participated [3]. In addition, insulin resistance, hyperglycemia and dyslipidemia in people with T2DM may play in the development of atherosclerotic disorders [4]. On the other hand, metabolic disorders in T2DM lead

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to more severe inflammation and overproduction of free radicals, which in turn play a major function in vascular events [5].

A few studies have showed that circulating melatonin levels are altered in people with T2DM and CHD. For example, the nighttime melatonin concentrations were changed in subjects with diabetes and proliferative diabetic retinopathy [6]. Moreover, an independent relationship between nocturnal levels of oxidized low-density lipoprotein and melatonin in people with myocardial infarction was reported [7]. Some studies have demonstrated the beneficial effects of melatonin supplementation on biomarkers of oxidative stress and inflammation, blood pressures, glycemic control and lipid profiles in animal models and people with T2DM and MetS [8–10]. Dominguez-Rodriguez et al. [7] observed that melatonin administration for 6 weeks in Zucker diabetic fatty (ZDF) rats ameliorated the pro-inflammatory state and oxidative stress. Similarly, melatonin supplementation (6 mg/day) for 40 days to obese women significantly improved inflammatory and oxidative stress factors [11]. In addition, melatonin administration for 12 weeks improved insulin sensitivity in old obese rats [12].

Given the antioxidant and anti-inflammatory effects of melatonin, we hypothesized that melatonin might be beneficial in diabetic people with CHD. The present study was, therefore, performed to evaluate the effects of melatonin supplementation on biomarkers of oxidative stress and cardio-metabolic risk in diabetic patients with CHD.

## 2. Subjects and methods

### 2.1. Participants and ethics statements

This randomized, double-blind, placebo-controlled trial, registered in the Iranian registry of clinical trials (<http://www.irct.ir>: IRCT2017051333941N1), was carried out at a cardiology clinic affiliated to Kashan University of Medical Sciences (KUMS), Kashan, Iran, between May 2017 and August 2017. Overweight people (BMI  $\geq 25$  kg/m<sup>2</sup>) with T2DM, aged 50–85 years old with 2- and 3-vessel CHD were included. Diagnosis of T2DM and CHD was conducted based on the criteria of the American Diabetes Association and American Heart Association, respectively. Those consuming melatonin supplements within the last 3 months, having an acute myocardial infarction and cardiac surgery within the past 3 months, and the night shift workers were not included in this study. This study was done according to the principals of the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to the intervention.

### 2.2. Study design

At first, all participants were categorized according to age (<65 and  $\geq 65$  y), BMI (25–29.9 and  $\geq 30$  kg/m<sup>2</sup>), gender, the severity of CHD (2- and 3-vessel disease), and the dosage and kind of medications. Then, participants were randomly allocated into two groups to take either 10 mg melatonin (2 melatonin capsules, 5 mg each) ( $n = 30$ ) or placebo ( $n = 30$ ) once a day 1 h before bedtime for 12 weeks. Melatonin and its placebo (paraffin) were produced by NUTRALab Pharmaceutical Company (Scarborough, Canada) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. Both melatonin supplements and placebo capsules had similar packaging and people and investigators were unaware of the content of the package until the end of study. Randomization assignment was conducted using computer-generated random numbers as blindness by a trained staff at the cardiology clinic. Compliance with the intake of supplements and placebos was determined by examining the capsule containers. In addition, participants received a daily reminder message on their cell

phones to take their supplements regularly. All participants completed 3-d dietary records at weeks 1, 4, 8 and 12 of the trial. Participants also monitored about their usual diet, the time of melatonin assumption each day and their number of sleep hours. To obtain nutrient intakes of participants according to 3-d food records, we applied Nutritionist IV software (First Databank, San Bruno, CA).

### 2.3. Assessment of anthropometric measures

Anthropometric measures (Seca, Hamburg, Germany) were measured at baseline and after intervention. BMI was calculated as weight in kg divided by height in meters squared.

### 2.4. Assessment of outcomes

Biomarkers of oxidative stress were considered as the primary outcomes and other metabolic profiles, blood pressures and parameters of mental health were defined as the secondary outcomes. Ten milliliter fasting blood samples were collected at the beginning and after the 12-week intervention at Kashan reference laboratory, Kashan, Iran. Plasma total antioxidant capacity (TAC) concentrations using the method of ferric reducing antioxidant power developed by Benzie and Strain [13], total glutathione (GSH) using the method of Beutler and Gelbart [14] and malondialdehyde (MDA) concentrations by the thiobarbituric acid reactive substances spectrophotometric test were determined with coefficient of variations (CVs) lower than 5%, respectively. Plasma protein carbonyl (PCO) levels were quantified using a spectrophotometric method [15] with inter- and intra-assay CVs of lower than 5%. Serum high-sensitivity C-reactive protein (hs-CRP) by commercial ELISA kit (LDN, Nordhorn, Germany) and insulin levels by the use of an ELISA kit (DiaMetra, Milano, Italy) with inter- and intra-assay CVs of lower than 7%. The plasma nitric oxide (NO) levels were determined using Griess method. The homeostasis model of assessment-insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were determined according to the standard formula. Enzymatic kits (Pars Azmun, Tehran, Iran) were applied to evaluate fasting plasma glucose (FPG) and lipid profiles with inter- and intra-assay CVs less than 5%. Systolic (SBP) and diastolic blood pressure (DBP) was determined via a sphygmomanometer (ALPK2, Zhejiang, China). Blood pressures were quantified between 08:00 and 09:00 AM by the same investigator each time.

### 2.5. Clinical assessment

Beck Depression Inventory (BDI) was assessed using a self-compiled questionnaire [16]. Anxiety measured by Beck Anxiety Inventory (BAI) that developed by Beck et al. [17].

### 2.6. Statistical methods and sample size

Type one ( $\alpha$ ) and type two errors ( $\beta$ ) were defined as 0.05, and 0.20 (power = 80%), respectively. According to the previous trial [11], we used 0.29 nmol/L as the SD and 0.23 nmol/L as the change in mean ( $d$ ) of MDA as a primary outcome. Based on the formula, we needed 25 people in each group; after allowing for 5 dropouts in each group, the final sample size was 30 persons in each group.

To determine the normal distribution of variables, the Kolmogorov–Smirnov test was used. The intention-to-treat (ITT) analysis was applied for all randomly allocated subjects. To detect differences in the general characteristics and dietary intakes between the two groups, independent samples *t*-test was used. Pearson Chi-

square test was used for comparison of categorical variables. To determine the effects of biochemical parameters, we used repeated measures analysis of variance. P values less than 0.05 were considered as significant. All statistical analyses were performed by the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

### 3. Results

One subject in the supplemented group and 2 patients in the placebo group dropped out for personal reasons. However, all 60 participants were included in the final analysis based on the ITT principle (Fig. 1). The compliance ranged between 90% and 100% throughout the study in both groups.

There were no significant differences between the two groups in terms of mean age, height, baseline weight, baseline BMI, baseline metabolic equivalents and mean changes in weight and BMI throughout the trial (Table 1). The frequency of smoking, consumption of antidiabetic and antilipidemic drugs, hypertension rate, consumption of angiotensin converting enzymes inhibitors, aldosterone receptor blockers drugs and blocker drugs ( $\beta$ -blocker and calcium channel blocker) of study participants were not statistically different between the two groups.

We observed no significant changes in macro- and micro-nutrients throughout the intervention between the two groups (Table 2).

Compared with the placebo, melatonin supplementation resulted in significant increases in plasma GSH ( $+64.7 \pm 105.7$  vs.  $-11.1 \pm 137.6$   $\mu\text{mol/L}$ ,  $P = 0.02$ ) and NO ( $+0.9 \pm 4.7$

vs.  $-3.3 \pm 9.6$   $\mu\text{mol/L}$ ,  $P = 0.03$ ), and significant reductions in MDA ( $-0.2 \pm 0.3$  vs.  $+0.1 \pm 0.5$   $\mu\text{mol/L}$ ,  $P = 0.007$ ), PCO ( $-0.12 \pm 0.08$  vs.  $+0.03 \pm 0.07$  mmol/mg protein,  $P < 0.001$ ) and serum hs-CRP levels ( $-1463.3 \pm 2153.8$  vs.  $+122.9 \pm 1230.4$  ng/mL,  $P = 0.001$ ). In addition, taking melatonin, compared with the placebo, significantly decreased FPG ( $-29.4 \pm 49.0$  vs.  $-5.5 \pm 32.4$  mg/dL,  $P = 0.03$ ), insulin concentrations ( $-2.2 \pm 4.1$  vs.  $+0.7 \pm 4.2$   $\mu\text{IU/mL}$ ,  $P = 0.008$ ), HOMA-IR ( $-1.0 \pm 2.2$  vs.  $+0.01 \pm 1.6$ ,  $P = 0.04$ ), total-/HDL-cholesterol ratio ( $-0.18 \pm 0.38$  vs.  $+0.03 \pm 0.35$ ,  $P = 0.02$ ), SBP ( $-4.3 \pm 9.6$  vs.  $+1.0 \pm 7.5$  mmHg,  $P = 0.01$ ) and DBP ( $-2.8 \pm 7.3$  vs.  $+0.1 \pm 3.6$  mmHg,  $P = 0.04$ ), and significantly increased QUICKI ( $+0.006 \pm 0.01$  vs.  $-0.004 \pm 0.01$ ,  $P = 0.01$ ) and serum HDL-cholesterol ( $+2.6 \pm 5.5$  vs.  $-0.01 \pm 4.4$  mg/dL,  $P = 0.04$ ). Additionally, melatonin supplementation significantly improved BDI ( $-2.7 \pm 1.8$  vs.  $-0.7 \pm 0.9$ ,  $P < 0.001$ ) and BAI ( $-2.0 \pm 3.4$  vs.  $-0.4 \pm 1.1$ ,  $P = 0.02$ ) compared with the placebo. Supplementation with melatonin had no significant effect on other metabolic profiles compared with the placebo (Table 3).

### 4. Discussion

We found that melatonin supplementation after 12 weeks to diabetic people with CHD had beneficial effects on plasma GSH, NO, MDA, PCO, serum hs-CRP levels, glycemic control, HDL-cholesterol, total-/HDL-cholesterol ratio, blood pressures and parameters of mental health. To our knowledge, this is the first report of melatonin intake on biomarkers of oxidative stress and inflammation, glycemic control, lipid profiles, blood pressures and parameters of mental health among diabetic people with CHD.

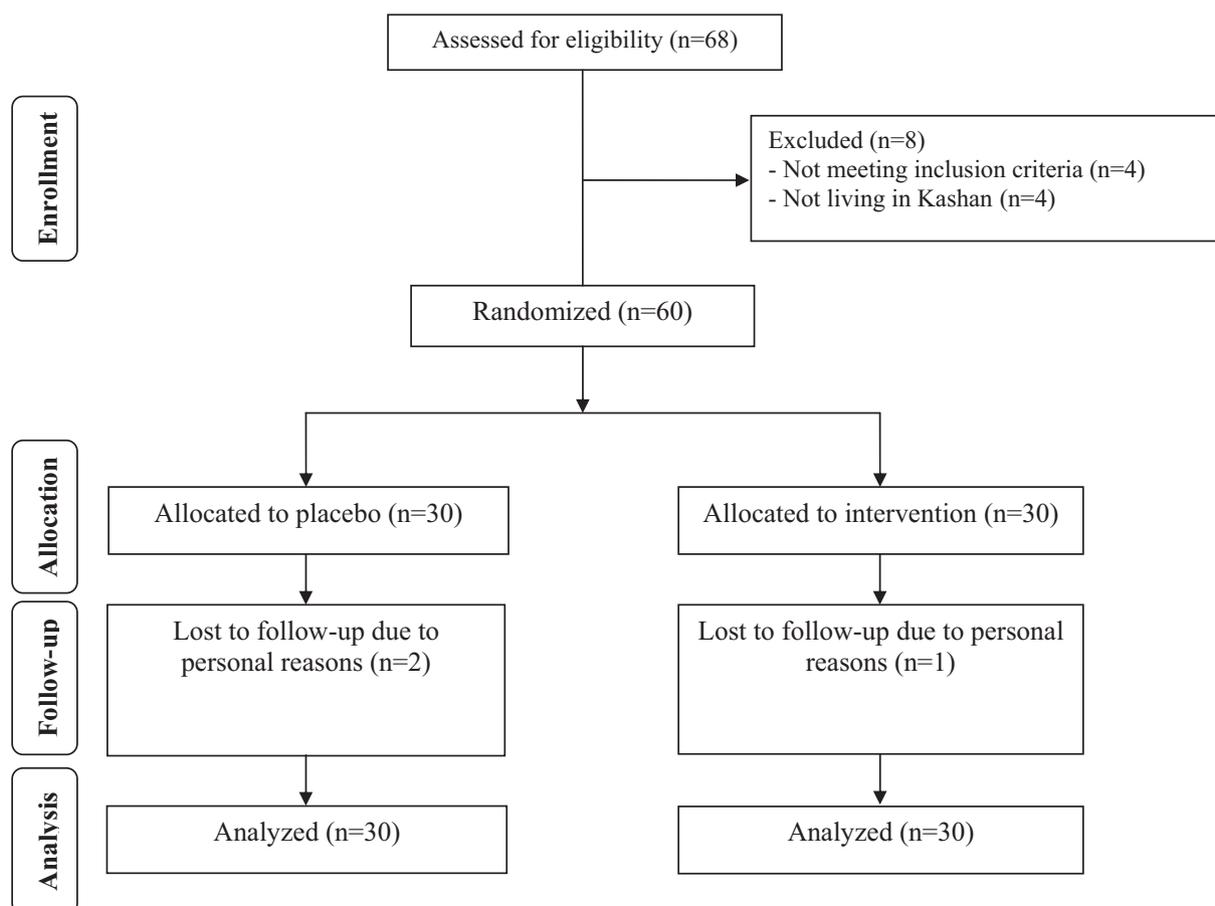


Fig. 1. Summary of patient flow diagram.

**Table 1**  
General characteristics of study participants at baseline study.

	Placebo group (n = 30)	Melatonin group (n = 30)	P <sup>a</sup>
Age (y)	65.3 ± 10.1	67.7 ± 11.4	0.40
Gender			
Female	17 (56.7)	16 (53.3)	0.79 <sup>b</sup>
Male	13 (43.3)	14 (46.7)	
Height (m)	161.3 ± 9.3	157.1 ± 12.6	0.14
Weight at study baseline (kg)	77.1 ± 11.2	75.4 ± 15.2	0.62
Weight at end-of-trial (kg)	77.2 ± 11.8	75.8 ± 15.9	0.71
Body weight change (kg)	0.1 ± 2.5	0.5 ± 1.8	0.50
BMI at study baseline (kg/m <sup>2</sup> )	29.7 ± 4.4	30.4 ± 4.3	0.53
BMI at end-of-trial (kg/m <sup>2</sup> )	29.7 ± 4.4	30.6 ± 4.4	0.46
BMI change (kg/m <sup>2</sup> )	0.01 ± 1.0	0.2 ± 0.7	0.49
MET-h/d at study baseline	25.9 ± 2.1	26.1 ± 2.4	0.68
MET-h/d at end-of-trial	25.7 ± 2.1	26.1 ± 2.4	0.45
MET-h/d change	-0.2 ± 0.7	-0.004 ± 0.7	0.30
The severity of CHD			
2-vessel disease	12 (40)	11 (36.7)	1.00 <sup>b</sup>
3-vessel disease	18 (60)	19 (63.3)	
Smoking (%)	2 (6.7)	2 (6.7)	1.00 <sup>b</sup>
Aspirin 80 mg (%)	30 (100)	30 (100)	1.00 <sup>b</sup>
Statin (%)	30 (100)	30 (100)	1.00 <sup>b</sup>
Insulin therapy (%)	10 (33.3)	9 (30.0)	1.00 <sup>b</sup>
Antidiabetic drugs (%)			
Monotherapy	17 (70.8)	18 (72.0)	
Combination therapy	7 (29.2)	7 (28.0)	1.00 <sup>b</sup>
Hypertension (%)	22 (73.3)	22 (73.3)	1.00 <sup>b</sup>
ACEI/ARB drugs (%)	30 (100)	30 (100)	1.00 <sup>b</sup>
Blocker drugs (%)			
β-blocker	28 (93.3)	27 (90.0)	
Calcium channel blocker	2 (6.7)	3 (10.0)	1.00 <sup>b</sup>

Data are means ± SDs.

ACEI, angiotensin converting enzymes inhibitors; ARB, aldosterone receptor blockers; CHD, coronary heart disease; METs, metabolic equivalents.

<sup>a</sup> Obtained from independent samples *t*-test.

<sup>b</sup> Obtained from Fisher's exact test.

The current study documented that compared with the placebo, taking melatonin for 12 weeks by diabetic people with CHD significantly increased plasma GSH and NO, and significantly decreased plasma MDA, PCO and serum hs-CRP levels, but plasma

**Table 2**  
Dietary intakes of type 2 diabetic patients with coronary heart disease at study baseline and throughout the study.<sup>a</sup>

	Placebo group (n = 30)	Melatonin group (n = 30)	P <sup>b</sup>
Energy (kcal/d)	2349 ± 188	2360 ± 247	0.85
Carbohydrates (g/d)	328.3 ± 31.5	321.1 ± 63.5	0.58
Protein (g/d)	81.9 ± 12.7	88.2 ± 16.8	0.10
Fat (g/d)	82.8 ± 10.6	83.9 ± 14.5	0.74
SFA (g/d)	24.7 ± 3.9	26.6 ± 5.2	0.12
PUFA (g/d)	25.3 ± 5.8	26.1 ± 6.3	0.60
MUFA (g/d)	23.2 ± 6.3	23.7 ± 6.1	0.74
Cholesterol (mg/d)	221.7 ± 127.0	214.2 ± 102.2	0.80
TDF (g/d)	20.5 ± 4.3	18.8 ± 4.6	0.15
Calcium (mg/d)	1149.8 ± 181.8	1167.7 ± 160.8	0.68
Zinc (mg/d)	10.1 ± 2.1	10.9 ± 2.3	0.14
Magnesium (mg/d)	287.9 ± 60.6	284.5 ± 55.3	0.81
Manganese (mg/d)	2.5 ± 0.7	2.3 ± 0.9	0.47
Selenium (μg/d)	56.3 ± 6.3	55.2 ± 6.0	0.53
Vitamin D (μg/d)	2.8 ± 0.7	2.8 ± 0.8	0.91
Vitamin C (mg/d)	74.0 ± 12.5	72.9 ± 13.6	0.74
Vitamin B2 (mg/d)	1.8 ± 0.3	1.7 ± 0.2	0.72
Vitamin B6 (mg/d)	1.6 ± 0.7	1.4 ± 0.3	0.15
Folate (μg/d)	297.8 ± 79.1	279.8 ± 95.5	0.43
Vitamin B12 (μg/d)	3.9 ± 1.0	4.2 ± 1.0	0.17

MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; TDF, total dietary fiber.

<sup>a</sup> Values are means ± SDs.

<sup>b</sup> Obtained from independent samples *t*-test.

TAC levels did not influence by melatonin supplementation. Earlier, observational and interventional studies have documented that melatonin supplementation has anti-inflammatory effects and antioxidant actions [18], but the findings have not always consistent. Dominguez-Rodriguez et al. [7] demonstrated that melatonin supplementation for 6 weeks in ZDF rats improved the pro-inflammatory state and reduced oxidative stress. In addition, melatonin supplementation (6 mg/day) for 40 days to obese women significantly improved biomarkers oxidative stress and inflammatory factors [11]. Melatonin supplementation at a dosage of 5 mg/day for 8 weeks among people with metabolic syndrome also reduced MDA and increased catalase activity [10]. Moreover, taking melatonin at a dosage of 5 mg/day for 30 days to patients with T2DM resulted in a significant increase in superoxide dismutase activity and a significant reduction in MDA concentrations [19]. Increased lipid peroxidation products; especially MDA is possibly correlated with many diseases such as cancer, cardiovascular disease (CVD) and diabetes mellitus [20]. Moreover, lipid peroxidation products have an important role in the pathogenesis of diabetes [20]. Previous studies demonstrated that the endothelium of patients with diabetes do not produce sufficient amount of NO [21]. Therefore, decreasing oxidative stress and inflammation with melatonin may decrease diabetes and CVD events. Melatonin inhibits oxidation reactions catalyzed by scavenges reactive oxygen species, thereby decreasing lipid peroxidation [22]. Likewise, taking melatonin may reduce oxidative stress through regulating the activities of antioxidative enzymes [23] and stimulating glutathione synthesis via promoting glucose-6-phosphate dehydrogenase activity [24].

We found that 12-week melatonin supplementation to diabetic people with CHD was associated with significant reductions in FPG, serum insulin, HOMA-IR, and a significant rise in QUICKI compared with the placebo. In previous two studies by Peschke et al. [8,9] a significant reduction in insulin values and an elevated expression of melatonin receptors after enteral intake was reported in both normal Wistar and type 2 diabetic Goto-Kakizaki rats. In addition, melatonin supplementation in combination with exercise significantly improved insulin resistance, hypertension, and inflammatory cytokines via up-regulation of glucose transporter type 4, peroxisome proliferator-activated receptor gamma coactivator 1α and mitochondrial biogenesis in T2DM rats [25]. Melatonin administration due to its glucose-lowering effect might be effective to decrease diabetic complications. The inhibitory effects of melatonin intake on insulin metabolism may be explained via two G-protein-coupled receptors, MT1 (cAMP signaling pathway) and MT2 (cGMP signaling pathway) [26].

The current study demonstrated that compared with the placebo, the 12-week melatonin supplementation to diabetic patients with CHD resulted in a significant reduction in HDL-cholesterol and significant drop in total-/HDL-cholesterol ratio and blood pressures, but serum triglycerides, VLDL-, total- and LDL-cholesterol levels did not affect by melatonin supplementation. Agil et al. [27] found that melatonin intake in ZDF rats significantly increased HDL-cholesterol, and decreased LDL-cholesterol; but it had no effect on total cholesterol levels. However, supplementation with 6 mg/day of melatonin for 2 weeks increased VLDL-cholesterol, but did not influence LDL- and HDL-cholesterol concentrations in postmenopausal women [28]. Dyslipidemia in diabetic and CVD people is characterized by moderately increased triglycerides and reduced HDL-cholesterol concentrations; these changes contribute to atherosclerotic cardiovascular diseases [29]. Melatonin may increase HDL-cholesterol through augmented cholesterol esterification mediated by higher lecithin-cholesterol acyltransferase activity [30].

**Table 3**

Biomarkers of oxidative stress and cardio-metabolic risk at baseline and after the 12-week intervention in type 2 diabetic patients with coronary heart disease.

	Placebo group (n = 30)			Melatonin group (n = 30)			P <sup>a</sup>
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
TAC (mmol/L)	876.4 ± 190.9	884.0 ± 363.4	7.6 ± 271.6	734.8 ± 68.4	748.8 ± 93.7	14.0 ± 63.9	0.90
GSH (μmol/L)	546.2 ± 148.4	535.1 ± 181.1	-11.1 ± 137.6	604.0 ± 79.3	668.7 ± 103.5	64.7 ± 105.7	0.02
MDA (μmol/L)	2.9 ± 0.8	3.0 ± 0.7	0.1 ± 0.5	2.6 ± 0.2	2.4 ± 0.2	-0.2 ± 0.3	0.007
PCO (nmol/mg protein)	2.17 ± 0.15	2.20 ± 0.16	0.03 ± 0.07	2.15 ± 0.17	2.03 ± 0.17	-0.12 ± 0.08	<0.001
hs-CRP (ng/mL)	3619.2 ± 3416.2	3742.2 ± 3406.9	122.9 ± 1230.4	4040.0 ± 2318.4	2576.7 ± 1763.9	-1463.3 ± 2153.8	0.001
NO (μmol/L)	41.9 ± 5.2	38.6 ± 8.1	-3.3 ± 9.6	46.1 ± 3.8	47.0 ± 5.0	0.9 ± 4.7	0.03
FPG (mg/dL)	156.5 ± 60.1	151.0 ± 55.0	-5.5 ± 32.4	170.9 ± 60.3	141.5 ± 49.2	-29.4 ± 49.0	0.03
Insulin (μU/mL)	12.2 ± 7.3	12.9 ± 7.2	0.7 ± 4.2	13.7 ± 6.5	11.5 ± 4.8	-2.2 ± 4.1	0.008
HOMA-IR	4.8 ± 3.7	4.8 ± 3.4	0.01 ± 1.6	6.0 ± 3.9	5.0 ± 2.9	-1.0 ± 2.2	0.04
QUICKI	0.31 ± 0.03	0.31 ± 0.02	-0.004 ± 0.01	0.30 ± 0.02	0.31 ± 0.02	0.006 ± 0.01	0.01
Triglycerides (mg/dL)	145.7 ± 53.1	143.8 ± 46.9	-1.9 ± 31.6	142.0 ± 73.8	140.4 ± 70.1	-1.6 ± 35.6	0.97
VLDL-cholesterol (mg/dL)	29.1 ± 10.6	28.8 ± 9.4	-0.3 ± 6.3	28.4 ± 14.8	28.1 ± 14.0	-0.3 ± 7.1	0.97
Total cholesterol (mg/dL)	141.4 ± 19.5	142.6 ± 19.3	1.2 ± 15.4	129.7 ± 24.1	129.7 ± 22.4	0.1 ± 5.4	0.70
LDL-cholesterol (mg/dL)	66.4 ± 18.0	68.0 ± 17.5	1.6 ± 15.5	59.8 ± 24.7	57.5 ± 21.2	-2.2 ± 9.6	0.25
HDL-cholesterol (mg/dL)	45.9 ± 8.1	45.9 ± 8.7	-0.01 ± 4.4	41.5 ± 7.3	44.2 ± 7.9	2.6 ± 5.5	0.04
Total-/HDL-cholesterol ratio	3.16 ± 0.63	3.19 ± 0.66	0.03 ± 0.35	3.18 ± 0.61	2.99 ± 0.59	-0.18 ± 0.38	0.02
SBP (mmHg)	129.9 ± 11.1	130.9 ± 11.8	1.0 ± 7.5	134.0 ± 11.5	129.7 ± 7.5	-4.3 ± 9.6	0.01
DBP (mmHg)	80.2 ± 7.0	80.3 ± 6.1	0.1 ± 3.6	78.5 ± 6.7	75.7 ± 6.0	-2.8 ± 7.3	0.04
BDI score	21.8 ± 3.8	21.1 ± 3.9	-0.7 ± 0.9	21.1 ± 4.4	18.3 ± 4.3	-2.7 ± 1.8	<0.001
BAI score	15.3 ± 4.8	14.9 ± 5.2	-0.4 ± 1.1	14.8 ± 5.4	12.8 ± 4.1	-2.0 ± 3.4	0.02

All values are means ± SDs.

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NO, nitric oxide; PCO, protein carbonyl; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TAC, total antioxidant capacity.

<sup>a</sup> P values represent the time × group interaction (computed by analysis of the one-way repeated measures ANOVA).

The current study had some limitations. We did not evaluate the effects of melatonin supplementation on serum and/or urinary melatonin. In addition, this study was adequately powered for examining the primary objective.

Overall, 10 mg/day of melatonin supplementation after 12 weeks among diabetic people with CHD had beneficial effects on plasma GSH, NO, MDA, PCO, serum hs-CRP levels, glycemic control, HDL-cholesterol, total-/HDL-cholesterol ratio, blood pressures and parameters of mental health. A longer treatment or a supplementation with a higher dose of melatonin may affect TAC or other cardio-metabolic markers.

### Authors' contributions

ZA contributed in conception, design, statistical analysis and drafting of the manuscript. FR, VO and FB, contributed in conception, data collection and manuscript drafting. Dr Russel J. Reiter, who reviewed the proposal and manuscript, and offered critical comments. The final version was confirmed by all authors for submission.

### Conflict of interest

None.

### Clinical trial registration number

<http://www.irct.ir:IRCT2017051333941N1>.

### Acknowledgments

The present study was supported by a grant from the Vice-chancellor for Research, KUMS, Kashan, and Iran (grant no. 96025).

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2017.12.004>.

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