

Original Article

## The impact of obesity on hypertension and diabetes control following healthy Lifestyle Intervention Program in a developing country setting

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### Abstract

**BACKGROUND:** The aim of this study was to evaluate the impact of obesity and overweight on diabetes mellitus (DM) and hypertension (HTN) control in a healthy lifestyle intervention program in Iran.

**METHODS:** Within the framework of the Isfahan Healthy Heart Program (IHHP), a community trial that was conducted to prevent and control cardiovascular disease and its risk factors, two intervention counties (Isfahan and Najafabad) and one reference county (Arak) were selected. Demographic information, medical history, anti-diabetic and anti-hypertensive medications use were asked by trained interviewers in addition to physical examination and laboratory tests for 12514 adults aged more than 19 years in 2001 and were repeated for 9572 adults in 2007.

**RESULTS:** In women, the frequency of HTN control change significantly neither in normal weight nor in those with high body mass index (BMI), waist circumference (WC) or waist to hip ratio (WHR). In men, the frequency of HTN control was only significant among those with high WHR, whereas the interaction between changes in intervention compared to reference area from 2001 to 2007 was significant in men with normal or high WC or WHR. In intervention area, the number of women with high BMI who controlled their DM increased significantly from 2001 to 2007 ( $p = 0.008$ ), however, this figure decreased in men. In reference area, obesity indices had no significant association with DM control. The percentage of diabetic subjects with high WC who controlled their DM decreased non-significantly in intervention area compared to reference area in 2007. A non-significant increase in controlled DM among men and women with high WHR was observed between intervention and reference areas.

**CONCLUSIONS:** Our lifestyle interventions did not show any improving effect on HTN or DM control among obese subjects based on different obesity indices. Other lifestyle intervention strategies are suggested.

**KEYWORDS:** Hypertension, Diabetes, Obesity, Control, Prevention, Iran.

JRMS 2011; 16(Special Issue): 368–376

Obesity and overweight are now considered to be serious health problems, with an increasing prevalence worldwide,<sup>1</sup> such that the World Health Organization (WHO) reported overweight and obesity

to be a rising epidemic.<sup>2</sup> Previous studies in Iran showed a high prevalence of obesity.<sup>3</sup> Overall, 11.2% of men and 28.1% of women are obese and 36.6% of Iranian men and 35.9% of women are overweight.<sup>4,5</sup>

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Obesity is an independent risk factor for a number of chronic diseases, including hypertension (HTN), Diabetic Mellitus (DM), cardiovascular disease (CVD) and some cancers.<sup>6</sup> It seems that obesity, DM and HTN are known to increase in both developed and developing populations.<sup>1</sup> Decrease in adiposity is one of the most effective preventive measures in decreasing not only the overall cardiovascular risk but also blood pressure (BP) and increasing DM control.<sup>7</sup>

Many studies suggested that lifestyle modification has great potential to reduce obesity and other related cardiovascular risk factors.<sup>8</sup> Some have recently shown effects of lifestyle modification on control of BP and decrease in the incidence of DM in individuals with impaired glucose tolerance.<sup>9</sup> Efforts which focus on changing lifestyle can lead to change people's attitude, behavior and practice in the whole population as well as high risk groups.<sup>10,11</sup>

In this regard, Isfahan Healthy Heart Programme (IHHP), a community-based intervention program, was designed and implemented to prevent CVD and control its related risk factors by promoting healthy lifestyle. IHHP used both the general population and high risk intervention strategies for CVD prevention.<sup>12,13</sup> In this study, we intended to evaluate the impact of obesity and overweight on DM and HTN control following IHHP interventions.

## Methods

IHHP was conducted in three counties, Isfahan and Najaf-Abad as intervention areas and Arak as reference area, all located in central Iran. Arak, placed 375km north-west of Isfahan was selected because of the similarity with the intervention areas regarding socio-economic, demographic and health profile and good cooperation.<sup>13</sup> Started in 2000, the programme was consisted of three phases, the first as baseline survey for situational analysis, the second included implementing interventional activities in the intervention areas (late in 2001-2005), and the third as post-intervention survey (2007) to evaluate the programme out-

comes.<sup>12,13</sup> Having resided for more than five years in these cities, not being pregnant for females and being mentally healthy were the criteria for participating in this study. After the baseline survey, a five-year intervention programme was started both in urban and rural areas of Isfahan and Najaf-Abad. Arak was evaluated during this period as the reference area.

During the intervention phase, we took a systematic approach to undertake process, impact and outcome evaluation.<sup>14</sup> Interventional activities were designed in ten projects based on different target groups to address the whole community and specific target populations and subjects in an integrated package.<sup>10</sup> The main themes of interventions included healthy nutrition, tobacco control, physical activity and coping stress. Public and health professionals training, intersectoral collaboration and legislation were among main strategies. Specific educational companion for hypertensive and diabetic patients were implemented.<sup>10</sup>

IHHP team conducted the baseline survey on 12514 adults, aged  $\geq 19$  years in 2000-2001 and carried out the same survey on 9572 adults in 2007 as outcome evaluation. Multistage random sampling was used to select participants in both studies in intervention and reference areas. The sample distribution was consistent with sex and age distribution in the population of three counties. Written informed consents were obtained from all subjects. The methods were in accordance with ethical standards of the Ethical Committee of Isfahan University of Medical Sciences. Full details of the program have been reported elsewhere.<sup>12,13</sup>

Initially, a questionnaire was completed at each subject's home by trained interviewers. It contained demographic information, smoking, nutritional and physical activity habits, medical history, and consumption of relevant medications especially anti-diabetic and anti-hypertensive agents. Subsequently, participants were invited to certain health centers, where physical examination and blood sampling after 12 to 14 hour fasting were carried out. Subjects were weighed with light clothes

and no shoes, and their height was measured and body mass index (BMI) was calculated.<sup>15</sup> Waist circumference (WC) was measured at the midpoint between the iliac crest and the lower margin of the rib to the nearest half-centimeter. Hip circumference (HC) was measured and waist to hip ratio (WHR) was calculated too.<sup>15</sup> The BP was measured twice with a standardized method, with the subjects in a sitting position, and the average BP was taken into consideration.<sup>12</sup>

In addition to fasting plasma glucose (FPG), 2-hour plasma glucose (2hpp) after consumption of 75 gram of glucose was also measured. Serum lipids, including total cholesterol (TC), triglyceride (TG), and HDL-cholesterol (HDL-C) were measured using enzymatic method. LDL cholesterol (LDL-C) was measured using Friedwald formula.<sup>13</sup> All blood sampling procedures were performed in the central laboratory of the Isfahan Cardiovascular Research institute, which is standardized with the Department of Epidemiology, K.U. Leuven, Belgium and Lab Quality External Assessment Services, Helsinki, Finland. Both quality controls showed good correlations.

The subjects of the present study were either hypertensives or diabetics who were selected among all participants of the IHHP study from the baseline and the post-intervention surveys. Controlled HTN was defined as SBP < 140 mmHg or DBP < 90 mmHg and currently taking antihypertensive medications.<sup>16</sup> Controlled DM was defined as FPG < 140 mg/dl and currently taking anti-diabetic treatment.<sup>16</sup> Normal weight was defined as BMI < 25 kg/m<sup>2</sup> and overweight plus obese as BMI ≥ 25 kg/m<sup>2</sup>.<sup>17</sup> Abdominal obesity was defined as WC ≥ 102 cm for men and ≥ 88 cm for women. Gender specific cut-off points of ≥ 0.95 cm and ≥ 0.80 cm for WHR were used for men and women respectively.<sup>15</sup>

### Statistical Analysis

Data entry was carried out using EPI info™. All data were analyzed by SPSS (SPSS Inc, Chicago, IL, USA; Version 15). The averages were reported as Mean ± SD and frequencies

as percents. For all analyses, statistical significance was assessed at a level of 0.05 (2-tailed). T-test and Mann-Whitney tests were used to compare continuous variables. The groups were compared using the chi-square test (or Fisher's exact test if required) for the categorical variables. In addition, area (intervention vs. reference)\* time (2001 vs. 2007) interaction term was tested in logistic regression model. Differences between 2001 and 2007 values were compared between intervention and reference areas among males and females separately using two-way ANOVA.

### Results

The mean values of risk factors in men and women with controlled hypertension in 2001 and 2007 are presented in Table 1. In the intervention area, from 2001 to 2007 the WC declined significantly among controlled hypertensive women (102.22 ± 12.77 vs. 98.30 ± 11.36), and non-significantly among men (99.17 ± 8.49 vs. 97.82 ± 10.14). However, it increased significantly among women (95.94 ± 13.55 vs. 99.92 ± 11.898) and men (92.5 ± 10.75 vs. 98.28 ± 10.75) in the reference area. In women, WHR did not show any difference from 2001 to 2007 in intervention area, however, it increased significantly in reference area (p < 0.05). No significant change was seen regarding lipoproteins level in intervention and reference areas.

Table 2 shows the basic characteristics of controlled diabetic men and women in 2001 and 2007. There was no significant difference in the mean age in both sexes. In the intervention area, WC decreased in women; whereas it increased significantly among men and women in reference area. No significant change was observed in men or women regarding systolic or diastolic BP, TC, TG, HDL-C and LDL-C.

The frequency of HTN control by obesity indices in 2001 and 2007 are presented in table 3. In women, the frequency of HTN control did not change significantly in normal weight or in those with high BMI, WC or WHR. The p-value for interaction was significant only for controlled HTN in women with high WC. In men, the frequency of HTN control was only

**Table 1.** Basic Characteristics of controlled hypertensive men and women following Isfahan Healthy Heart Program in intervention and reference areas in 2001 and 2007

	Women				Interaction p-value	Men				Interaction p-value
	Intervention Area Mean(SD)		Reference area Mean(SD)			Intervention Area Mean(SD)		Reference area Mean(SD)		
	2001 N = 82	2007 N = 116	2001 N = 80	2007 N = 97		2001 N = 24	2007 N = 51	2001 N = 39	2007 N = 50	
Age (Year)	57.31 (14.32)	57.68 (13.19)	57.85 (12.63)	57.55 (15.45)	0.82	57.17 (13.52)	58.98 (12.26)	57.17 (16.12)	58.82 (16.71)	0.97
BMI (Kg/m <sup>2</sup> )	28.44 (4.71)	29.05 (4.32)	27.32 (4.38)	27.60 (4.75)	0.73	27.20 (3.59)	27.40 (3.26)	25.29 (3.68)†	26.75 (4.46)	0.32
WC (cm)	102.22 (12.77)	98.30 (11.36)*	95.94 (13.55)†	99.92 (11.898)*‡	0.003	99.17 (8.49)	97.82 (10.14)	92.5 (10.75)†	98.28 (12.21)*	0.049
WHR (cm)	0.96 (0.07)	0.96 (0.09)	0.93 (0.10)†	0.97 (0.08)*	0.086	0.9 (0.06)	0.94 (0.06)	0.93 (0.11)	0.96 (0.09)	0.07
FPG (mg/dl)	99.99 (45.81)	96.55 (24.26)	85.85 (20.62)†	97.87 (28.21)*	0.017	92.67 (25.01)	110.01 (41.21)*	103.47 (57.48)	96.98 (26.13)	0.07
Total Cholesterol (mg/dl)	239.67 (51.06)	217.12 (41.69)*	228.97 (52.07)	210.09 (43.25)*	0.71	218.54 (65.04)	203.74 (42.94)	208.34 (69.59)	199.78 (35.72)	0.72
Triglyceride (mg/dl)	217.21 (109.39)	190.98 (166.26)	188.42 (73.56)	170.56 (80.74)	0.73	219.29 (102.31)	197.63 (112.22)	207.84 (134.83)	202.20 (114.99)	0.68
hdl-c (mg/dl)	49.26 (9.93)	46.54 (12.04)	48.77 (9.86)	46.11 (10.83)	0.98	46.37 (9.33)	38.96 (10.62)*	44.50 (9.81)	40.54 (11.69)	0.33
ldl-c (mg/dl)	147.70 (38.57)	132.18 (33.05)*	143.21 (42.61)	128.93 (35.98)*	0.88	121.76 (47.33)	125.74 (32.86)	116.72 (46.30)	119.01 (30.99)	0.89

BMI: Body Mass Index, WC: waist circumference. WHR: waist-to-hip ratio, FPG: Fasting plasma glucose, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol.

\* Indicates p-value between 2001 and 2007 by t- test or Mann-Whitney test.

† Indicates p-value between intervention and reference areas in 2001 by t-test or Mann-Whitney test.

‡ Indicates p-value between intervention and reference areas in 2007 by t-test or Mann-Whitney test.

P-value for Interactions obtained from two-way ANOVA.

**Table 2.** Basic Characteristics of controlled diabetic men and women following Isfahan Healthy Heat Program in intervention and reference areas in 2001 and 2007

	Women				Interaction P-Value	Men				interaction p-value
	Intervention Area Mean(SD)		Reference area Mean(SD)			Intervention Area Mean(SD)		Reference area Mean(SD)		
	2001 N = 27	2007 N = 37	2001 N = 24	2007 N = 34		2001 N = 21	2007 N = 27	2001 N = 17	2007 N = 19	
Age (Year)	53.48 (13.74)	54.29 (12.99)	54.62 (12.91)	52.88 (9.97)	0.57	55.14 (12.95)	59.71 (13.06)	55.76 (15.13)	55.00 (13.21)	0.38
BMI (Kg/m <sup>2</sup> )	29.06 (5.10)	30.14 (3.61)	28.21 (4.67)	28.47 (3.62)	0.59	27.91 (4.36)	26.71 (4.32)	27.38 (5.13)†	25.98 (3.77)	0.92
WC (cm)	103.98 (10.78)	99.62 (11.33)	96.61 (13.96)‡	102.21 (11.37)	0.031	102.19 (14.37)	95.52 (11.56)	91.88 (13.20)‡	95.71 (7.99)	0.07
WHR(cm)	0.96 (0.06)	0.97 (0.09)	0.92 (0.07)‡	0.96 (0.098)	0.18	0.96 (0.06)	0.95 (0.06)	0.91 (0.097)‡	0.93 (0.06)	0.23
Systolic Blood Pres- sure (mmHg)	128.14 (22.67)	125.07 (16.79)	132.71 (22.24)	131.03 (29.58)	0.87	133.81 (23.92)	131.94 (23.43)	135.88 (27.35)	128.42 (20.55)	0.59
Diastolic Blood Pres- sure (mmHg)	81.11 (14.71)	79.66 (11.72)	85.10 (2.97)	78.52 (12.40)*	0.28	79.52 (13.52)	78.31 (17.29)	83.67 (11.89)	78.02 (8.60)	0.47
Total Cho- lesterol (mg/ dl)	190.03 (75.87)	177.24 (99.40)	180.04 (88.82)	208.52 (109.32)	0.24	208.61 (74.46)	196.37 (158.11)	225.47 (163.65)	192.68 (130.24)	0.73
Triglyceride (mg/ dl)	224.19 (46.91)	213.86 (44.59)	206.58 (46.42)	223.23 (44.28)	0.11	206.52 (41.94)	185.37 (28.77)*	203.53 (52.14)	197.89 (30.63)	0.36
hdl-c (mg/ dl)	53.48 (9.63)	44.72 (9.44)*	47.04 (9.34)‡	45.09 (14.12)	0.1	46.47 (9.54)	37.14 (9.91)*	48.18 (12.18)	42.47 (12.54)	0.46
ldl-c (mg/ dl)	130.37 (38.93)	133.37 (35.86)	128.42 (43.48)	136.92 (35.94)	0.71	120.61 (43.55)	112.78 (24.99)	106.50 (29.16)	118.02 (31.22)	0.2

BMI: Body Mass Index, WC: waist circumference. WHR: waist-to-hip ratio, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol.

\* Indicates p-value between 2001 and 2007 by t-test or Mann-Whitney test.

† Indicates p-value between intervention and reference areas in 2001 by t-test or Mann-Whitney test.

‡ Indicates p-value between intervention and reference areas in 2007 by t-test or Mann-Whitney test.

P-value for Interactions obtained from two-way ANOVA.

**Table 3.** Sex-based hypertension controlled by obesity indices following Isfahan Healthy Heart Program in intervention and references areas in 2001 and 2007

		Women				Interaction P-value	Men				Interaction P-value	Total				Interaction P-value
		Intervention Area		Reference area			Intervention Area		Reference area			Intervention Area		Reference area		
		2001 n(%)	2007 n(%)	2001 n(%)	2007 n(%)		2001 n(%)	2007 n(%)	2001 n(%)	2007 n(%)		2001 n(%)	2007 n(%)	2001 n(%)	2007 n(%)	
BMI kg/m <sup>2</sup>	Normal	16 (20.0)	19 (17.0)	26 (32.5)	31 (32.0)	0.72	6 (25.0)	12 (24.5)	21 (53.8)	18 (36.0)	0.33	22 (21.2)	31 (19.3)	47 (39.5)	49 (33.3)	0.71
	Obese	64 (80.0)	93 (83.0)	54 (67.5)	66 (68.0)		18 (75.0)	37 (75.5)	18 (46.2)	32 (64.0)		82 (78.8)	130 (80.7)	72 (60.5)	98 (66.7)	
WC (cm)	Normal	9 (11.0)	24 (21.2)	22 (27.5)	16 (18.2)	0.019	12 (50.0)	33 (66.0)	31 (81.6)	24 (61.5)	0.022	21 (19.8)	57 (35.0)*	53 (44.9)	40 (31.5)	0.001
	Obese	73 (89.0)	89 (78.8)	58 (72.5)	72 (81.8)		12 (50.0)	17 (34.0)	7 (18.4)	15 (38.5)		85 (80.2)	106 (65.0)	65 (55.1)	87 (68.5)	
WHR (cm)	Normal	2 (2.4)	6 (5.3)	5 (6.3)	2 (2.3)	0.12	5 (20.8)	25 (50.0)	20 (52.6)	17 (43.6)	0.021	7 (6.6)	31 (19.0)*	25 (21.2)	19 (15.0)	0.003
	Obese	80 (97.6)	107 (94.7)	75 (93.8)	86 (97.7)		19 (79.2)	25 (50.0)*	18 (47.4)	22 (56.4)		99 (93.4)	132 (81.0)	93 (78.8)	108 (85.0)	

BMI: body mass index (kg/m<sup>2</sup>) Normal: < 25 kg/m<sup>2</sup>; Overweight and obese ≥ 25.

WC: waist circumference. Normal: in women 88 <, in men 102 <; Obese: in women 88 ≥, in men 102 ≥ cm.

WHR: waist-to-hip ratio. Normal: in women < 0.8, in men < 0.95; Obese: in women ≥ 0.8, in men 0.95 ≥ cm.

Controlled hypertension is defined as SBP < 140 mmHg or DBP < 90 mmHg or currently taking blood pressure medications.

\* Indicates p-value between 2001 and 2007 by chi-Square or Fisher Exact test.

P-value for Interactions obtained from two-way ANOVA.

significant among those with high WHR, whereas the p-value for interaction between changes in intervention compared to reference area from 2001 to 2007 was significant in men with normal or high WC or WHR.

The prevalence of controlled DM based on obesity indices in both intervention and reference areas at baseline and after interventions are presented in table 4. In intervention area, the number of women with high BMI who controlled their DM increased significantly from 2001 to 2007 ( $p = 0.008$ ), however, this figure decreased in men. In reference area obesity indices had no significant association with control of DM. The percentage of diabetic subjects with high WC who controlled their DM decreased non-significantly in intervention area compared to reference area in 2007. However, the p-value for interaction was significant in

women. A non-significant increase in controlled DM among men and women with high WHR was observed between intervention and reference area.

## Discussion

We observed non-significant changes in DM control in both sexes and HTN control in women following IHHP lifestyle intervention strategies. It can be explained by the non-significant changes in obesity in diabetic and hypertensive patients in intervention areas. On the contrary, our results demonstrated that WC decreased significantly in hypertensive women and non-significantly in hypertensive men in intervention area and increased significantly in both sexes in reference areas. In diabetic patients, WC decreased significantly in men in intervention area and increased

**Table 4.** Sex-based diabetes controlled by obesity indices following Isfahan Healthy Heart Program in intervention and reference areas in 2001 and 2007

Obesity indexes	Women				Interaction P-value	Men				Interaction P-value	Total				Interaction P-value	
	intervention area		reference area			intervention area		reference area			intervention area		reference area			
	2001 n(%)	2007 n(%)	2001 n(%)	2007 n(%)		2001 n(%)	2007 n(%)	2001 n(%)	2007 n(%)		2001 n(%)	2007 n(%)	2001 n(%)	2007 n(%)		
BMI kg/m <sup>2</sup>	Normal	7 (25.9)	1 (2.7) *	7 (29.2)	6 (17.6)	0.14	5 (23.8)	10 (37.0)	7 (41.2)	7 (36.8)	0.39	12 (25.0)	11 (17.2)	14 (34.1)	13 (24.5)	0.99
	Obese	20 (74.1)	36 (97.3)	17 (70.8)	28 (82.4)		16 (76.2)	17 (63.0)	10 (58.8)	12 (63.2)		36 (75.0)	53 (82.8)	27 (65.9)	40 (75.5)	
WC (cm)	Normal	1 (3.7)	7 (18.9)	8 (33.3)	4 (17.4)	0.041	11 (52.4)	18 (66.7)	14 (82.4)	11 (78.6)	0.44	12 (25.0)	25 (39.1)	22 (53.7)	15 (40.5)	0.057
	Obese	26 (96.3)	30 (81.1)	16 (66.7)	19 (82.6)		10 (47.6)	9 (33.3)	3 (17.6)	3 (21.4)		36 (75.0)	39 (60.9)	19 (46.3)	22 (59.5)	
WHR (cm)	Normal	0 (0)	0 (0)-	1 (4.2)	0 (0)	0.99	8 (38.1)	10 (37.0)	13 (76.5)	9 (64.3)	0.59	8 (16.7)	10 (15.6)	14 (34.1)	9 (24.3)	0.58
	Obese	27 (100.0)	37 (100.0)	23 (95.8)	2 (100.0)		13 (61.9)	17 (63.0)	4 (23.5)	5 (35.7)		40 (83.3)	54 (84.4)	27 (65.9)	28 (75.7)	

BMI: body mass index (kg/m<sup>2</sup>) Normal: < 25 kg/m<sup>2</sup>; Overweight and obese ≥ 25.

WC: waist circumference. Normal: in women 88 <, in men 102 <; Obese: in women 88 ≥, in men 102 ≥ cm.

WHR: waist-to-hip ratio. Normal: in women < 0.8, in men < 0.95; Obese: in women ≥ 0.8, in men 0.95 ≥ cm.

Controlled diabetes was defined as FPG < 140 mg/dl and currently taking anti-diabetic treatment.

\* Indicates p-value between 2001 and 2007 by chi-Square or Fisher Exact test.

P-value for Interactions obtained from two-way ANOVA.

significantly in women living in reference area. Previous findings from IHHP showed no significant difference regarding BMI and significant decrease of WC between intervention and reference areas at the population level. It is known that weight loss can not only prevent or reverse blood pressure elevations but also have a favorable impact on other obesity-related CVD risk factors such as DM and hyperlipidemia.<sup>7,18,19</sup> The interaction between body weight and HTN is very complex and both environmental and genetic factors are involved.<sup>20</sup> Non-pharmacological treatments including lifestyle behaviours have an important role on HTN and DM control. We reported the impact of IHHP on lifestyle behaviours improvement before.<sup>10</sup> Furthermore HTN control reached 15.8% following the implementation of IHHP interventions.<sup>21</sup>

Large studies have shown that central obesity has powerful relationship with CVD risk factors.<sup>22-24</sup> Strong relationship between decreasing abdominal obesity and BP control has been reported specifically in women. In addition to lifestyle behaviors, it could be related to more complex interaction of female hormones with blood pressure. Although lifestyle behaviours have been improved following the implementation of IHHP interventions,<sup>10</sup> however, HTN and DM control was not improved in patients with high BMI or WC who lived in intervention areas.

## Conclusion

This study demonstrated that IHHP as a community trial targeting behaviours and CVD risk factors control did not show improving effect on HTN or DM control among obese subjects

based on their different obesity indices. Our results suggested that other interventional strategies aiming at decreasing obesity might have an impact on HTN and DM control.

### Limitations

As an important limitation, we acknowledge the low rate of controlled hypertensive and diabetic patients in the studied population. This might have affected the difference between two groups based on different obesity indices.

### Conflict of Interests

Authors have no conflict of interests.

### Authors' Contributions

AAT designed the study and drafted this manuscript, MG carried out the study and drafted the manuscript, NT drafted the manuscript and tables, NS designing the study, revising the manuscript procedures and drafting the manuscript, AK helped in designing the study, BZ helped in statistical analysis, SZ revising the manuscript. All authors read and approved the final manuscript.

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### Acknowledgment

This study was a part of Isfahan Healthy Heart Program that was supported by Grant No. 31309304 from the Iranian Budget and Programming Organization in the Department of Health, Ministry of Health and Medical Education, Isfahan Cardiovascular Research Centre and Isfahan Provincial Health Centre, both affiliated to Isfahan University of Medical Sciences. We like to express our sincere thanks to the big team who conducted IHHP in Isfahan, Najafabad and Arak.



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